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REVIEW

Elucidation of the primary events in photosynthesis. Novel insights based on enolisation and iminium involvement with pheophytin in photosystem 2

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Abstract

Pheophytin (Pheo) is structurally constituted as to make possible certain reactions, previously given very little attention, which appear to play crucial roles in the initial electron transfer (ET) processes. The transformations involve enolisation with subsequent formation of mono- and di-iminium cations at the Pheo core. The important impact of these ions on ET from chlorophyll (Chl) to Pheo and then to quinone are evaluated. These insights rationalise the long-standing enigmas of fast transfer, across gap ET, activation-less aspect, and essential lack of reversibility. Comparisons are made to other important areas of iminium involvement, *e.g.* chemistry of vision, polyaniline doping, and DNA alkylation.

Additional key words: chlorophyll; electron transfer; electrostatics; enigma elucidation.

Introduction

If the question were posed to life scientists: "Which single chemical reaction is most fundamental to life on Earth?" what might the choice be? Photosynthesis would likely come in first. There have been large advances in the subject dealing with various facets, including the primary steps and determination of the structure of a bacterial photosynthetic reaction centre (Deisenhofer and Michel 1989). However, a number of crucial aspects of the early stages of electron transfer (ET) remain puzzling and unresolved, *e.g.* the very fast rate, irreversibility, across gap ET, activation-less character, and spectroscopic observations (Friesner and Won 1989). Possible reasons for the state may be invalid assumptions as to initial receptor structure and charge separation, the dearth of plausible mechanistic hypotheses, and insufficient application of interdisciplinary approaches. The research, both theoretical and experimental, is becoming more and more esoteric.

Discussion

The main classes of ET agents employed in Nature are quinones, metal complexes, and conjugated imines or iminium species (Kovacic and Jacintho 2001). The last category has received scant consideration as an organised group, which may explain lack of more attention and progress in a number of pertinent areas. Examples are iminium ions (Fig. 1*B,C*) of pheophytin (Pheo) (Fig. 1*A*), rhodopsin, and alkylated DNA. Flavin enzymes, which are conjugated imines, are exceptions in that the chemistry has been rather well delineated. Examination of the porphyrin structures from this vantage point reveals conjugated imines in chlorophyll (Chl) as well as in Pheo

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Abbreviations: Chl, chlorophyll; ET, electron transfer; G, guanine; glu, glutamic acid; OS, oxidative stress; Pheo, pheophytin; PS2, photosystem 2; Q, quinone; RC, reaction centre.

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which can act as precursor of conjugated mono- and di-iminium ions on protonation of the latter (Kovacic *et al.* 1991).

Various electrochemical studies have been performed with compounds in this general class (Seely 1966, Wilson and Neri 1973, Felton 1978, Kazakova *et al.* 1989). Pheo gave $E_{1/2}$ values of –0.5 to –0.7 V for the first one-electron reduction. Significantly from this mechanistic viewpoint, in acid solution the reduction potential increases markedly by about 0.4 V to a value of –0.2 V (Seely 1966, Kazakova *et al.* 1989) which is favourable for *in vivo* ET reactions. The increase is not surprising since the positive charge provides beneficial energetics for attraction of the negative electron.

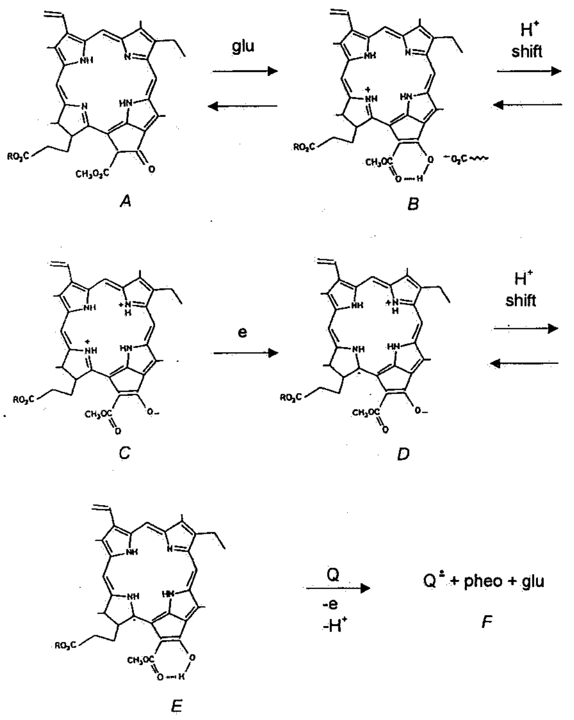
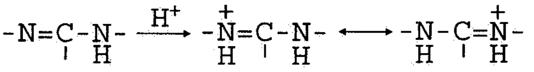


Fig. 1. Reaction sequence: (*A*) Pheo, (*B*) monoprotonation to form mono-iminium, (*C*) diprotonation to form di-iminium, (*D*) one electron uptake to form radical cation, (*E*) proton shift to form radical, (*F*) one-electron loss to form semiquinone with regeneration of Pheo by proton loss. R = phytyl.

About ten years ago, we proposed involvement of enolisation as a key contributing factor (Kovacic *et al.* 1991). Starting with the early literature, there are numerous reports on Pheo type compounds serving as substrates for facile enolisation (Rabinowitch 1945, 1951). However, with few exceptions, little attention is paid to this property. Usually Nature is efficient and the various structural constituents serve a purpose as determined by evolution. Pheo exists in the keto form in the crystallised reaction centre (Deisenhofer and Michel 1989). The β-ketoester arrangement is particularly well suited to undergo tautomerisation to the enol form (Fig. 1*B*) by acid catalysis. The nearby glu side chain of protein, apparently in un-ionized form (Lubitz *et al.* 1989), reasonably provides the requisite enol hydrogen (Deisenhofer and Michel 1989, Nabedryk *et al.* 1990). The glu residue is conserved in all reported L-subunits in purple bacteria (Deisenhofer and Michel 1989).

The proton released from the tert-carbon site is near electron-rich locales as reported for chlorins (Scheer and Inhoffen 1978), and can readily migrate throughout the highly conjugated system, in accord with related systems (Cram 1965). Two imine nitrogens at the central core provide basic sites for protonation. Since they are conjugated imines of the vinylogous amidine class, there should be enhanced basicity due to cation de-localisation as illustrated for the amidine skeleton (Eq. 1).



$$-N{=}\underset{|}{C}{-}\underset{H}{N}- \xrightarrow{H^+} -\overset{+}{\underset{H}{N}}{=}\underset{|}{C}{-}\underset{H}{N}- \longleftrightarrow -\underset{H}{N}{-}\underset{|}{C}{=}\overset{+}{\underset{H}{N}}- \qquad (1)$$

An appreciable amount of work has been done on protonation of Pheo and analogous compounds, with mono- and di-cations being the usual products. The results for Pheo including conditions and degree of protonation are the following: CF_3CO_2H-CCl_4, di; CF_3CO_2H, di and tri (Chl precursor, apparent carbonyl involvement in tri) (Oettmeir *et al.* 1977); acetone - $HClO_4$, mono (Delaporte and Laval-Martin 1972); acetone-H_2O-HCl, mono and di (Zanker *et al.* 1977). Doubt has been expressed concerning validity of the monocation claims (Oettmeir *et al.* 1977). The energy barrier for addition of the first proton may be greater than for the second. The proposal was advanced that formation of the unstable monocation results in greater exposure of the non-bonding electrons on the second nitrogen, thereby facilitating diprotonation. Higher energy for the non-planar monocation due to loss of aromaticity appears to be a factor in comparison with the aromatic system in the planar dication. *In vivo*, glu appears to furnish the initial proton for *B* in Fig. 1, and the source of the second is likely the proximate enol hydrogen, resulting in a zwitterion (Fig. 1*C*). Structurally, 1*C* is a vinylogous analogue of the ionic contributor to an amide resonance hybrid. In connection with subsequent mechanistic discussion, ease of diprotonation in the Pheo core should be kept in mind as a vital element. The dication appears to be better suited as the initial electron acceptor, rather than the monocation that was previously assigned to that role (Kovacic *et al.* 1991).

The differing basicities and stabilities, plus degree of protonation, may play significant roles in ET to and from Pheo. Thus, cogent reasons can be discerned for the absence of magnesium. Elementary organic chemistry provides the fundamentals for understanding. In the first step (Fig. 1) photo-activated Chl conveys an electron to dication 1*C*. The high degree of positive charge in the receptor relates to the observed very rapid velocity of the transfer. Due to de-localisation of the charges on the four nitrogens, the positive electrostatic field extending toward Chl could be cylinder-like, attracting the excited electron and conveying it across the gap, thus illuminating the aspect of through space ET. The resultant radical cation 1*D* serves to deter back donation of the electron due to the presence of positive charge, in addition to repulsion between the two generated de-localised radical cations. The Chl radical cation may possess unusual stability, perhaps by association in the special pair (Deisenhofer and Michel 1989), also discouraging back transfer. These aspects clarify the enigma of essential lack of reversibility. The scheme is in accord with direct reduction of Pheo by activated Chl without intermediate steps, as had been entertained earlier (Deisenhofer and Michel 1989). Part of the process involves neutralisation that is considered exothermic. In essence, the initial event entails electron unpairing, but not separation of unlike charges, hence negating the widespread belief in charge separation. This reasoning can also be applied to the aspect of activation-less ET (Krishtalik 1989). The common error rests on the assumption that 1*A* is the entity directly involved in ET, often with resultant flaws in design and interpretation, both in calculations and experiments. Since monocations of type 1*D* appear to be quite unstable, loss of proton would readily ensue generating the radical 1*E*. This event aids in preventing back donation in the subsequent transfer to quinone. The final transformation involves transfer of an electron from 1*E* to the quinone receptor. In the process, a cation might conceivably remain as a hindrance. However, since the cation is presumably unstable, concomitant proton loss yielding neutral Pheo would obviate this potential deterrent. In addition, the electrostatic repulsive effect of the adjacent Chl radical cation should assist in expulsion of the two protons from the positive core of the Pheo radical iminium species. For the second step, favourable energetics pertain to aromatisation in the semi-quinone and to absence of unlike charges. A number of these steps may transpire in a synchronous fashion. The reactions occur at very fast rates, each step acting to drive the next in a co-ordinated manner. Electric fields and protein counter ions may be playing important roles in stabilisation. A deficiency in prior theoretical studies may be the assumption that Pheo rings remain coplanar during reaction (Lubitz *et al.* 1989).

There are more recent reports that lend a degree of support to the proposed theoretical framework:

(*1*) "Stabilisation of the charge separated state by subsequent charge shift reactions as occurs *in vivo* and in larger size particles does not occur in the isolated PS2 RC. The isolation procedure removes the quinones that are the secondary electron acceptors" (Greenfield and Wasielewski 1996).

(*2*) A prior mechanism involved two weakly coupled electrons and a strongly hyperfine coupled nucleus to explain the polarised nucleus in the RC (Polenova and McDermott 1999).

(*3*) In PS2, initial separation is much less strongly driven than in the bacterial system, resulting in increased energy conversion efficiency (Blomberg *et al.* 1998).

(*4*) The primary ET state is further stabilised by subsequent ET. Recent data point to a viable mechanism of regulation that has not been addressed (Merry *et al.* 1998).

(*5*) New elements of ET theory, disregarded in the usual theoretical approaches, are needed (Kharkats *et al.* 1995).

Various important factors play crucial roles as evidenced by prior work entailing geometry and the nature of constituents adjacent to the electronic pathway. Electrostatic effects (Kharkats *et al.* 1995), magnetic fields (Sevdimaliev *et al.* 1993), dielectrics (Blomberg *et al.* 1998), hydrogen bonding and protein interaction (Blomberg *et al.* 1998), as well as solvation, represent additional considerations, some of which have been addressed. Also, PS2 may differ significantly from purple bacteria with regard to the nature of electron acceptors (van Mieghem *et al.* 1989). There is a striking resemblance to ET reactions involving iminium in the chemistry of vision (Salem 1979), polyaniline doping (MacDiarmid *et al.* 1985), and DNA alkylation (Kovacic and Jacintho 2001).

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