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REVIEW

Evaluation of the specific roles of anions in electron transport and energy transfer reactions in photosynthesis

A. JAJOO*, S. BHARTI*, and P. MOHANTY**

*School of Life Sciences, Vigyan Bhawan, Khandwa Road, Devi Ahilya Vishwavidyalaya, Indore-452 001, India**
*LBG House, Orissa Housing Board Colony, Phase I, Block 11, H/11/6, CSPUR, Bhubaneshwar 751016, Orissa, India***

Abstract

Ionic environment is important in regulating photosynthetic reactions. The roles of cations, Mn^{2+}, Mg^{2+}, Ca^{2+}, Na^+, and K^+ as cofactors in electron transport, energy transfer, phosphorylation, and carbon assimilation are better known than the roles of anions, except for chloride and bicarbonate. Only a limited information exists on the roles and effects of nitrite, formate, sulphate, and phosphate. In this review, we evaluate and highlight the roles of some specific anions on electron transport as well as on excitation energy transfer processes in photosynthesis. Anions exert significant effects on thylakoid membrane conformation and membrane fluidity, possibly by redistributing the thylakoid membrane surface charges. The anion/cation induced phase transitions in the hydrophilic domains of the thylakoid membranes are probably responsible for the various structural and co-related functional changes under stress. Anions are also important in regulation of energy distribution between the two photosystems. Anions do not only divert more energy from photosystem (PS) 2 to PS1, but can also reverse the effect of cations on energy distribution in a valence-dependent manner. Anions affect also the structure of the photosynthetic apparatus and excitation energy distribution between the two photosystems.

Additional key words: bicarbonate; cations; chloride; chloroplast structure; oxygen evolution; photosystems 1 and 2; thylakoid; water oxidation.

Process of photosynthesis

Photosynthesis is a physico-chemical process by which plants, algae, and photosynthetic bacteria use radiant energy to drive the synthesis of organic compounds. The photosynthetic process depends on a set of complex protein molecules that are located in and around a highly organised membrane. Through a series of energy-transducing reactions, the photosynthetic machinery transforms radiant energy into saccharides. A key element in photosynthetic energy conversion is electron transport within and between protein complexes and simple organic molecules. The electron transfer reactions are rapid (some of them as fast as a few picoseconds) and highly specific. The photosynthetic reactions are traditionally divided into two stages—the "light reactions" which consist of electron and proton transfer reactions and the "dark reactions" which consist of the biosynthesis of saccharides from CO_2.

The first step in the "light reaction" is the conversion of a photon to an excited electronic state (an exciton) of an antenna pigment molecule. Some excitons are converted back into photons and emitted as fluorescence, some are converted to heat, and a reaction centre (RC) protein traps some. Excitons trapped by a RC provide energy for the primary photochemical reaction of photosynthesis—the transfer of an electron from a donor molecule to the acceptor molecule. In oxygenic photosynthetic organisms, two different RCs, known as photosystem 1 (PS1) and photosystem 2 (PS2), work in series. Intermediate carriers transfer electrons from PS2 to PS1. PS2 uses radiant energy to drive two chemical reactions—the oxidation of water and the reduction of plastoquinone. Photochemistry of PS2 is initiated by charge separation between P680 and pheophytin creating $P680^+/Pheo^-$. Subsequent electron transfer steps prevent the primary

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Abbreviations: ABS, anion binding site; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, dichlorophenol indophenol; ETC, electron transport chain; MV, methyl viologen; OEC, oxygen evolving complex; P700, primary electron donor of PS1; PQ, plastoquinone; PS, photosystem; Q_A, primary quinone electron acceptor of PS2; Q_B, secondary quinone electron acceptor of PS2; RC, reaction centre; Tris, tris-(hydroxymethyl) amino methane; WOC, water oxidising complex.

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charge separation from recombining. PS2 is the only known protein complex that can oxidise water, resulting in the release of oxygen into the atmosphere. How electrons are transferred from water to $P680^+$ remains a mystery (Whitmarsh and Govindjee 1999). The PS1 complex catalyses the oxidation of plastocyanin, a small soluble Cu-protein, and the reduction of ferredoxin, a small Fe-S protein. Primary photochemistry is initiated by a chlorophyll (Chl) *a* dimer, P700. Primary charge separation occurs between primary electron donor P700, and a Chl monomer (A_o). Fig. 1 shows the various components of the electron transport chain.

The part of photosynthesis described above is the focus of the present review in which we have critically examined the physiological and biophysical effects of anions.

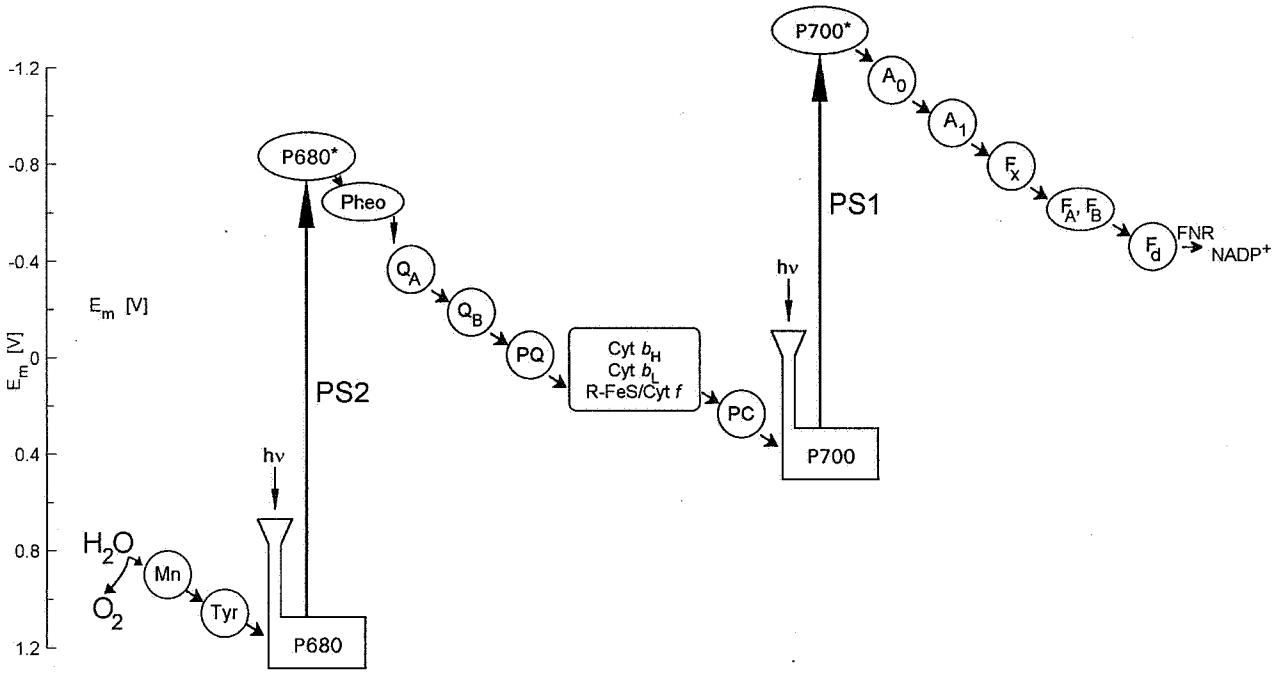

Fig. 1. Electron transport pathways of plants (oxygenic photosynthesis). Membrane-bound electron transport protein complexes involved in transferring electrons are the photosystem 2 and 1 reaction centres (PS2 and PS1) and the cytochrome *bf* complex (Cyt *bf*). Tyr, a specific tyrosine on the D1 protein; P680 and P700, the reaction centre chlorophyll of PS2 and PS1, respectively; Pheo, pheophytin; Q_A, and Q_B, bound plastoquinones; PQ, plastoquinone; Cyt b_L and Cyt b_H, different forms of *b*-type cytochromes; FeS, iron-sulphur centres; Cyt *f*, cytochrome *f*; PC, plastocyanin; A_0, chlorophyll; A_1, phylloquinone; F_x, F_A and F_B, iron-sulphur centres; Fd, ferredoxin; FNR, ferredoxin/$NADP^+$ oxidoreductase; $NADP^+$, nicotineamide adenine dinucleotide phosphate (reduced form).

Roles of ionic environment

Ionic environment regulates a variety of metabolic and physiological functions of plants (Izawa and Good 1966a,b, Homann 1969). Oxygenic photosynthesis is a unique metabolic process of plant, algal, and cyanobacterial cells, wherein the ionic environment of thylakoid membranes influences and regulates many functions, such as electron transport, energy transfer, photophosphorylation, photosynthetic carbon reduction, photorespiration, and other associated metabolic reactions. The effect of ions on electron transport, catalysed by PS1 or PS2 or both, and excitation energy transfer between the two interacting photosystems have been extensively studied. Most of the studies of ions on these processes were directed at evaluating the effects of specific cations and on their ionic charge (mono-, di-, or polyvalent nature) and size (for examples see Table 1).

Mono- and divalent cations affect several primary photoprocesses in thylakoids: initial energy distribution between the two pigment systems, excitation energy redistribution, or 'spillover' from PS2 to PS1, rate constant of thermal dissipative transitions, and activation of RC 2. Some of these effects may occur concurrently with the largest effect on the excitation energy redistribution process. Braun and Malkin (1990) studied the regulation of imbalance in irradiance excitation between PS2 and PS1 by cations and by the energised state of the membrane. They concluded that: (*a*) High cation concentrations change the allocation of excitation energy, probably *via* the screening of negative charges on the thylakoid surface. (*b*) The effect of uncouplers and ionophores in the presence of high cation concentrations most probably reflects, at least in part, the effect on external surface charges, their exposure by membrane energisation, and their screening by cations.

Table 1. Some specific functions regulated by cations in electron transport and energy transfer reactions.

Cation	Assay/technique used	Effect	Reference
Salts such as NaCl, $MgCl_2$	Electron microscopy (pea, spinach, maize, *etc.*)	Stacking of the lamellae	Izawa and Good (1965)
$MgCl_2$ (3.3 mM)	Hill activity, Chl *a* fluorescence, ferricyanide photo-reduction	All increased in presence of $MgCl_2$	Homann (1969)
$MgCl_2$	Hill reaction, NADP reduction	Suppressed spill-over of excitation energy from PS2 to PS1	Murata (1969)
Monovalent cations such as NaCl	Fluorescence emission spectra at room temperature and 77 K	Suppressed spill-over of excitation energy from PS2 to PS1	Murata (1969)
KCl, $MgCl_2$, K-acetate, Na-acetate	Chl *a* fluorescence in *Chorella* cells	Quenching of fluorescence	Mohanty *et al.* (1974)
$CaCl_2$	Polarisation of Chl *a* fluorescence in the presence of DCMU	Reverses the effects of 5 mM NaCl	Vandermeulen and Govindjee (1974)
$MgCl_2$ (3 mM)	Low temperature fluorescence emission spectra	Increase in F_{695}/F_{685} ratio	Wydrzynski and Govindjee (1975)
Ca^{2+} and Mg^{2+}	Chl *a* fluorescence at room temperature and at 77 K, electron microscopy	Cations control energy distribution and alteration in membrane conformation as seen in changes in radiation scattering and thylakoid stacking	Telfer *et al.* (1975)
$MgCl_2$ (5 mM)	Polarisation of Chl *a* fluorescence in the presence of DCMU	Reverses the effects of 5 mM NaCl	Wong and Govindjee (1979)
$MgCl_2$, Ca^{2+}, $MgSO_4$	Ascorbate, DCPIP, TMPD used as electron donors	Stimulated dark recovery of P700	Gross (1979)
$MgCl_2$ (5 mM)	Polarisation of Chl *a* fluorescence in the presence of DCMU	Increase in the degree of polarisation	Wong and Govindjee (1979)
$MgCl_2$ (*ca.*10 mM)	$H_2O \rightarrow$DCIP electron transport rates	Increased electron transport rates in presence of Mg^{2+} under irradiance-limited conditions	Wong *et al.* (1980)
NaCl, $MgCl_2$, TEC.Cl_3	Digitonin method	Induced stacking of thylakoids in grana	Barber and Chow (1982)
Mg^{2+}, Ca^{2+}	$DCIPH_2 \rightarrow$MV reaction at saturating irradiance	PS1 rate decreased as the degree of stacking increased	Ramanujam and Bose (1983)
Mono- and di-valent cations	Room temperature Chl *a* fluorescence, absorption spectra of spheroplasts in *Synechococcus*	Cations alter membrane pigment-protein interactions	Wavare and Mohanty (1985)
NaCl (10 mM) NaCl (100 mM) $MgCl_2$ (5 mM) TEC (100 μM)	Modulated fluorescence method	No imbalance in favour of PS2 Energy transfer in favour of PS2	Braun and Malkin (1990)

When cations (3-5 mM Mg^{2+} or 200 mM Na^+) are added to isolated chloroplasts, the rate of PS1 electron transfer under limiting irradiance decreases by about 20-30 % (Murata 1969). This observation is generally interpreted as due to redistribution of excitation energy in favour of PS2 at the cost of PS1 (Bonaventura and Myers 1969, Murata 1971, Mohanty *et al.* 1973, Jennings *et al.* 1979, 1981, Wong *et al.* 1979). The rate of PS1 electron transport ($DCIPH_2 \rightarrow MV$) under saturating irradiance may decrease by 20-30 % upon addition of 5 mM Mg^{2+} or 200 mM Na^+ (Ramanujam and Bose 1983). This effect of cations cannot be explained in terms of the well-known cation effect on regulation of excitation energy distribution because the rate at saturating irradiance should not be influenced by alteration in distribution of excitation energy. In all these studies, the thylakoids were suspended in a medium containing NaCl and therefore the role of anions was not recognised. Jajoo and Bharti (1995) argued that if cations can cause a distribution of excitation energy more in favour of PS2, then counter anion should cause redistribution of excitation energy more in favour of PS1, and PS2 should get less excitation energy.

Cations exert multiple effects on chloroplast structure and function (Barber 1980, 1982). Cations influence and regulate electron flow catalysed by PS1 and PS2. In particular, the divalent cation Mg^{2+} promotes the interaction of plastocyanin (and other negatively charged electron donors) with P700 (Itoh 1978, 1979a,b, Gross 1979). Cations also act on the reducing side of PS1 (Haehnel *et al.* 1980). They regulate chloroplast structure and function including the grana stacking (Izawa and Good 1966a, Murakami and Packer 1971, Gross and Prasher 1974) and

excitation energy transfer (Murata 1969, Gross and Hess 1973, Mohanty *et al.* 1973, Marsho and Kok 1974). Some of these effects also occur in sub-chloroplast particles. Cations cause a conformational change in the P700-Chl *a* protein complex amounting to a 12 % increase in the alpha helical content. This, in turn, causes (*a*) an increase in the transfer of excitation energy from the light-harvesting Chl *a* molecules of the P700-Chl *a*-protein complex to P700, and (*b*) an increase in the steady state rate of the electron transport observed at high irradiances. Magnesium and other cations stimulate the dark recovery of P700 by various reduced PS1 electron donors such as ascorbate, $DCPIPH_2$, and $TMPDH_2$.

Salts induce significant changes in many reactions in the primary processes of photosynthesis. Many of these effects are probably related to the changes in the thylakoid membrane structure. Salts accelerate P700 reduction after flash irradiation (Tamura *et al.* 1980). In the above studies anions have been assumed to have a non-specific or no effect, as changing one cation salt to another, yielding similar results. Often these studies overemphasised the role of cations and ignored the participation of the anions in the chloroplast photofunctions. Cations, particularly Mg^{2+}, cause an increase in PS2 electron transport rate and yield of Chl *a* fluorescence as well as a decrease in PS1 mediated electron transport rate in a low salt suspension of thylakoids (Murata 1969). Moreover, cation-induced changes are associated with structural organisation of thylakoid membrane (Ramanujam and Bose 1983). The effect of a number of divalent and monovalent cations on the PS1 mediated electron transport activity has also been analysed. PS1 reaction shows more response to cations when $DCIPH_2$ is used as an electron donor, rather than $TMPD_{red}$ or DAD_{red} (Sabat and Mohanty 1989). Similarly salts affected the interaction of MV with PS1. Both anion and cation selectivity was observed (Tripathy *et al.* 1984). Barber and Chow (1979) suggested a correlation between thylakoid stacking and cation-induced decrease in PS1 electron transport.

Discoveries of the anion effects

There has been recurring interest on characterising the role of anions in the photosynthetic reactions, especially in the photosynthetic electron transport. For complete understanding, we shall briefly review some of the studies on anion effects. Pioneering works of Warburg and Lüttgens (1944, 1946) and Warburg (1948) showed that various anions (Cl^-, Br^-, I^-, and NO_3^-) stimulated oxygen evolution by the Hill reaction in water-washed broken chloroplasts. Chloride was the most effective anion, but SCN^-, SO_4^{2-}, and HPO_4^{2-} were quite ineffective. Thus, they discovered the requirement of monovalent anions, of which the Cl^- works best, for the electron transport chain. Anions received many stimuli by the observations of Good (1962). He reported uncoupling of the Hill reaction from photophosphorylation by anions. The reduction of oxidants by irradiated chloroplasts is greatly stimulated by the presence of high concentration of many anions, among which phosphate, pyrophosphate, arsenate, citrate, malate, and malonate are particularly effective. Acetate and butyrate uncouple, but also produce rapidly increasing inhibitions, while oxalate, tartarate, and bicarbonate are intermediate uncouplers.

Good (1963) was the first to examine the ability of various anions to induce bicarbonate dependence and found that small monovalent anions, particularly chloride and acetate, were most effective. Izawa and Good (1966a,b) explained the effect of salts on the conformation of isolated chloroplasts. Chloroplasts isolated in low salt media lose their grana without losing any Chl. Despite these interesting discoveries, research on the role of anions in photosynthetic electron transport mechanisms did not receive immediate recognition. Later on, Hind *et al.* (1969) brought the problem back into focus.

The chloride effect

Hind *et al.* (1969) systematically studied stimulation of electron transport with added Cl^- over that measured in its absence. Stimulation of electron transport was specific for Cl^- and largely independent of the cation used. The depletion of Cl^- has several effects on oxygen evolution, and in some cases, the requirement of Cl^- is not completely specific and anions of other strong acids can partially substitute for the Cl^- (Critchley *et al.* 1982). The effectiveness of anions followed the order: $Cl^- > Br^- > NO_3^- > I^-$. The ionic volume may be critical factor in dictating the effectiveness of an anion, *i.e.*, the smaller the anion, the greater the activity (Hind *et al.* 1969, Kelley and Izawa 1978). Critchley *et al.* (1982) suggested that ionic volume is not the only factor because F^-, OH^-, and acetate (Ac^-) that have smaller volumes than Cl^- gave low or no stimulation of electron flow. Multivalent ion (especially anion) salts strongly suppress the reactivation of O_2 evolution by Cl^- in Cl^--depleted PS2 membrane fractions in a competitive manner. The effectiveness of anions in the suppression of Cl^--supported O_2 evolution was in the order of trivalent > divalent > monovalent. Multivalent anions strongly compete with Cl^- but whether they directly bind to the site of action in place of Cl^- or simply work to decrease the effective Cl^- concentration in the vicinity of the action site, without direct binding, was unclear. Monovalent anions, such as HCO_2^- and particularly HCO_3^-, can control the rate of electron flow between PS1 and PS2 (Warburg and Krippahl 1960, Good 1963, Stemler and Govindjee 1973, Stemler and Jursinic 1983). Some monovalent anions such as N_3^- and $Au(CN)_2^-$ might share a common inhibitory site on PS2, that differs from the HCO_3^- binding site (Stemler and Murphy 1985). Jursinic and Stemler (1988) reported multiple anion effects on PS2 in chloroplast membranes. They investigated the activity of several anions at various sites on PS2, in particular those associated with the Cl^-

effect (anion binding site I, ABS I) and the HCO_3^- effect (anion bind-ing site II, ABS II). Anions such as NO_3^-, HCO_3^-, HCO_2^-, F^-, NO_2^-, and acetate can, depending on the conditions, bind to either ABS I, ABS II, or both sites simultaneously. The anions N_3^- and $Au(CN)_2^-$ are exceptions. ABS II may be heterogeneous, with two or three distinct bicarbonate binding sites. There are still controversies regarding the number and location of the bicarbonate binding sites and the bicarbonate affinities at each site. Systematic studies need to be performed to clarify these topics.

The bicarbonate effect

Bicarbonate was discovered to stimulate electron flow in the Hill reaction by Warburg and Krippahl (1958). Stemler and Govindjee (1973) were the first to suggest that bicarbonate may function on the water oxidising side of PS2. However, very soon a major effect on the acceptor side of PS2 was discovered, specifically at the two-electron gate (Wydrzynski and Govindjee 1975). It was speculated that bicarbonate is involved not so much in the electron flow *per se*, but in the protonation steps. The bicarbonate-binding site is close to (Khanna *et al.* 1981), but not identical to that where certain herbicides bind and act (Velthuys 1981). Bicarbonate ions are required for the maximum activity of PS2 (Van Rensen *et al.* 1999). Roles of bicarbonate in regulating electron flow and/or water oxidation have been reviewed by Govindjee and Van Rensen (1978), Klimov *et al.* (1997), or Stemler (1998). Terbutryn inhibits and kills plants by displacing Q_B from its binding site, and bicarbonate seems to be involved in the protonation of the reduced form of Q_B. One of the binding sites of bicarbonate is the non-heme iron (Diner *et al.* 1991) and the other near an arginine residue, D1-R-257 (Xiong *et al.* 1998). It is not yet clear how this binding at the acceptor side would provide another major bicarbonate action on the oxygen evolving complex expounded by O. Warburg, H. Metzner, and A. Stemler, and now being investigated in great detail by Klimov and coworkers. It calls for further research.

Effects of anions on overall PS2 reactions

Anions have profound effects on both the structure and function of PS2. *In vitro*, anions can remove proteins (Kuwabara and Murata 1982) or influence surface charges (Richter and Homann 1984) with polycation-inhibited thylakoids. Apart from these gross effects, there exists a number of specific anion binding sites in PS2. Jursinic and Stemler (1988) have pointed out the complexity of anion interactions. Anions affect various reactions associated with PS2. These anion effects can be put into two classes: those that directly alter the oxygen evolution mechanism and those that change the rate of charge transfer in the quinones. At least two different binding sites apparently are involved: ABS I binds Cl^- and other anions with widely differing affinities and is associated with the O_2 evolution mechanism. The actual number of these sites is not known but there may be more than one specific binding site of this bind per PS2 complex. ABS II has a fairly high affinity for bicarbonate, but also binds other monovalent anions. One or more of these sites modulates the rate of electron transfer between quinone acceptor of PS2. There are at least two ABS II for each PS2 complex. Depending on the experimental conditions, a given anion may interact at either ABS I or II or both sites at once. A few generalisations regarding anion interactions have also been made. The affinity of PS2 for most of the anions appears greater at ABS I than at ABS II. At low concentration, anions induce a response at ABS I, while at higher concentration they affect ABS II more. Anions exchange at ABS I more easily at pH > 7 and at ABS II more easily at pH < 7. Irradiation is also a critical factor in determining affinities for anions. Relative to HCO_3^-, most anions bind more tightly to ABS II in the light (Stemler and Murphy 1983). While monovalent anions were more effective against ABS II (Stemler and Murphy 1985), trivalent and divalent anions were more effective against ABS I (Itoh and Uwano 1986).

Effect of anions on water oxidation and oxygen evolution

There exist numerous studies on the possible role of Ca^{2+} and Cl^- as putative cofactors of the water oxidising complex (WOC; for reviews see Debus 1992, Rutherford *et al.* 1992, Yocum 1992). Compared to the role of Ca^{2+} the role of Cl^- is not very clear. The loss of oxygen evolution activity by different treatments (*e.g.*, Na_2SO_4, high pH) can be reversed after addition of Cl^- and several other monovalent anions (for reviews see Debus 1992, Rutherford *et al.* 1992). However, these findings do not necessarily prove that Cl^- is a cofactor of WOC because secondary effects can not be excluded (*vide supra*). Furthermore, the effect of Cl^- is not very specific also. The possible mode of blocking a definite redox step in the WOC upon Cl^- depletion is still a matter of controversy (Wincencjusz *et al.* 1997). There are two main observations that are not easily reconcilable with the direct role of Cl^-. Firstly, the partial restoration of the oxygen evolution capacity by iodide (Rashid and Homann 1992) raises a question on a close proximity of a functional halide ion to high valence manganese because I^- is a potent electron donor that is expected to reduce rapidly S_2 and S_3. Secondly, based on a very careful study on Cl^- binding (using the radioactive isotope ^{36}Cl) the WOC was inferred to retain full oxygen evolution capacity in almost completely Cl^--depleted PS2 membrane fragments. Only the rate of the overall process is slowed down (Lindberg and Andreasson 1996). These authors presented a one-site, two-state model for the binding of anions in PS2. They propose that Cl^- binding, responsible for high oxygen evolution activity and normal EPR properties of the S2

state, occurs either as high affinity (Kd = 20 μM) and slowly exchanging ($t_{1/2}$ = 1 h), or low affinity (Kd = 0.5 mM) and rapidly exchanging ($t_{1/2}$ < 15 s). Br^- but not F^- have a mode of binding similar to that of chloride. The high-affinity state is the normal state of binding, but once Cl^- has been removed, it will first rebind as low affinity, rapidly exchanging followed by a conversion into a high affinity, slowly exchanging mode of binding.

In light of the latter observations all considerations on a specific role of Cl^- are disputable and therefore at this state of knowledge only indirect structural or charge compensation effects can be discussed (Renger 1999). Cl^- is an essential cofactor for photosynthetic oxygen evolution (Critchley 1985), although its mode of action is still uncertain. The anion specificity of O_2 evolution in spinach thylakoids was observed by studying O_2 evolution rates *versus* anion volume. The Cl^- volume of 0.025 nm^3 is optimal for activity in spinach thylakoids. Steric hindrance at the Cl^- binding site itself or of accessibility could account for the decreasing effectiveness of larger ions, whereas smaller ions such as F^- and Ac^- may act by displacing Cl^- or by binding to the water site (Critchley *et al.* 1982). Replacement of Cl^- by other halide ions has been attempted (Hind *et al.* 1969, Kelley and Izawa 1978, Critchley *et al.* 1982) in an effort to investigate the Cl^- effect. Of these anions, only bromide has been found to significantly replace Cl^- in function, F^- being inhibitory. The membrane surface in the vicinity of the Cl^- action site is probably positively charged and attracts Cl^- electrostatically. This site is almost freely accessible to other anions (Itoh and Uwano 1986). These authors also suggest that multivalent anions strongly compete with Cl^- probably by decreasing the effective concentration of Cl^- in the vicinity of the action site. The effectiveness of anions in decreasing the O_2 evolution rates is dependent on the anion valence of the salts and on the concentration of co-existing Cl^-. It confirms the results obtained with the Cl^--depleted PS2 particles and may support the conclusion that multivalent anions decrease the local Cl^- concentration at its active site through electrostatic interaction. Depletion of Cl^- has several effects on O_2 evolution. It makes the oxygen evolving complex (OEC) more sensitive to inhibition by hydroxylamine (Kelley and Izawa 1978), Tris (Izawa *et al.* 1983), and heat (Coleman *et al.* 1984).

The relative effectiveness of anions which support water oxidation is as follows: $Cl^- \sim Br^- > NO_3^- >> I^- \sim ClO_2^- \sim CNS^- > HCO_2^- \sim ClO_3^- > F^- \sim BrO_3^- \sim CH_3CO_2^-$. Bicarbonate, that appears to be required on the electron acceptor site of PS2, is not very effective in replacing Cl^-. The relative effectiveness as activators of O_2 evolution is changed by the removal of extrinsic 17 and 23 kDa polypeptides. Anions in a similar, but not identical, order of effectiveness prevent dissociation of 17 and 23 kDa extrinsic polypeptides. The size, ionic field, and hydration energies of the anions seem to be significant.

Homann (1985) analysed the association of the site of water oxidation with Cl^- and other activating anions. A model is developed that takes into account the known requirement of more than one anion per centre for maximum activity. It assumes a finite and anion dependent capacity of the water-oxidising site for activating anion and that the rate of photosynthetic oxygen evolution is proportional to the number of anions at the water-oxidising enzyme. Significant insight into the mechanism of water photo-oxidation by the WOC of PS2 has come from studies of its non-physiological reactions, such as the catalase reaction. The enzyme catalase dismutates hydrogen peroxide in all aerobic cells as a defence mechanism against oxidative damage. Maintaining a low peroxidase concentration is critical for retaining PS2 centres with normal water oxidase activity. Anions such as KCN (NaCN), NaN_3, and KF compete with H_2O_2 for binding to the WOC. Furthermore, the chloride-binding site involved in stimulation of water oxidation differs from the anion site that inhibits the catalase reaction. There is no competition observed between these sites, as further established by the inability of NaCl to block the strong inhibition by NaCN. On the basis of above observations, a new binding site for anions that inhibit the WOC of PS2 in spinach has been identified on the basis of inhibition of the catalase reaction of PS2 by anions (Mano *et al.* 1993).

The other important anionic cofactor involved in photosynthetic water cleavage is bicarbonate. Some results led to the conclusion that HCO_3^- is not only essential for PS2 acceptor side (for reviews see Xiong *et al.* 1996, Stemler 1998), but also affects the reaction pattern of the WOC (Allakhverdiev *et al.* 1997). Bicarbonate accelerates the assembly of the inorganic core of the water oxidising complex in manganese-depleted PS2 (Baranov *et al.* 2000). Bicarbonate also can protect the donor side of PS2 against photoinhibition and thermoinactivation in oxygen-evolving pea sub-chloroplast membrane fragments (Klimov *et al.* 1997).

Thermoluminescence properties of the S2 state in Cl^--depleted WOC before and after reconstitution treatments with various monovalent anions have been studied. Homann (1993) suggested that the structural environment of the charge accumulating Mn centre is influenced by the ionic conditions encountered by the PS2 membranes after Cl^- removal, further enforced by the binding of compatible anions, and then stabilised by the 17 and 23 kDa extrinsic polypeptides. The anion-binding site may be located at or near the functional Mn. The potentially electron donating I^- anion serves as an activator and stabilises rather than destabilises the S_2 state. The photosynthetic oxygen-evolving complex requires Cl^- for its redox state $S_2 \rightarrow S_3$ transitions but not for $S_0 \rightarrow S_1$ or $S_1 \rightarrow S_2$ transitions (Wincencjusz *et al.* 1997). This requirement of Cl^- only on the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions can be rationalised by the hypothesis that Cl^- is required for electron transfer

between manganese ions within the OEC.

In the absence of functional anions, the susceptibility of the WOC to stress such as heat, NH_2OH, and OH^- is increased and the association of the extrinsic 17 and 23 kDa polypeptides is destabilised. Homann and Inoue (1985) have shown that the anion selectivity in the activation of O_2 evolution depends on the polypeptide status of the sample. The changing anion effectiveness suggests that the actions of different anions are modulated by changes in the selectivity of the binding sites, which, in turn, depend on the presence or absence of various polypeptides.

Sinclair (1984) showed that NO_3^- (and Ac^-) inhibit PS2 by selectively slowing the S state transition, $S_3 \rightarrow S_0$. These anions also produce a phase lag in O_2 production on modulated radiation. The results were interpreted as an effect of anions on the O_2 evolving mechanism. The effect of NO_3^- resembled that of HCO_2^- described by Stemler (1980, 1982). Stemler and Murphy (1985) indicate that the anions F^-, NO_2^-, HCO_2^-, and Ac^- are all acting at the HCO_3^- binding site. Moreover, reversibility of their inhibition by added HCO_3^- indicated that the HCO_3^- binding site is the main rate-determining factor for the binding of other anions. They also showed that not all monovalent anions inhibit at the HCO_3^- binding site. Some linear anions such as $Au(CN)_2^-$, N_3^-, *etc.* probably bind to another site.

Effect of anions on PS2 acceptor side

Electron transfer at the level of the two-electron gate is inhibited by the presence of formate and nitrite (Govindjee *et al.* 1976, Robinson *et al.* 1984, Eaton-Rye *et al.* 1986). Whether this effect is due to the removal of bound bicarbonate or a direct inhibitory effect, is not yet clear. However, this inhibition is uniquely reversed by the addition of bicarbonate (Good 1963, Stemler and Murphy 1985). Furthermore, a wide range of monovalent anions are competitive inhibitors of HCO_3^- binding (Stemler and Murphy 1985). These findings suggest the existence of an anion binding site on PS2 that, when occupied by HCO_3^-, facilitates electron transport to the PQ pool. There is a possibility that acetate, formate, and nitrite inhibit the electron flow by displacing bicarbonate. An alternative may be that the stimulation of electron transport by HCO_3^- is simply because of the removal of inhibitory anion (Stemler and Murphy 1983, Snel and Van Rensen 1984).

Bicarbonate-reversible anion inhibition of PS2 is a real phenomenon and has already proved itself an important gateway into the complex reactions of the acceptor side of PS2. Thus the proposed role of HCO_3^- is that it is required for stability and efficient functioning of PS2 and plays a highly significant role in the protonation events. Whether HCO_3^- is an essential activator (Cao *et al.* 1992, Govindjee 1993) remains, perhaps, an academic question because the non-essentiality is claimed to exist only when the binding sites are empty of all monovalent anions (Jursinic and Stemler 1992), a condition that can never be fulfilled *in vitro*. In fact, Jajoo and Bharti (1993) have shown that Cl^- may even aid in the stimulation by bicarbonate. The inhibitory effect of several anions (Stemler and Murphy 1985) as well as nitric oxide (Diner and Petrouleas 1990) is overcome by the addition of HCO_3^-. There are at least two locations at which anions affect electron flow through PS2: in the region of Q_B, where HCO_3^- depletion or HCO_2^- addition inhibits electron flow (Govindjee *et al.* 1983), and in the OEC, where Cl^- is necessary for steady state O_2 evolution (Hind *et al.* 1969, Kelley and Izawa 1978, Govindjee *et al.* 1983, Izawa *et al.* 1983, Critchley 1985). In the latter case, the requirement is not completely specific for Cl^-; anions of other strong acids may partially substitute for it. It is believed that HCO_2^- and other monovalent anions do not inhibit electron flow by removing bound HCO_3^-, but by binding to the empty sites (Stemler 1989). On the other hand, Govindjee *et al.* (1991) suggest that HCO_2^- inhibit electron flow by replacing HCO_3^-. However, controversy still remains regarding the mode of action of other anions in the presence of bicarbonate.

Examining the effects of a number of carboxylated anions on the EPR signals associated with the non-heme iron, Petroleas *et al.* (1994) observed that glycollate, glyoxylate, and oxalate compete with NO, formate, and bicarbonate for binding to the iron. The anions inhibited diversely the electron flow rates from Q_A to Q_B (or to Q_B^-), which was measured by following the rates of relaxation, in the sub-ms to ms time scale, of the Chl fluorescence yield in isolated chloroplasts after single turnover saturating actinic flashes. Glycollate was the strongest inhibitor, glyoxylate being intermediate, and the oxalate being the weakest inhibitor of the PS2 electron flow. Thus many anions are able to bind as dissociable ligands to the non-heme iron of PS2 (Van Rensen *et al.* 1999).

Effect of anions on the donor side of PS2

The influence of bicarbonate on the donor side occurs at lower concentrations or earlier in time than the effect on the acceptor side. The role of bicarbonate on the donor side of PS2 is an active area of research and much remains to be examined before a new picture is expected. The reason is that most of the donor side effects have been shown in the DT-20 particles, but not in thylakoids (Van Rensen *et al.* 1999). Most of the earlier data on thylakoids contradict the proposed effects between water and the primary electron donor P680 (Jursinic *et al.* 1976, Govindjee *et al.* 1989) although an influence between Z and Q_A may occur in leaves and intact cells (El-Shintinawy and Govindjee 1990, El-Shintinawy *et al.* 1990). Hutchison *et al.* (1996) and Xiong *et al.* (1996) have shown that a mutation of the arginine at D1-269 into a glycine at the acceptor side of PS2 significantly alters the structure and function of both the acceptor and the donor

sides of the PS2 complex. This may perturb the binding of bicarbonate and formate. Following explanations for the involvement of bicarbonate in the events on the donor side of PS2 have been considered by Klimov and Baranov (2000): (*1*) bicarbonate serves as an electron donor (alternative to water or as a way of involvement of water molecules in the oxidative reactions) to the Mn-containing O_2 centre; (*2*) bicarbonate facilitates re-assembly of the WOC from apo-WOC and Mn^{2+} due to formation of the complexes $Mn(HCO_3)^+$ and $Mn(HCO_3)_2$ leading to an oxidation of Mn^{2+} with PS2; (*3*) bicarbonate is an essential integral component of the WOC essential for its function and stability; it may be considered a direct ligand to the Mn cluster; (*4*) the WOC is stabilised by bicarbonate through its binding to other components of PS2.

However, there is an urgent need of extensive investigations in the area of requirement for bicarbonate on the donor side of thylakoids and cells. Role of other anions on the donor side is a totally unexplored area for further research.

Effects of anions on intersystem electron transport system

Thylakoid surfaces are negatively charged because the number of exposed anionic groups on them exceeds the number of exposed cationic groups. The surface charge forces redistribution of ions in the liquid phase in contact with the membrane. Papageorgiou (1989) examined the effect of the chemical nature of the anions on the stimulation or inhibition of photoinduced electron transport in *Anacystis* permeaplasts by 0.6 M of K^+ ions. It depends on the associated anion whether K^+ ions will stimulate or inhibit DCIP photoreduction. In terms of their effects, the examined anions rank in the order as $SO_4^{2-} = HPO_3^{2-} < Cl^- < Br^- < NO_3^- < SCN^-$. The structural perturbation of thylakoid membrane by the anions may be a physical cause for the observed inhibition of the photoinduced electron transport.

Jajoo and Bharti (1993) suggested an entirely new role of Cl^-. They found that the inhibition induced by HCO_2^- or NO_2^- is overcome by HCO_3^- more in the presence of Cl^-. This proposes participation of Cl^- in the HCO_3^- effect. Since these effects are observed equally well in heat-shocked thylakoids that have lost the ability to evolve O_2 (Yamashita and Butler 1968), it is suggested that Cl^- has an additional site of action, other than on the water oxidation side. This site of Cl^- action may be perhaps between Q_A^- and the PQ pool. This suggests that there is a Cl^--binding site, which, in the absence of Cl^-, is occupied by anions such as HCO_2^- and NO_2^-. The observations point to the fact that HCO_3^- and NO_2^-/HCO_2^- apparently compete for binding sites which may be in close proximity to each other. However, the above results were obtained in the thylakoids isolated in a chloride-deficient medium. There are probabilities that experiments carried out in thylakoids depleted of chloride may give better idea of the role of chloride in the electron transport chain, in the presence or absence of various other anions. The binding affinities of different anions must also be calculated in the chloride deficient and chloride depleted thylakoids. This will help immensely to know the role and mode of action of other anions.

Effect of anions on PS1 activity

Not much work has been done in this field. Earlier studies on the effects of anions on PS1 electron transport include the work of Murai and Katoh (1975) on the blue green alga, *Anabaena variabilis*. Tripathy *et al.* (1984) found that salts affect the interaction of methyl viologen (MV) with PS1, and both anion and cation selectivity was observed. Anions such as acetate were more effective than Cl^- ions in promoting fluorescence quenching. A fraction of PS1 becomes unavailable for electron transport in the presence of Mg^{2+} (Bose and Ramanujam 1984). Moreover, PS1-A fraction is involved in cation regulation of excitation energy distribution that becomes unavailable to $DCIPH_2$ for electron donation in the presence of cations. Since anions promote PS1 rates even in the presence of Mg^{2+}, it is likely that anions also affect the PS1-A fraction (Jajoo and Bharti 1995). Mg^{2+} probably alters the ratio of positive and negative charges on PS1, thereby causing inhibition of the rate of electron transport through PS1. Addition of anion may restore the balance of positive and negative charges resulting in an increased rate of electron transport through PS1.

Since many anions stimulate PS1-mediated electron transport, the stimulation may be explained by the increased negative surface charge potential. The enhanced negative charge due to anions may accelerate the rate of electron transport because the negatively charged anions may not allow the radical of MV to penetrate the membrane, thus decreasing the speed of back reaction. Sulphonated MV dissolved in the form of its negative ions enhances the speed of the reaction (Krasnovsky 1985). This may partly explain the acceleration of PS1 electron transport by anions. Plastocyanin is a negatively charged soluble protein. $P700^+$ may interact electrostatically with negatively charged plastocyanin facilitating electron transport. Anions that stimulate electron transport may substitute for the requirement of the negative charges in the system that may otherwise be fulfilled by the plastocyanin in intact chloroplast. This is supported by the observation that the rates of P700 reduction by anionic electron donors are determined by their concentration at the surface of the thylakoid membrane (Itoh 1979a,b). As proposed by Barber and Chow (1979), electrostatic forces can control the integrity of chloroplast membrane structure. Anions, in theory, might alter electrostatic forces between the membrane components by acting as counter ions at localised regions of the chloroplast membrane bearing a fixed net positive charge. Increase in PS1 ac-

tivity by anions may partly be explained by changes in the electrostatic interaction of plastocyanin and P700 and electrostatic effect on the ability of $DCPIH_2$ to donate electron to P700. Moreover, anions may also increase the density of negative charges causing a force of repulsion acting between Chl-protein complexes and causing them to redistribute within the plane of the chloroplast membrane. This may divert more energy in favour of PS1 (Sinclair 1987).

Effects of anions on electron transport-coupled processes

Changes in spinach thylakoid activity due to nitrite ions have been studied. Nitrite induces inhibition of electron transport between the two photosystems (Spiller and Böger 1977, Stemler and Murphy 1985, Sinclair 1987). Inorganic sulfate or selenate inhibits photophosphorylation in lettuce fragments (Pick and Avron 1973). The inhibition was competitive with inorganic phosphate in all systems studied (photophosphorylation, post-irradiation ATP formation, acid-base phosphorylation). Effect of nitrite on primary photosynthetic processes in isolated chloroplasts of wheat grown under various nitrogen supply has also been studied. In wheat chloroplasts, NO_2^- (5 mM) inhibited non-cyclic photophosphorylation coupled to the linear electron flow through both photosystems but stimulated cyclic photophosphorylation with phenazine methosulphate (*plus* diuron) and electron flux from the artificial electron donor through PS1 and MV to O_2. During energisation of chloroplasts, NO_2^- increased the F_{740}/F_{695} ratio in the low temperature Chl *a* fluorescence spectra, thus suggesting that the energy of absorbed quanta is redistributed in favour of PS1. Nitrite also stimulated the activity of Mg^{2+}-dependent H^+-ATPase. Changes in the slow component of the induction curve of delayed fluorescence also suggest that NO_2^- affects energy transformation processes that are coupled to electron transport in the chloroplasts. Nitrite had no effect on these functional characteristics of thylakoids and chloroplasts isolated from plants grown under nitrogen deficiency in the medium (Makarova *et al.* 1998).

Sulfite stimulated the ATP hydrolysis by the ATP synthase in chromatophores of *Rhodobacterium capsulatus* and interfered with its activation by delta μH^+ (Cappellini *et al.* 1997). Phosphate at mM concentrations could reverse the inhibition by sulfite. Salt-induced alterations of the fluorescence yield and of emission spectra were noticed in *Chlorella pyrenoidosa* (Mohanty *et al.* 1974). Mc Swain *et al.* (1976) reported the effects of Mg^{2+} and Cl^- on irradiation-induced electron transport in membrane fragments from a blue green alga, *Nostoc muscorum*.

Carbonic anhydrase is an enzyme that catalyses the inter-conversion between CO_2 and bicarbonate, and changes in its activity parallel photosynthetic activity. It rapidly inter-converts CO_2 and bicarbonate in the stroma and may influence carbon fixation. Monovalent anions inhibit both carbonic anhydrase and PS2, with similar pH dependence (Stemler and Jursinic 1983). Carbonic anhydrase activity in Cl^--depleted thylakoids was stimulated by low (10 mM) concentration of added Cl^- and Br^-, but not by F^- or SO_4^{2-} (Stemler 1985). There are at least two anion binding sites on PS2 and carbonic anhydrase each, which may interact with each other.

Effect of anions on structure of photosynthetic apparatus

The thylakoid membranes remain normally in a highly fluid state. The organisation of the thylakoid membrane at ambient temperature in higher plants, and probably in green algae, including the spatial relationships between various intrinsic protein complexes in the membrane, are governed by the interplay of electrostatic and electrodynamic forces. The dramatic change in organisation of thylakoid membrane induced by varying the ionic composition of the bathing medium is an extreme example of perturbing the electrostatic forces (Barber 1989). The stacking and un-stacking of thylakoids depends upon the ionic conditions of the suspension medium (Izawa and Good 1966b, Murakami and Packer 1971, Gross and Prasher 1974).

Effect of anions on membrane conformation

Coupled with salt-induced changes in the quantum efficiency of PS2 and PS1 reactions are gross membrane conformational changes (Barber 1982). Barber *et al.* (1974) showed that the associated changes in spill-over were a reflection of changes in the electrostatic properties of the membrane. The salt-induced grana formation is caused by electrostatic screening and involves the lateral movement of electrical charge on the membrane surface (Barber 1976, 1980). As a consequence of this, the charge density on the membrane is non-homogeneously distributed so that the membrane appression occurs in those regions where the surface charge density (σ) is low and not between membranes where there is significant short-range coulombic repulsion due to a high surface charge density. This segregation of charges and associated membrane-membrane interaction is probably a co-operative effect and is dependent on the fluidity of the membrane lipids (Barber *et al.* 1980). Salt-induced changes have been clearly linked with changes in the space charge density adjacent to the membrane that in turn is dependent on the fluidity of the membrane lipids. Thylakoid membranes in suspension show different characteristics depending on the medium composition: in the so-called 'low-salt' medium, the granal structure of the photosynthetic membrane disintegrates and the membrane becomes completely un-stacked (Telfer *et al.* 1975). In the 'high salt' medium, the thylakoids preserve or reform the granal structure (Homann 1969, Murata 1969).

As these studies were performed in thylakoids suspended in a low-salt medium, the role of anions went unnoticed. Jajoo *et al.* (1994) observed significant effects of anions on Mg^{2+}-treated thylakoid membrane. They showed that Mg^{2+}-induced rigidity of thylakoid membrane could be reversed by various anions such as HCO_3^- and Cl^-. Thus, cation/anion induced phase transition in the lipophilic domains may be responsible for the various structural and functional changes observed. Cations bind to the negatively charged phosphate groups of the phospholipids as well as to the carboxyl groups of the proteins resulting in altered membrane fluidity and phase separation of the bilayer (Radda 1975, Papahadjopoulos *et al.* 1977). Thus, it is possible that anions may increase the fluidity of thylakoid membranes by redistributing the surface charges.

Effect of anions on thermal inactivation (heat stress)

The O_2 evolving capacity of isolated chloroplasts is inactivated by heat treatment (Katoh and San Pietro 1967). Mild heat treatment depletes chloride from chloroplasts (Coleman *et al.* 1984). Krishnan and Mohanty (1984) demonstrated that inactivation of Hill activity caused by mild heat treatment is reversible. Reactivation of Hill activity requires Cl^-. Other anions such as NO_2^- or SO_4^{2-} cannot substitute for this action of Cl^-. Homann *et al.* (1983) postulated that osmotic status of the thylakoids influences protonation and Cl^- association with the O_2 evolving centres.

The heat treatment of chloroplasts not only uncouples electron transport activity but also induces some specific alterations in structural reorganisation of membrane pigment-protein complexes. Photosynthetic O_2 evolution is abolished easily by mild heating. The 18, 24, and 33 kDa extrinsic proteins are released from the thylakoid membrane by heat treatment (Yamamoto and Nishimura 1983, Franzén and Andréasson 1984, Nash *et al.* 1985). Indications that bound Cl^- and Ca^{2+} ions are essential for OEC function (Homann 1987a,b) make it necessary to determine whether they also contribute to the stability of the complexes. In the case of Cl^-, a new perspective on this problem was provided by the discovery that Cl^- depletion increases the sensitivity of O_2 evolution to thermal inactivation (Homann 1988).

Electron transport reactions supported by artificial electron donors in chloroplasts show high activities even after heat treatment (Katoh and San Pietro 1967). It indicates that the PS2 RC is more stable than the O_2 evolving system. However, the primary photochemical reactions of PS2 such as the photooxidation of P680 and the photoreduction of pheophytin in a PS2 preparation were also inhibited to about 50 % by heating at 40 °C (Klimov and Krasnovskiĭ 1981). Inoue *et al.* (1987) suppose that heat inactivation involves a reversible change of the membrane assembly and suggests the existence of divalent cation binding sites in the PS2 RC.

The heat-induced reorganisation of PS2 and its light-harvesting apparatus is strongly dependent on the ionic properties of the medium in which the thylakoid membranes are suspended. Sundby and Andersson (1985) stated that monovalent cations are required. As far as the role of cations in heat treatment of the isolated chloroplasts is concerned, Boucher *et al.* (1990) observed a heat stress stimulation of electron flow in a PS1 sub-membrane fraction. This stimulation was enhanced by specific cations (Mg^{2+}, Na^+, K^+) but not by Mn^{2+} or Ca^{2+}.

Anions stimulate the PS1-mediated electron transport both in control and in heat-shocked chloroplasts. Ivanov and Velitchkova (1990) reported heat-induced changes in the increased efficiency of P700 photooxidation. Heat treatment induces an efficient electron donation at a locus characterised by a low K_m for $DCIPH_2$ (Thomas *et al.* 1986, Sabat and Mohanty 1989). Addition of anions further stimulated the PS1 rate both in control and heat-shocked chloroplast, but to a larger extent in heat-shocked chloroplasts (Jajoo and Bharti 1993). This suggests that heat treatment exposes some anion action sites as well as $DCIPH_2$ donation sites. Since many anions stimulate PS1 mediated electron transport, it may not be a ion specific effect, but a general effect of anions due to their negative charge. Jajoo and Bharti (1993) observed a general anion effect on PS1 mediated electron transport. Anions seems to exert their effect by acting at two different sites beyond the diuron (DCMU) binding site in the photosynthetic electron transport chain. One site is between the DCMU binding site and the $HgCl_2$ binding site (or plastocyanin) and the other site is on the P700 itself.

No correlation between the presence of Na^+ ions and the heat-induced migration of PS2 to the stroma region was reported (Andersson *et al.* 1989). Only when relatively high concentrations of divalent anions HPO_4^{2-} were present, a significant change was seen. Hence HPO_4^{2-} ions may destabilise an electrostatic interaction between the outer and inner sub-populations of the light-harvesting chlorophyll *a/b* binding protein complex of PS2 and thereby allow them to dissociate when the temperature becomes sufficiently high. Whether other divalent anions can substitute for the HPO_4^{2-} ions has not yet been found.

Effect of anions on energy distribution between PS2 and PS1 and state shift

As discussed earlier, changes in Chl *a* fluorescence yield at room temperature and at 77 K in chloroplasts upon manipulation of their ionic environment or quality of irradiation (PS1 or PS2 radiation) reflect changes in the excitation energy transfer from the antennae of PS2 to the RC of PS1 (Homann 1969, Murata 1969) or in the initial distribution of the absorbed radiant energy between PS1 and PS2 (Bonaventura and Myers 1969). They demonstrated that a regulatory mechanism *in vivo* regulates the supply of quanta to PS2 and PS1 in order to maximise photosynthetic efficiency in *Chlorella* cells. By using

fluorescence spectroscopy and oxygen polarography, they suggested that under limiting irradiance, the organism adjusted to various wavelengths so as to maintain a more even distribution of excitation to PS2 and PS1. Changes in the F_{730}/F_{685} ratio may indeed reflect changes in the organisation of Chl among the pigment-protein complexes comprising the photosynthetic units.

Barber (1982) proposed the role of surface charges in controlling the regulation of energy transfer between the pigments of PS2 and PS1. Weis (1985) reported the influence of radiation quality and temperature on the distribution of the absorbed quanta between PS1 and PS2 in spinach. Bennett (1983) assumed that state changes could produce a specific imbalance in the rate of excitation of the photosystems. According to Sinclair (1987) nitrite causes a change in the distribution of absorbed radiant energy in favour of PS1. It may be due to some conformational change in the thylakoid (in the light) which permits a greater degree of nitrite binding thus causing diversion of radiant energy towards PS1.

The link between redistribution of absorbed excitation energy and salt concentrations has been studied extensively by different methods (Barber 1980), including Chl fluorescence, electron flow, and redox-state of the plastoquinone pool.

Activity distribution between PS1 and PS2 of oxygenic photosynthesis under limiting irradiance is regulated in response to the ambient conditions. Photosynthetic organisms have the capacity to regulate the distribution of excitation energy between PS2 and PS1 *via* a mechanism termed light state transition (Bonaventura and Myers 1969, Murata 1969). At least two "states" of energy distribution are known *in vivo*: "state 1", achieved by adaptation to far-red radiation (>690 nm) absorbed mostly by PS1 (radiation 1) and "state 2", achieved by adaptation to radiation of shorter wavelengths (radiation 2). In state 1, radiation absorbed by PS2 light-harvesting antenna is retained by PS2 and only weakly transferred to PS1. This induces high yield of Chl *a* fluorescence. In state 2, the radiation absorbed by PS2 is transferred to PS1 thereby resulting in low yield of Chl *a* fluorescence. In higher plants, state 1 to state 2 transition involves redistribution of pigment-protein complexes between PS2 and PS1. Associated with the low salt state 1 conditions were un-stacked membranes and a randomisation of membrane protein complexes. On the other hand, the salt-induced state 2 occurred when the membranes were stacked so as to form grana and stroma lamellae with different types of intra-membrane particles located in discrete regions. In this sense, the state 1-state 2 conditions induced by salts differed from the physiological process occurring *in vivo* since in the latter case, extensive un-stacking and stacking of membrane probably does not occur.

At liquid nitrogen temperatures the fluorescence emission spectrum of chloroplasts shows three peaks: F_{685}, F_{695}, and F_{735}. F_{685} and F_{695} arise primarily from PS2 while F_{735} arises primarily from PS1 (Govindjee 1995). The relative heights of these peaks have been used as an indicator of excitation energy distribution between the two photosystems. When more quanta are distributed to PS1, the F_{735} emission is relatively higher and the F_{685} emission relatively lower than when more quanta are preferentially distributed to the PS2.

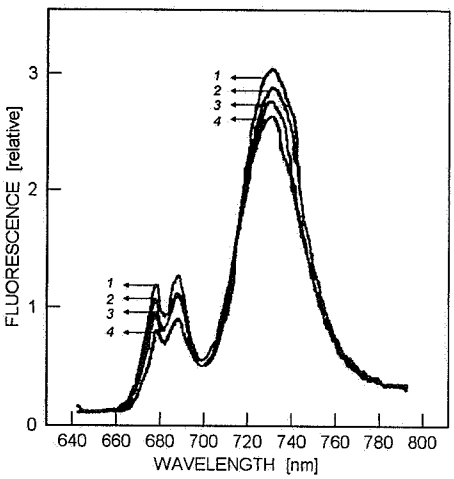

Fig. 2. Normalised fluorescence emission spectra measured at 77 K in sucrose-washed chloroplasts after various inorganic salt treatments. *1*: Control, *2*: 10 mM NaCl, *3*: 5 mM Na_2SO_4, *4*: 5 mM Na_2HPO_4 (from Jajoo *et al.* 1998).

Jajoo *et al.* (1998) proposed an important role of anions in regulating distribution of absorbed radiant energy between the two photosystems. Inorganic anions cause redistribution of energy more in favour of PS1 as judged from measurements of Chl *a* fluorescence transient, rate of electron transport at low irradiance, and 77 K fluoresence emission spectra. The F_v/F_m ratio was decreased by inorganic anions even in the presence of DCMU, the PS2 electron transport was also decreased, whereas the PS1 electron transport and the F_{735}/F_{685} ratio were increased (Fig. 2). Such changes were observed with inorganic anions of different valences (Cl^-, SO_4^{2-}, PO_4^{3-}): The higher the valence of the inorganic anion, the more energy was transferred towards PS1. The interesting point is the correlation of anion valence with its effects. However, organic anions such as acetate, succinate, and citrate did not cause any significant effect. Change in the valence of inorganic anions definitely regulates the distribution of absorbed radiant energy between the two photosystems, by causing state changes in the PS2. Cations such as Mg^{2+} regulate redistribution of absorbed excitation energy mainly in favour of PS2. Effect of low concentration (less than 10 mM) of the monovalent cation Na^+ on Chl *a* fluorescence was completely overridden by the divalent cation Mg^{2+} (5 mM). Based on Chl *a* fluorescence yield and 77 K emission measurements, the role

Table 2. Normalised emission peak ratios F_{735}/F_{685} and F_{695}/F_{685} measured at 77 K in sucrose-washed chloroplasts after giving various treatments at Chl concentration of 7 g m^{-3}. Concentrations of used salts were 5 mM $MgCl_2$, 10 mM NaCl, 5 mM Na_2SO_4, and 5 mM Na_2HPO_4. Each value represents an average of five different samples.

Treatment	F_{735}/F_{685}	F_{695}/F_{685}
Control	1.00 ± 0.02	1.00 ± 0.02
$MgCl_2$	0.86 ± 0.01	1.24 ± 0.02
NaCl	1.15 ± 0.01	1.18 ± 0.01
Na_2SO_4	1.24 ± 0.01	1.16 ± 0.01
Na_2HPO_4	1.42 ± 0.02	1.15 ± 0.01
A. Anion and $MgCl_2$ added together		
$MgCl_2$ + NaCl	0.89 ± 0.02	1.23 ± 0.02
$MgCl_2$ + Na_2SO_4	0.93 ± 0.01	1.23 ± 0.02
$MgCl_2$ + Na_2HPO_4	0.95 ± 0.005	1.22 ± 0.01
B. Anion added prior to the addition of		
NaCl + $MgCl_2$	0.98 ± 0.01	1.22 ± 0.01
Na_2SO_4 + $MgCl_2$	1.05 ± 0.02	1.20 ± 0.02
Na_2HPO_4 + $MgCl_2$	1.16 ± 0.02	1.19 ± 0.02

and effectiveness of anions (Cl^-, SO_4^{2-}, PO_4^{3-}) in lowering the Mg^{2+}-induced PS2 fluorescence was explained. The higher the valence of the anion, the less was the expression of the Mg^{2+} effects (Table 2). Anions may thus overcome the Mg^{2+} effects to a certain extent in a valence-dependent manner, thereby diverting more energy to PS1 even in the presence of $MgCl_2$ (Jajoo and Bharti 1999). Anions also reverse Mg^{2+}-induced rigidity of thylakoid membrane that might help in the attainment of state 2. The change in thylakoid membrane conformation by anions may result in a reversal of Mg^{2+}-induced state changes. Anions also reverse Mg^{2+}-induced effects on excitation energy distribution and membrane fluidity to some extent.

Conclusion and future perspective

Anions are important in the regulation of several interlinked photosynthetic processes. Although the role of various anions on the electron transport reactions has been confirmed, there are still doubts about the number and location of anion binding sites in the ETC. The binding affinities of different anions at various binding sites could not be calculated precisely. This calls for further research. After the establishment of roles of anions in PS1 and PS2 mediated electron transports (Fig. 3), other studies were naturally done in order to elucidate the role of anions in other photosynthetic processes. An important phenomenon observed is that anions change the distribution of absorbed excitation energy in PS2. Anion effects are valence dependent. The higher the valence of an anion, the more energy is transferred towards PS1. Since cations are important for diverting more energy towards PS2, it was obvious to study interaction of anions and cations in regulating energy distribution between the two photosystems. Again a valence-dependent action of anions was observed. The higher is the valence of the anion, the less is the expression of Mg^{2+} effect. These results may be interpreted keeping in mind two important changes brought about by anions. Firstly, anions cause an increase in the fluidity of the thylakoid membrane, probably by redistributing the surface charge. It is speculated that anion/cation induced phase transition in the lipophilic domains may be responsible for the various structural and functional changes observed. Secondly, cause an increase in the F_{735}/F_{685} ratio, the extent of increase of which defined the extent of the attainment of

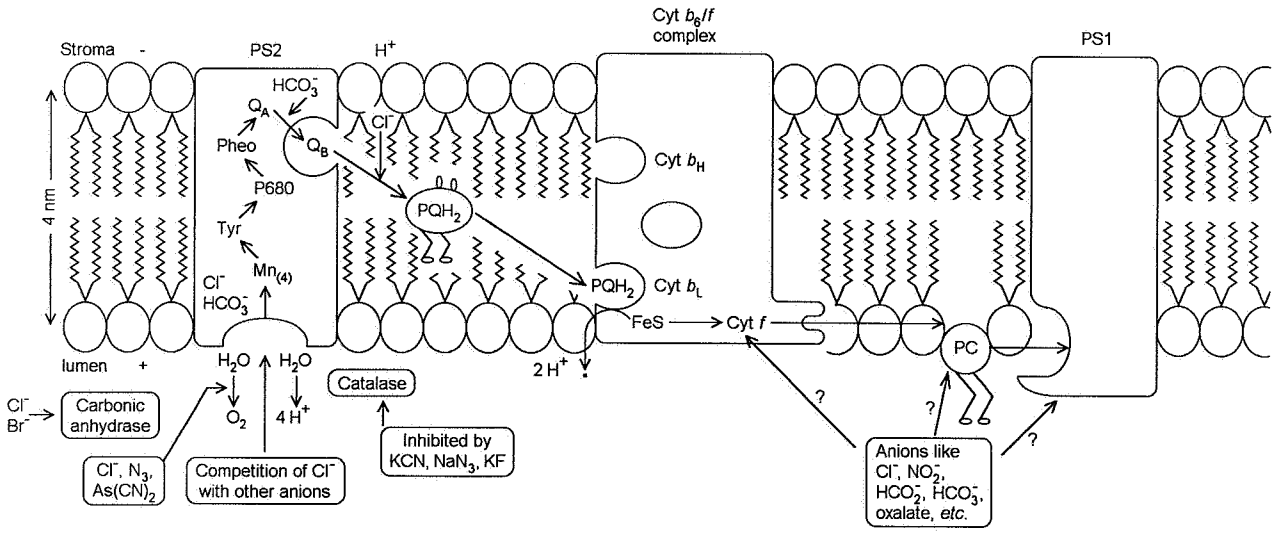

Fig. 3. Scheme showing the site of action of various anions in the photosynthetic electron transport chain. For abbreviations see legend to Fig. 1.

state II, the state usually caused by exposure to radiation absorbed in PS2. The state changes caused by anions are a phenomenon whose mechanism is not yet explained. Cations cause stacking of thylakoid membrane and thereby affect state changes. So far, there is no direct evidence to prove that being oppositely charged, anions may cause un-stacking of the thylakoid membranes. Indirect evidence, provided by fluorescence polarisation and membrane fluidity studies, suggests that some anions may have a role in causing un-stacking of the thylakoid membranes.

More attention is needed in defining the exact roles of anions in the above mentioned processes. Their mechanism and site of action is yet to be elucidated. Moreover, anion effects have not been explored fully in other photosynthetic reactions including the Calvin cycle, phosphorylation mechanism, proton translocation, *etc.* Several roles have been assigned to chloride and bicarbonate, and studies are still being carried out, but focus is also to be made on role of other anions such as nitrite, fluoride, oxalate, *etc.* Preliminary studies have given interesting results regarding their effects. The potential of these anions in regulating important photosynthetic processes is to be explored. The challenge for future research is to de-convolute these effects and characterise and explain them individually. In this review, the role of anions specifically on the electron transport chain and energy transfer has been emphasised, yet many other reactions are to be worked out. Anions protect against thermal inactivation and photoinhibition. The role of anions in protecting plants from other stresses such as drought, salinity, heavy metal stress, *etc.* is a promising area for further research. In this respect, the roles of anions in photosynthesis remain one of the most important and fascinating challenges in future photosynthesis research.

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