PHOTOSYNTHETICA 40 (1): 145-151, 2002

Mercury inhibits the activity of the NADPH:protochlorophyllide oxidoreductase (POR)

K. LENTI*, F. FODOR**, and B. BÖDDI***,+

Doctoral School of the Eötvös University, Departments of Plant Physiology** and Plant Anatomy***, Eötvös University, H-1117Budapest, Pázmány P. sétány 1/C, Hungary*

Abstract

The effect of Hg^{++} was studied on the arrangement and photoactivity of NADPH:protochlorophyllide oxidoreductase (POR) in homogenates of dark-grown wheat (*Triticum aestivum* L.) leaves. 77 K fluorescence emission spectra of the homogenates were recorded before and after the irradiation of the homogenates and the spectra were deconvoluted into Gaussian components. The mercury treatment caused a precipitation of the membrane particles, which was followed by a remarkable decrease of the fluorescence yield. 10^{-3} M Hg^{++} decreased the ratio of the 655 nm-emitting protochlorophyllide (Pchlide) form to the 633 nm-emitting form. 10^{-2} M Hg^{++} shifted the short wavelength band to 629-630 nm and a 655 nm form was observed which was inactive on irradiation. This inhibition may be caused by serious alteration of the enzyme structure resulting in the trans-localisation of NADPH within the active site of POR.

Additional key words: chlorophyll formation; fluorescence emission spectra; heavy metal; Hg; protochlorophyllide; *Triticum*; wheat.

Introduction

Etioplasts formed in chlorenchymatic tissues of dark-grown higher plants are characterised by specially arranged inner membranes, the prolamellar bodies (PLBs), and/or prothylakoids (PTs). Protochlorophyllide (Pchlide), the chlorophyll (Chl) biosynthesis precursor, is localised mainly in the PLBs (Ryberg and Sundqvist 1982, Böddi *et al.* 1989) as well as the majority of the NADPH:protochlorophyllide oxidoreductase (POR) enzyme the function of which is the photoreduction of Pchlide into chlorophyllide (Chlide). The subunits of POR are ternary complexes of the enzyme-protein, the coenzyme NADPH, and the substrate Pchlide (Griffiths 1978). These subunits, however, are built into large complexes (Wiktorsson *et al.* 1993). The arrangement of the POR subunits enables excitonic interactions among Pchlide molecules (Böddi *et al.* 1989), which results in a spectral heterogeneity of the pigment (Böddi *et al.* 1993). Four main fluorescence emission bands—corresponding to 0-0 transitions—were found universal in 77 K spectra of etiolated leaves. These bands had maxima at 633, 644, 655-657, and 669 nm (Böddi *et al.* 1992) and an additional maximum was found at 628 nm in the 10 K spectra (Kis-Petik *et al.* 1999). 629 and 636 nm emitting emission bands were described in 77 K fluorescence emission spectra of non-leaf organs (Böddi *et al.* 1998). The complexes having these emission bands are called "forms" mainly because of the insufficient description of their molecular structures. Along with the spectral variations, there are differences in the photoactivities of these complexes: the 644 and 655-657 nm emitting forms are flash-photoactive, *i.e.* they transform into 684 and 696 nm emitting Chlide forms (Böddi *et al.* 1991) in a ms time-scale (Franck and Mathis 1980). The shorter wavelength forms are not photoconvertible under flash irradiation, they can transform on hour time scale (Böddi *et al.* 1996).

The description of the three different iso-forms of POR, designated PORA, PORB (Holtorf *et al.* 1995), and PORC (Osawa *et al.* 2000) increased the uncertainty about the molecular structures of the spectral forms. Most of the information available concerns the PORA, which is abundant in etioplasts of leaves of dark-germinated seedlings (for review see Fujita 1996). The knowledge of protein structure is important in the understanding of the catalytic action of POR. On the basis of the nucleotide sequence, the POR subunit was reported to be built up of 314 amino acid residues among which 4 cysteins were detected (Darroh *et al.* 1990). The Val_2-Cys_{31} region was suggested to bind NADPH, the –SH group of Cys_{296} was identified as the Pchlide binding site in the active centre of POR isolated from wheat (Teakle and Griffiths 1993).

Received 26 November 2001, *accepted* 4 April 2002.
+Corresponding author; e-mail: bbfotos@ludens.elte.hu

However, the Cys_{281} and Cys_{308} were suggested to be responsible for the catalytic activity (Lebedev and Timko 1998). The dehydrogenases seem to be similar in their structures: they usually consist of six β-sheets and α-helices, and about 130-150 residues are conservative (for review see Hendrich and Bereza 1993).

In the enzyme reaction, the NADPH is the primary proton donor; the other proton derives from the environment (Valera *et al.* 1987, Begley and Young 1989). The reduction of the C-17–C-18 double bond is stereospecific (Helfrich *et al.* 1996).

After flash irradiation, POR:$NADP^+$:chlorophyllide complex is formed, but the $NADP^+$ is re-reduced within a few seconds. Under further irradiation, the NADPH is oxidised and in this way the so-called Chlide micro-cycle functions (Franck and Inoue 1984). Chlide is finally released from the complex and the enzyme can function in several cycles (Oliver and Griffiths 1982, for reviews see Beale 1999, Franck *et al.* 1999).

The enzyme activity depends also on the native arrangement and molecular environment of the POR subunits. In this way, stress factors such as heavy metals may influence it. The inhibitory effect of mercury was studied on numerous NADPH-utilising enzymes. Despite the great variety of the research materials [for instance, pig heart lipoamid dehydrogenase, human glutation reductase, and trypanothione reductase (Fox and Walsh 1983, Shames *et al.* 1986)], the mode of action of inhibition seems to be very similar. Therefore a redox reaction between the Hg^{++} ions and NADPH was suggested (for review see Foster 1983, Summers 1986) as follows:

$$Hg(SR)_2 + NADPH + H^+ \rightarrow Hg(0) + NADP^+ + 2\,HSR$$

Mercury reacts also with the protein, *i.e.* with certain amino acid residues. In experiments with different amino acids, the reaction of Hg^{++} with L-cystein was found (Barlton and Smith 1973, Lau and Sarkar 1979). As a result, the denaturation of the protein has occurred the extent of which depended on the number of thiol groups (in human haemoglobin) (Barlton and Smith 1973).

In this work, we studied the effect of mercury on the activity of POR. Leaves of dark germinated wheat were homogenised; the spectral properties and the photoactivities of POR complexes in the homogenates were identical to those of intact leaves. $HgCl_2$ of different concentrations was added to the homogenates and the 77 K fluorescence spectra were measured before and after irradiation. A comparison of the properties of control and Hg treated homogenates allowed us to draw conclusions about the native arrangement of the POR enzyme and the mode of action of the mercury.

Materials and methods

Leaves of 8-d-old dark-germinated wheat (*Triticum aestivum* L.) were used. 1 cm of the leaf tip was discarded and homogenates were prepared from the 4-cm segments of the leaves. Usually 20 g (fresh mass) of leaf material was homogenised in 30 cm^3 isolation medium. Before homogenisation, the leaves were kept on ice for 10 min and the isolation medium was cooled to 0 °C. The isolation medium contained 0.5 % (v/m) sucrose, 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), and 50 % (v/v) glycerol. The pH of the solution was adjusted to 7.2 with KOH. The homogenates were filtered through 4 layers of gauze. In case of mercury treatment, $HgCl_2$ was added to the homogenates (from a stock solution) with final concentrations of 10^{-2}, 10^{-3}, and 10^{-4} M. Simultaneously, isolation buffer was added to the control samples not to have differences between the control and treated samples due to the dilution. The homogenates were incubated in the dark at 0 °C for longest 3 h. Samples were stirred during the incubation and at different incubation periods were frozen in liquid nitrogen in the dark. Other samples were irradiated with "white light" of 450 μmol m^{-2} s^{-1} for 10 s and were frozen. This irradiance was checked not to cause bleaching. The 10-s irradiation period prevented the quenching effect of the photochemical intermediates (Dujardin and Sironval 1970).

The 77 K fluorescence emission spectra were recorded with a *FluoroMax-2 Jobin Yvon-Spex* spectrofluorometer. The emission spectra were recorded with 440 nm excitation between 580 and 780 nm with 0.5 nm frequency. Three spectra were recorded in each case and their averages were automatically calculated. The spectra were corrected for the wavelength-dependent sensitivity variations of the fluorometer and baseline correction was done to eliminate light-scattering effects. The fluorescence spectra were deconvoluted into Gaussian components (software *SPSERV V3.14*, copyright Cs. Bagyinka, Institute of Biophysics and Biological Research Center of the Hungarian Academy of Science, Szeged, Hungary) with procedures described in Böddi *et al.* (1992).

To test if NADPH molecules solved in the isolation medium were directly oxidised by Hg^{++}, the absorption spectra of the NADPH solved in the medium with or without Hg^{++} were compared. The concentration of NADPH and Hg^{++} were both 10^{-2} or 10^{-3} M. The absorption spectra were recorded with a *Shimadzu 2101-PC* spectrophotometer.

All measurements and calculations were done on five separate membrane preparations. Since the membrane contents of the preparations and thus the POR/Hg^{++} ratio varied in the homogenates, the spectra of the separate experiments were not averaged. Figures show representative spectra of only one series of experiments. The results of other experiments were qualitatively the same as presented here.

Results

The 77 K fluorescence emission spectra of the homogenates were very similar to those of intact etiolated wheat leaves; they showed a dominating band at 655.5 nm and there was a band of lower intensity at 632.5 nm. The amplitude ratio of these bands [A(amplitude)$_{632.5}$/A$_{655.5}$] was 0.3 in average after the preparation, and became 0.4 after 3 h of incubation at 0 °C (Fig. 1, curve *a*). These band position and amplitude ratio values were similar to those of young etiolated wheat leaves (Böddi *et al.* 1992).

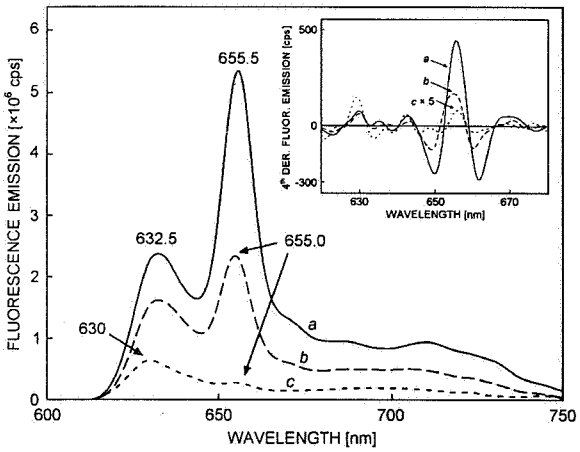

Fig. 1. 77 K fluorescence emission spectra of etiolated wheat leaf homogenates. Excitation wavelength: 440 nm. *Inset*: 4th derivatives of the spectra. *a*: control, *b*: treated with 10^{-3} M $HgCl_2$, *c*: treated with 10^{-2} M $HgCl_2$. The homogenates were incubated in the dark for 2 h at 0 °C.

The Hg^{++} treatment caused significantly different effects at various concentrations. 10^{-4} M or lower concentration of Hg^{++} did not change the shape of the fluorescence spectra, nor did influence the photochemical activity of the Pchlide forms even after 3 h of incubation. No position changes of the emission bands were found at 10^{-3} M Hg^{++} treatment, however, the A_{633}/A_{655} value gradually increased. This value became 0.8 after 2-h incubation (Fig. 1, curve *b*). A remarkable phenomenon was the decrease of the fluorescence yield, the integral of the emission spectrum of this sample decreased to 53.3 % of the control. 10^{-2} M Hg^{++} caused very fast and drastic changes in the fluorescence spectra. Already after 5-min incubation, the fluorescence signal significantly decreased (the integral of the spectrum was only about 20 % of that of the control) and the 633 nm band shifted to 631 nm. In parallel with the decrease of the fluorescence yield, strong baseline distortions appeared and the light scattering of the sample increased. Surprisingly, the position of the 655.5 nm band did not change; its relative amplitude decreased only. To demonstrate the phenomena described above, the spectra of control or treated with 10^{-3} and 10^{-2} M Hg^{++} are plotted together (Fig. 1). The emission band positions were almost identical in the 4th derivatives of the spectra; only the position change of the short-wavelength band from 633 to 631 nm was found at 10^{-2} M Hg^{++}. The positions of the other bands did not change or could not be detected due to the overlap of the bands (Fig. 1, *inset*).

Using the 4th derivatives, the 600-670 nm region of the spectra was de-convoluted into Gaussian components and their integral values were compared. Three components with maxima at around 633, 645, and 655 nm were evaluated because they can be considered as 0-0 transitions. The fourth component around 670 nm, which overlaps with the shorter wavelength bands, is complex; it belongs to a vibronic band of the 0-0 transition at 655 nm and to a 0-0 transition at 672 nm (Kis-Petik *et al.* 1999). Longer wavelength emission bands above 670 nm correspond to vibronic multiplicities of the 633, 645, and 655 nm transitions (Böddi *et al.* 1993); they were not analysed in this work. The sum of the integral of the above described four Gaussian components was taken as 100 % and the relative contributions of the Gaussians were calculated (Table 1). These values showed that the increasing concentration of the Hg^{++} gradually increased the relative contribution of the 632 and 644 nm components, while that of the 655 nm band gradually decreased. The half-bandwidth values and maximum positions of the 644 and 655 nm components did not change remarkably but the maximum of the 632 nm shifted to the vicinity of 630 nm and its half-bandwidth value decreased. To demonstrate the deconvolution, the spectra of the control and of the 10^{-2} M Hg^{++}-treated homogenates are shown (Fig. 2*A,B*, respectively).

Since the spectral changes were completed within 5 min at 10^{-2} M Hg^{++}, the time course of the reaction was studied at 10^{-3} M Hg^{++} concentration. Samples were collected from the control and Hg^{++}-treated samples after 0.5-, 1-, 2-, and 3-h incubation. The fluorescence spectra were recorded and the spectra were deconvoluted into Gaussian components. There was a continuous decrease in the fluorescence yield: the integral of the spectra decreased to 80, 60, 45, and 40 % (after 0.5-, 1-, 2-, and 3-h incubation, respectively) of the integral of the spectrum measured before the incubation. The position and the half-bandwidth values were similar in the case of control samples, however, in spectra of the treated samples a continuous blue shift was found in the position of the 633 and 644 nm bands, they shifted to 631 and 642 nm, respectively. The integrals of the components were calculated and the spectral changes were demonstrated with the integral ratios of the components (633/655 and 644/655 for the control and 631/655 and 642/655 for the treated samples). These values showed that the changes of spectra of the control homogenates were not significant but the Hg^{++}-treatment remarkably increased the relative contributions of the 631 and 642 nm forms (Fig. 3).

Table 1. Characteristics of the Gaussian components of 77 K fluorescence emission spectra of control, 10^{-3} M, and 10^{-2} M $HgCl_2$ treated etiolated wheat leaf homogenates. The spectra were measured after 2-h incubation with 440 nm excitation. The sum of the integrals of the 629-633, 642-644, 655, and 669-670 nm Gaussian components was taken as 100 %.

	Control	10^{-3} M $HgCl_2$	10^{-2} M $HgCl_2$
Maximum position of the Gaussian components [nm]	632.0	632.0	629.5
Half-bandwidth [nm]	14.5	14.5	14.0
Integral value [%]	25.2	32.0	47.5
Maximum position of the Gaussian components [nm]	644.0	644.0	642.2
Half-bandwidth [nm]	14.5	14.5	14.5
Integral value [%]	12.7	16.3	21.8
Maximum position of the Gaussian components [nm]	655.0	655.0	655.4
Half-bandwidth [nm]	11.0	11.0	11.5
Integral value [%]	41.6	33.7	13.7

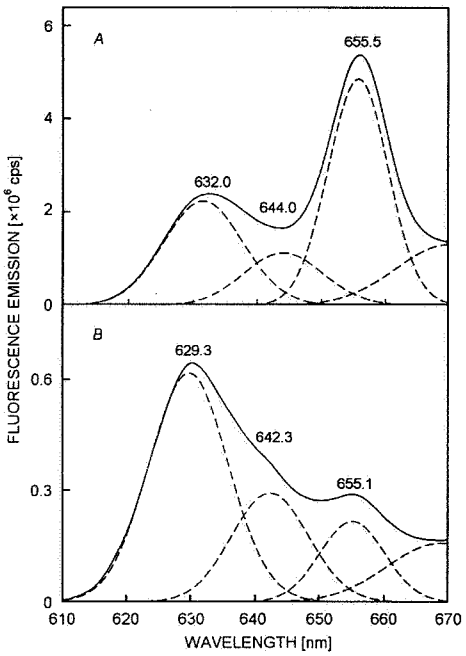

Fig. 2. Gaussian components of the 77 K fluorescence emission spectra of etiolated wheat leaf homogenates. Excitation wavelength: 440 nm. *A*: control, *B*: treated with 10^{-2} M $HgCl_2$. *Solid lines*: experimental spectra, *dashed lines*: Gaussian components. The de-convolution was done in wavenumber function of spectra and the result was converted into wavelength function. The error of the fit was less than 1 %.

Irradiation resulted in the conversion of the 655 and 644 nm Pchlide forms and as a result, the production of a 690 nm emitting Chlide form in the control homogenates (Fig. 3, curve *a*). Similar photoconversion was found also in the case of the homogenate incubated with 10^{-3} M Hg^{++} for 2 h. In this case, however, the decrease of the fluorescence yield was found in the whole spectrum and a shoulder was present in the spectra of irradiated samples at 655 nm, indicating the presence of a photoinactive Pchlide form (Fig. 3, curve *b*). No photoconversion was found in homogenates incubated with 10^{-2} M Hg^{++} for 5 min or longer, bands about 630 and at 655 nm were evident in the spectra and no bands appeared which would

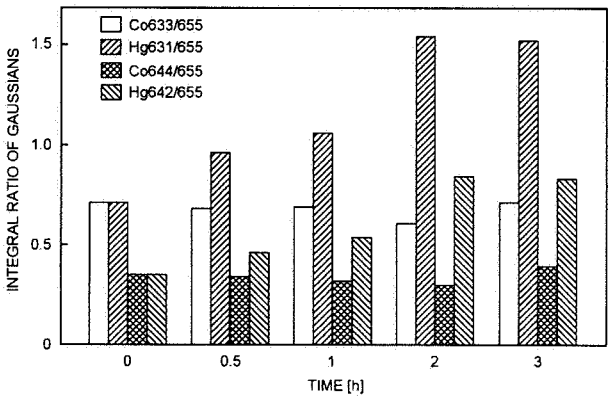

Fig. 3. Changes of the integral ratios of Gaussian components found in 77 K fluorescence emission spectra of etiolated wheat leaf homogenates incubated with 10^{-3} M $HgCl_2$ for different time periods. The samples were kept in the dark, the incubation temperature was 0 °C, the homogenates were shaken during the incubation.

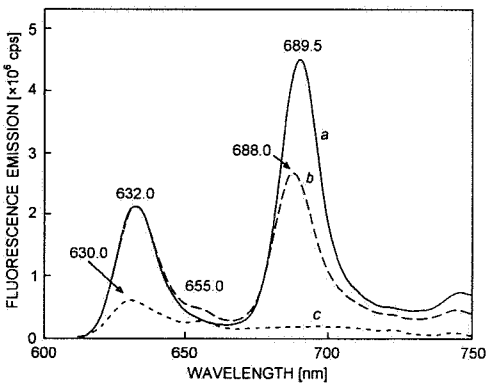

Fig. 4. 77 K fluorescence emission spectra of etiolated wheat leaf homogenates after 10 s irradiation. Excitation wavelength: 440 nm. *a*: control, *b*: treated with 10^{-3} M $HgCl_2$, *c*: treated with 10^{-2} M $HgCl_2$. The homogenates were incubated in the dark for 2 h at 0 °C before irradiation.

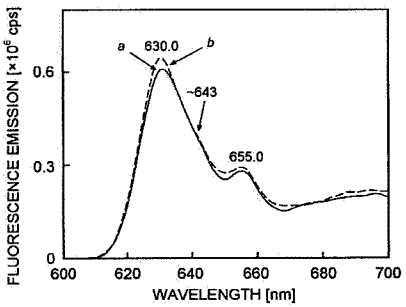

Fig. 5. Comparison of 77 K fluorescence emission spectra of dark-kept and irradiated wheat leaf homogenates treated with 10^{-2} M $HgCl_2$. Excitation wavelength: 440 nm. *a*: spectrum of the dark sample, *b*: spectrum recorded after 10 s irradiation.

Discussion

Mercury is a widely studied element in works dealing with environment pollution (Šeršeň and Kráľová 2001). Our results, however, belong to basic research because of the high concentration of mercury; only 10^{-3}-10^{-2} M concentrations affected the leaf homogenates. Since mercury may react with many native molecules, it is not probable that mercury ions could directly react with POR molecules if intact plants were treated. Therefore, this system should be considered as a model in which the connections between the structure and activity of POR can be studied. The control homogenate changed slightly during the 3-h incubation time as reflected by the relatively low amplitude of the 633 nm band, and the photoactivity did not change either (Fig. 3, curve *a*).

A striking phenomenon was the decrease of the fluorescence yield (indicated by the decrease of the integral of the spectra) caused by mercury in concentration of 10^{-3} M or higher. Precipitation of the membrane particles or proteins of the homogenate can explain this decrease, which is indicated by the opalescence and base-line distortions, too. In parallel with this precipitation, the molecular re-arrangement of the etioplast inner membranes took place as shown by the change in the ratios of the maxima of the Pchlide forms; the relative amplitudes of the 633 and 644 nm bands remarkably increased. A second step of the membrane alteration was shown by the blue shift of the short-wavelength maximum from 632-633 to 629-630 nm and that of the 644 nm band to 642 nm. The relative amplitude of the 655 nm band gradually decreased but its position and half-bandwidth value did not change even at high, 10^{-2} M mercury concentration (Fig. 1, *inset*, and Fig. 2).

Despite the unchanged spectral properties, the photoactivity of the 655 nm form was fully inhibited at 10^{-2} M Hg^{++}. This inhibition could be caused by the direct redox reaction of Hg^{++} with NADPH, similarly as suggested for Cd^{++} ions (Böddi *et al.* 1995). As a result, POR-Pchlide-$NADP^+$ complexes are formed which are inactive at irradiation. The Pchlide-$NADP^+$ complexes exhibit specific spectral properties: in case of Cd^{++} treatment, narrow emission band appeared at 641.5 nm (Böddi *et al.* 1995). This emission band, however, did not appear in the case of Hg^{++}-treatment. The ion size of Hg^{++} must be considered when discussing the possible mode of the inhibition: it is 110 pm (larger that that of Cd^{++} ion with its size of 97 pm) which allows a smaller mobility. Also, the high affinity of Hg to sulfur (Wright *et al.* 1990), and the presence of S-containing amino acid residues in the POR (Darroh *et al.* 1990) must be considered. Consequently, the Hg^{++} ions could not reach all of the NADPH molecules in the reaction centre of POR. They could react with NADPH molecules connected at different sites of the prolamellar body membranes or with the amino acid residues—probably with cystein—on the surface of the enzyme. The formation of –S-Hg-S– bonds cause remarkable structural changes because of the closely linear geometry of this bond (Bochmann *et al.* 1992). On the other hand, the reaction of the Hg^{++} with amino groups—where it can substitute the H—can also have an effect (Brodersen and Hummel 1987). This reaction could cause the precipitation of the membrane particles and modify the native geometry of the active site of POR. As a result, the NADPH could be removed from the direct vicinity of the C17-C18 double bond but could be localised close enough to the electronic cloud of Pchlide to keep the spectral properties unchanged. A further possibility is that the POR protein was fully denatured and the Pchlide molecules were released from the enzyme subunits. The "free" Pchlide molecules could form artificial aggregates with similar spectral properties to those observed in water containing model systems (Böddi *et al.* 1983), however, the fluorescence bands of the artificial aggregates are usually much broader than those of the forms observed in this work.

These results demonstrate that the spectral similarities are not absolute evidences for the structural identity, a combination of different methods is needed to characterise the precise molecular arrangement of POR or other enzymes.

correspond to newly formed Chlide (Fig. 4, curve *c*).

The complete inhibition of the photo-conversion is demonstrated in Fig. 5 in which the spectra of homogenates treated with 10^{-2} M Hg^{++} recorded before (Fig. 5, curve *a*) and after irradiation (Fig. 5, curve *b*), are plotted together. A slight amplitude decrease is only seen, which can be caused by slight bleaching.

To study if Hg^{++} effected directly on NADPH, absorption spectra of solutions were recorded in the 300-400 nm region. The solvent was the isolation medium to which NADPH and Hg^{++} were added in equimolar ratios with 10^{-3} and 10^{-2} M concentrations. In all cases, the 340 nm absorption band characteristic for the reduced NADPH disappeared within a few seconds.

References

Barlton, D., Smith, A.M.: Merury binding to human haemoglobin. – Experientia **29**: 1178-1179, 1973.

Beale, S.I.: Enzymes of chlorophyll biosynthesis. – Photosynth. Res. **60**: 43-73, 1999.

Begley, T.P., Young, H.: Protochlorophyllide reductase. 1. Determination of the regiochemistry and the stereochemistry of the reduction of protochlorophyllide to chlorophyllide. – J. amer. chem. Soc. **111**: 3095-3096, 1989.

Bochmann, M., Webb, K.J., Powell, A.K.: Synthesis and structure of $[Hg(SC_6H_2Bu^t_3)_2(py)]$: a T-shaped complex of mercury. – Polyhedron **111**: 513-516, 1992.

Böddi, B., Evertsson, I., Ryberg, M., Sundquist, C.: Protochlorophyllide transformation and chlorophyllide accumulation in epicotyls of pea (*Pisum sativum*). – Physiol. Plant. **96**: 706-713, 1996.

Böddi, B., Kis-Petik, K., Kaposi, A.D., Fidy, J., Sundqvist, C.: The two spectroscopically different short wavelength protochlorophyllide forms in pea epicotyls are both monomeric. – Biochim. biophys. Acta **1365**: 531-540, 1998.

Böddi, B., Lindsten, A., Ryberg, M., Sundqvist, C.: On the aggregational states of protochlorophyllide and its protein complexes in wheat etioplasts. – Physiol. Plant. **76**: 135-143, 1989.

Böddi, B., Oravecz, A.R., Lehoczki, E.: Effect of cadmium on organization and photoreduction of protochlorophyllide in dark-grown leaves and etioplast inner membrane preparations of wheat. – Photosynthetica **31**: 411-420, 1995.

Böddi, B., Rákász, É., Láng, F.: The effect of water on the aggregation of protochlorophyll in solid films. – Photobiochem. Photobiophys. **5**: 27-33, 1983.

Böddi, B., Ryberg, M., Sundqvist, C.: The formation of a short-wavelength chlorophyllide form at partial phototransformation of protochlorophyllide in etioplast inner membranes. – Photochem. Photobiol. **53**: 667-673, 1991.

Böddi, B., Ryberg, M., Sundqvist, C.: Identification of four universal protochlorophyllide forms in dark grown leaves by analyses of the 77 K fluorescence emission spectra. – J. Photochem. Photobiol. B **12**: 389-401, 1992.

Böddi, B., Ryberg, M., Sundqvist, C.: Analysis of the 77 K fluorescence emission and excitation spectra of isolated etioplast inner membranes. – J. Photochem. Photobiol. B **21**: 125-133, 1993.

Brodersen, K., Hummel, H.-U.: The mercury. – In: Comprehensive Coordination Chemistry. Vol. 5. Pp. 1047-1130. Pergamon Press, Oxford 1987.

Darroh, P.M., Kay, S.A., Teakle, G.R., Griffiths, W.T.: Cloning and sequencing of protochlorophyllide reductase. – Biochem. J. **265**: 789-798, 1990.

Dujardin, E., Sironval, C.: The reduction of protochlorophyllide into chlorophyllide. III. The phototransformability of the forms of the protochlorophyllide-lipoprotein complex found in the darkness. – Photosynthetica **4**: 129-138, 1970.

Foster, T.J.: Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. – Microbiol. Rev. **47**: 361-409, 1983.

Fox, B.S., Walsh, C.T.: Mercuric reductase: Homology to glutathione reductase and lipoamide dehydrogenase. Iodoacetamide alkylation and sequence of the active site peptide. – Biochemistry **22**: 4082-4088, 1983.

Franck, F., Bereza, B., Böddi, B.: Protochlorophyllide-$NADP^+$ and protochlorophyllide-NADPH complexes and their regeneration after flash illumination in leaves and etioplast membranes of dark-grown wheat. – Photosynth. Res. **59**: 53-61, 1999.

Franck, F., Inoue, Y.: Light-driven reversible transformation of chlorophyllide $P_{696,684}$ into chlorophyllide $P_{688,678}$ in illuminated etiolated bean leaves. – Photobiochem. Photobiophys. **8**: 85-96, 1984.

Franck, F., Mathis, P.: A short-lived intermediate in the photoenzymatic reduction of protochlorophyll(ide) into chlorophyll(ide) at physiological temperature. – Photochem. Photobiol. **32**: 799-803, 1980.

Fujita, Y.: Protochlorophyllide reduction: a key step in the greening of plants. – Plant Cell Physiol. **37**: 411-421, 1996.

Griffiths, W.T.: Reconstitution of chlorophyllide formation by isolated etioplast membranes. – Biochem. J. **174**: 681-692, 1978.

Helfrich, M., Schoch, S., Schäfer, W., Ryberg, M., Rüdiger, W.: Absolute configuration of protochlorophyllide and substrate specificity of NADPH:protochlorophyllide oxidoreductase. – J. amer. chem. Soc. **118**: 2606-2611, 1996.

Hendrich, W., Bereza, B.: Spectroscopic characterization of protochlorophyllide and its transformation. – Photosynthetica **28**: 1-16, 1993.

Holtorf, H., Reinbothe, S., Reinbothe, C., Bereza, B., Apel, K.: Two routes of chlorophyllide synthesis that are differentially regulated by light in barley. – Proc. nat. Acad. Sci. USA **92**: 3254-3258, 1995.

Kis-Petik, K., Böddi, B., Kaposi, A., Fidy, J.: Protochlorophyllide forms and energy transfer in dark-grown wheat leaves. Studies by conventional and laser excited fluorescence spectroscopy between 10 K–100 K. – Photosynth. Res. **60**: 87-98, 1999.

Lau, S., Sarkar, B.: Inorganic mercury(II)-binding component in normal human blood serum. – J. Toxicol. environ. Heart **5**: 907-916, 1979.

Lebedev, N., Timko, M.P.: Protochlorophyllide photoreduction. – Photosynth. Res. **58**: 5-23, 1998.

Oliver, R.P., Griffiths, W.T.: Pigment-protein complex of illuminated etiolated leaves. – Plant Physiol. **70**: 1019-1025, 1982.

Osawa, N., Masuda, T., Awai, K., Fusada, N., Shimada, H., Ohta, H., Takamiya, K.: Identification and light-induced expression of a novel gene of NADPH-protochlorophyllide oxidoreductase isoform in *Arabidopsis thaliana*. – FEBS Lett. **474**: 133-136, 2000.

Ryberg, M., Sundqvist, C.: Characterization of prolamellar bodies and prothylakoids fractionated from wheat etioplasts. – Physiol. Plant. **56**: 125-132, 1982.

Šeršeň, F., Kráľová, K.: New facts about $CdCl_2$ action on the photosynthetic apparatus of spinach chloroplasts and its comparison with $HgCl_2$ action. – Photosynthetica **39**: 575-580, 2001.

Shames, S.S., Fairlamb, A., Cerami, A., Walsh, C.T.: Purification and characterisation of trypanothione reductase from *Crithidia fasciculata*, a newly discovered member of the family of disulfide-containing flavoprotein reductases. – Biochemistry **25**: 3519-3526, 1986.

Summers, A.O.: Organization, expression, and evolution of genes for mercury resistance. – Annu. Rev. Microbiol. **40**:

607-634, 1986.

Teakle, G.R., Griffiths, W.T.: Cloning, characterization and import studies on protochlorophyllide reductase from wheat (*Triticum aestivum*). – Biochem. J. **296**: 225-230, 1993.

Valera, V., Fung, M., Wessler, A.N., Richards, W.R.: Synthesis of 4R- and 4S-tritium labeled NADPH for the determination of the coenzyme stereospecificity of NADPH:protochlorophyllide oxidoreductase. – Biochem. biophys. Res. Commun. **148**: 515-520, 1987.

Wiktorsson, B., Engdahl, S., Zhong, L.B. Böddi, B., Ryberg, M., Sundqvist, C.: The effect of cross-linking of the subunits of NADPH-protochlorophyllide oxidoreductase on the aggregational state of protochlorophyllide. – Photosynthetica **29**: 205-218, 1993.

Wright, J.G., Natan, M.J., MacDonell, F.M., Ralston, D.M., O'Halloran, T.V.: Mercury(II)-thiolate chemistry and the mechanism of the heavy metal. – Prog. inorg. Chem. **38**: 323-412, 1990.