

Hypothesis

A general model for regulation of photosynthetic unit function by protein phosphorylation

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We propose that regulatory effects of membrane protein phosphorylation in photosynthetic systems result in all cases from simultaneous phosphorylation by a single kinase of the polypeptides of two intrinsic pigment-protein complexes, with phosphorylation leading to their mutual electrostatic repulsion in a direction parallel to the membrane plane and therefore to decreased excitation energy transfer between them. One complex is a peripheral light-harvesting complex and the other is bound to the reaction centre and functions as a link in excitation energy transfer. Immediate effects of phosphorylation are therefore decreased absorption cross-section together with decreased cooperativity of photosynthetic units. This general model applies equally to photosystem II of green plants, algae and cyanobacteria, as well as to the single photosystem of purple bacteria. Special cases of this general model permit increased excitation energy transfer to one type of reaction centre at the expense of another, and this may occur even in laterally homogeneous membranes that are uniformly unappressed.

Photosynthetic unit Light harvesting Protein phosphorylation Excitation energy distribution
Membrane stacking 9 kDa phosphoprotein

1. INTRODUCTION

In green plants, phosphorylation of LHC-II is known to regulate distribution of absorbed excitation energy between PS I and PS II [1-3]. This regulation involves lateral migration of LHC-II from appressed regions of thylakoid membrane rich in PS II to unappressed regions rich in PS I [4-12], and the force initiating this migration is

thought to be electrostatic repulsion between the phosphorylated and therefore negatively charged LHC-II complexes on adjacent, appressed regions of membrane [4,5,7]. Regulation of the activity of the protein kinase by the redox level of plastoquinone [1,13] completes a negative feedback loop which maintains balanced excitation energy distribution under physiological conditions [1,4,14,15].

This model cannot apply to photosynthetic prokaryotes, however, since it depends absolutely on heterogeneous distribution of the two photosystems between appressed and unappressed regions of membrane. In cyanobacteria, for example, the photosystems are thought to be distributed homogeneously throughout unappressed thylakoids [16-21]. Cyanobacteria are also devoid of LHC-II, carrying out light harvesting by means of extrinsic phycobiliprotein complexes called phycocyanin.

This paper is dedicated by J.F.A. to the memory of his father, George Frederick Allen, 1908-1985

Abbreviations: LHI, light-harvesting complex I; LHII, light-harvesting complex II; LHC-II, light-harvesting chlorophyll *a/b* pigment-protein complex; LHi, intermediate light-harvesting complex; LHp, peripheral light-harvesting complex; PS, photosystem; RC, reaction centre

bilisomes [17–22]. Nevertheless, it has been clear for some time that cyanobacteria are able to perform a physiological regulation of excitation energy distribution which is similar in its overall properties to that encountered in green plants [23]. This is also true of red algae [24–26]. Although it has been argued that the absence of lateral heterogeneity rules out a protein phosphorylation mechanism for phycobilisome-containing organisms [27–31], cyanobacterial membrane protein phosphorylation has now been demonstrated both *in vitro* and *in vivo* [32–34] and is accompanied by changes in excitation energy distribution [34].

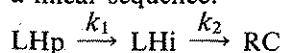
Phosphorylation of membrane proteins has also now been demonstrated in the purple photosynthetic bacteria *Rhodospirillum rubrum* [35–37] and *Rhodospseudomonas sphaeroides* [38], and in both cases phosphorylated polypeptides have been provisionally identified as light-harvesting polypeptides on the basis of mobility in polyacrylamide gel electrophoresis. Purple bacteria have a single type of reaction centre and the need to explain control of excitation energy distribution between PS I and II does not arise.

In this letter we propose a common mechanism for effects of protein phosphorylation on excitation energy transfer in these apparently diverse systems. This model provides a functional explanation of phosphorylation of membrane proteins in photosynthetic prokaryotes. It also requires a reappraisal of the role of thylakoid lateral heterogeneity in effects of protein phosphorylation in green plants: it predicts that large-scale lateral migration of light-harvesting complexes is neither a necessary nor a sufficient condition for regulation of excitation energy distribution in photosynthesis.

2. THE MODEL

There are eight postulates of this model, as follows.

- (1) Excitation energy transfer from peripheral light-harvesting complexes (LHp) to reaction centres (RC) occurs via one or more intermediate light-harvesting complexes (LHi) in a linear sequence:



- (2) Chlorophyll-binding polypeptides of both types of light-harvesting complex (LHp and LHi) are reversibly phosphorylated under physiological conditions.
- (3) Phosphorylation of LHp and LHi causes a change in the balance of charge on their polypeptide chains at the membrane surface. This results in their mutual electrostatic repulsion with the repulsive force acting in a direction parallel to the membrane plane.
- (4) The magnitude of the repulsive force between phosphorylated LHp and LHi exceeds the sum of the forces which otherwise hold the unphosphorylated complexes in close contact. The phosphorylated complexes therefore move apart, and this decreases the probability of excitation energy transfer between them, i.e. k_1 is decreased.
- (5) LHi is tightly bound to RC and excitation energy transfer from LHi to RC is unaffected by protein phosphorylation, i.e. k_2 is constant.
- (6) Where a number of reaction centres are connected by a common pool of LHp, the connectivity of these reaction centres is decreased by phosphorylation-induced mutual electrostatic repulsion of individual LHi and LHp complexes.
- (7) Phosphorylation-induced mutual electrostatic repulsion of LHp and LHi decreases the effective absorption cross-section of individual photosynthetic units.
- (8) Where two kinds of reaction centre compete for excitation energy from a pool of LHp, decreased excitation energy transfer to a reaction centre that has a tightly bound and phosphorylated LHi permits increased excitation energy transfer to a reaction centre that has no phosphorylated LHi.

Fig.1 shows a diagram representing this general model as it applies in the ideal case of two cooperating photosynthetic units. The individual components of each photosynthetic unit are represented by circles and the units are shown as if viewed in a direction perpendicular to the membrane plane. A protein kinase catalyzes phosphorylation of polypeptides of both LHp and

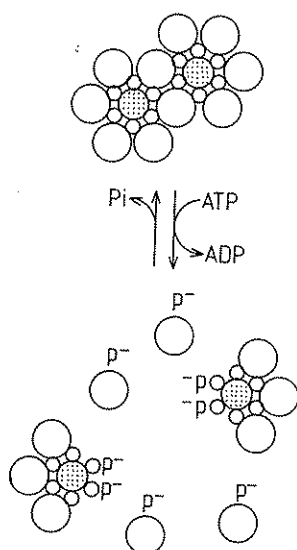


Fig.1. Effects of protein phosphorylation on the association of LHp (large circles) and LHi (small circles) in the ideal case of two cooperating photosynthetic units. A protein kinase catalyzes phosphorylation of both complexes which results in their mutual electrostatic repulsion. This in turn causes decreased absorption cross-section and decreased cooperativity. A protein phosphatase catalyzes dephosphorylation of the complexes which restores their original interaction. Medium, stippled circles; RC.

LHi, leading to their electrostatic dissociation. Total excitation energy transfer to the reaction centre is decreased, as is the cooperativity of the two photosynthetic units. Reassociation of LHp and LHi results from their dephosphorylation which is catalyzed by a protein phosphatase. This in turn causes reconnection of the two units together with increased energy transfer to each reaction centre. Fig.2 depicts altered cooperativity without complete detachment of LHp. Figs 3 and 4 depict special cases of the general model that apply where two different reaction centres compete for excitation energy from LHp in accordance with the eighth postulate.

3. SPECIAL CASE:

PURPLE PHOTOSYNTHETIC BACTERIA

The light-harvesting pigment-protein complexes of the Rhodospirillaceae can be of a number of different types, dependent on the biological species. Typically, a long-wavelength form, LHI (B875 or

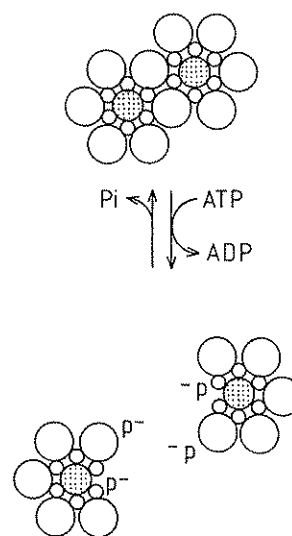


Fig.2. Effects of protein phosphorylation of LHp (large circles) and LHi (small circles) on cooperativity of two photosynthetic units of a purple photosynthetic bacterium. A protein kinase catalyzes phosphorylation of both complexes which in turn decreases cooperativity. A protein phosphatase catalyzes dephosphorylation which increases cooperativity. In the case of *R. rubrum*, the single light-harvesting species, B880, is likely to function both as LHp and as LHi.

B880), is closely associated with the reaction centre, and other species such as LHII (B800-850) donate absorbed excitation energy to the reaction centre via LHI [39]. They exist as mobile oligomers [40] and, for LHII at least, their aggregation state appears responsive to the ionic environment [41]. We suggest that light-harvesting components that act as LHi for any photosynthetic unit in a 'lake' of units also act as LHp for neighbouring units. We further suggest that the aggregation state of a number of photosynthetic units, i.e. the extent to which they function as a lake or as discrete 'puddles' [42], could then be regulated by phosphorylation of light-harvesting polypeptides. We therefore propose that polypeptide components of both LHI and LHII are phosphorylated and that this phosphorylation causes their electrostatic dissociation, leading in turn to decreased cooperativity. Phosphorylation of membrane polypeptides in the correct molecular mass region has been demonstrated in cells and chromatophores of *Rps. sphaeroides* [38]. Effects on absorption cross-

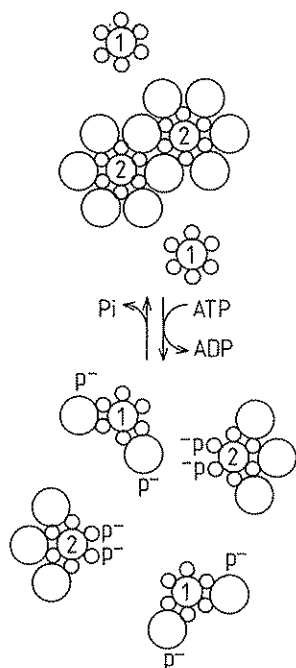


Fig.3. Effects of protein phosphorylation in causing dissociation of LHP from one photosynthetic unit and its reassociation with another. Cooperativity and absorption cross-section of PS II is decreased as in fig.1. Phosphorylated LHP (large circles) is dissociated from phosphorylated LHi (small circles) of PS II and reassociates with PS I. This process is proposed as the basis of the state 2 transition in systems that lack lateral heterogeneity (cyanobacteria, red algae, green algae such as *Euglena* and some higher plant chloroplasts). The state 1 transition is the reverse of this process and is catalyzed by the protein phosphatase.

section in purple photosynthetic bacteria are likely to be small or absent, since there is no evidence for a highly fluorescent LHP species completely decoupled from any reaction centre. A special case of the general model which takes into account and which we propose for the purple photosynthetic bacteria is shown in fig.2.

R. rubrum is a purple bacterium which has only LHI, B880 [43]. Phosphorylation of polypeptides of B880 has been reported [35-37], and conditions affecting this phosphorylation in whole cells have been shown also to be correlated with altered cooperativity of photosynthetic units [36,37]. Recent results [37] indicate that decreased cooperativity is associated with phosphorylation of

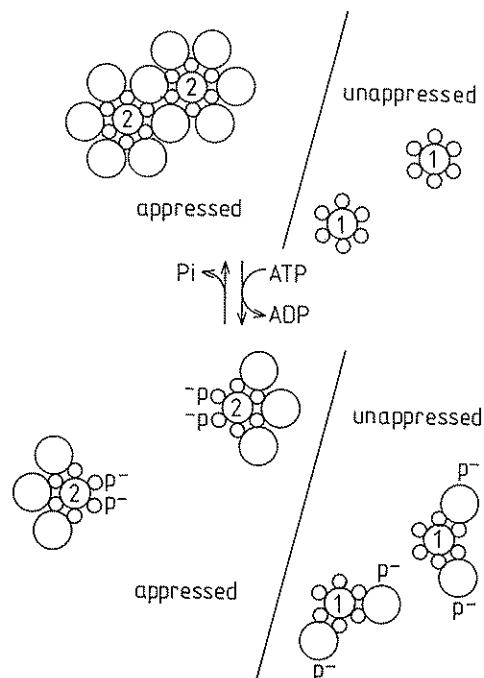


Fig.4. Effects of protein phosphorylation on photosynthetic unit function where PS I and PS II are laterally separated. The phosphorylated LHP (large circles) is dissociated from the phosphorylated LHi (small circles) as in fig.3, but LHP (LHC-II) must then migrate laterally in order to reassociate instead with PS I.

an 11 kDa polypeptide that is provisionally identified as the β -subunit of B880, while increased cooperativity is associated with phosphorylation of a 13 kDa polypeptide that may be the α -subunit of B880. Since the phosphorylation sites of the two subunits may be located on opposite sides of the membrane [37], it is proposed that phosphorylation of B880- β is the event leading to electrostatic dissociation of individual B880 units, consistent with the model described above (fig.2). During regulation of excitation energy transfer, the B880 of any given photosynthetic unit would then function as LHi for that unit/ while the B880 of neighbouring units would function as LHP. The role of phosphorylation of B880- α , possibly on the periplasmic side of the membrane, is unclear.

Redox control of protein phosphorylation has yet to be demonstrated in purple bacteria. By analogy with green plants, control by redox level of the ubiquinone pool is a possibility. This would

serve to couple excitation energy transfer to electron transport, perhaps providing a negative feedback control to maintain optimal redox levels despite changes in light intensity, electron donor and acceptor concentrations, and metabolic demand for ATP.

4. SPECIAL CASE: CYANOBACTERIA

The LH_p of PS II of cyanobacteria (blue-green algae) is an extrinsic phycobilin pigment-protein complex, the phycobilisome [18,20,22,44]. Phosphorylation in vivo of soluble or weakly membrane-bound polypeptides of 17–20 kDa has been reported for the cyanobacterium *Synechococcus* 6301 (*Anacystis nidulans*), with a major phosphorylated band appearing at 18.5 kDa [33,34]. It is suggested [33,34] that this band corresponds to a polypeptide of the phycobilisome core [20,43]. Both this 18.5 kDa polypeptide and a membrane-bound polypeptide of 15 kDa have been shown to be phosphorylated in *Synechococcus* 6301 in the light but not in the dark [33,34]. This light regulation is also found in isolated *Synechococcus* thylakoids, and is correlated with changes in excitation energy distribution between PS I and PS II such that phosphorylation of these two polypeptides is accompanied by an increase in excitation energy transfer to PS I at the expense of PS II [34].

These findings support a model for regulation of excitation energy distribution in cyanobacteria in which it is assumed that the 15 kDa phosphoprotein functions as an LH_i species. This model [34] proposes that regulation of excitation energy transfer from the phycobilisome to PS II results from phosphorylation of the 18.5 kDa phycobilisome component as well as of the 15 kDa intermediate, resulting in their mutual electrostatic repulsion. If this model is correct then excitation energy transfer in PS II of cyanobacteria is regulated by a special case of the general mechanism proposed here (fig.1). An important difference between cyanobacteria and the purple bacteria is the existence in the former of two photosystems. In order to explain redistribution of absorbed excitation energy in favour of PS I, it is necessary to assume that the phosphorylation-induced electrostatic decoupling of the phycobilisome from PS II is accompanied by a closer

association and increased interaction of the phycobilisome with PS I. A temperature-dependent decoupling of the phycobilisome from PS II has been observed [45] and resembles closely the phosphorylation-dependent decoupling that is predicted by our model.

Fig.3 depicts the general model for regulation of photosynthetic unit function as it applies in organisms, such as cyanobacteria, where two photosynthetic units compete for excitation energy from an LH_p complex, and where the two types of photosynthetic unit are homogeneously distributed throughout the membrane plane.

Control of the protein kinase by redox level of an interphotosystem electron transport component such as plastoquinone would enable distribution of excitation energy between the two photosystems to be regulated by their relative rates of turnover. Protein phosphorylation is therefore a plausible mechanism for state 1–state 2 transitions even in laterally homogeneous thylakoid membranes.

5. SPECIAL CASE: GREEN PLANTS

In most green plant chloroplasts, two types of photosynthetic unit are distributed heterogeneously throughout the thylakoid membrane. Lateral movement of the LH_p complex, LHC-II, from appressed regions of membrane rich in PS II to unappressed regions rich in PS I is now widely accepted as the basis of the state 2 transition, in which absorbed excitation energy is redistributed from PS II to PS I [4–12]. This lateral movement of LHC-II is in some way brought about by phosphorylation of a 25 kDa LHC-II polypeptide, and the consensus of opinion at present [4–12] is that electrostatic forces between adjacent, phosphorylated LHC-II complexes on neighbouring membranes act in a direction perpendicular to the membrane plane in order to initiate this lateral movement.

We propose instead that regulation of excitation energy distribution in green plants by phosphorylation of LHC-II is a third special case of our general model. This special case is depicted in fig.4. In our view, the initial event in the transition to state 2 in green plants is phosphorylation of not one but two membrane-bound pigment-protein complexes, one being the mobile LH_p complex, LHC-II, the other being an LH_i complex tightly bound to PS II. As

for all cases of the general model, mutual electrostatic repulsion of the phosphorylated forms of the two complexes acts in a direction parallel to the membrane plane. It thereby serves to detach LHP units, leading initially to decreased absorption cross-section and to decreased cooperativity. Decreased connectivity of PS II reaction centres is known to result from thylakoid membrane protein phosphorylation [46]. In green plants, lateral heterogeneity and thylakoid membrane stacking serve merely to increase the distance that has to be travelled by the LHP complex, LHC-II, before it can reassociate with PS I.

Although it is not critical for the formal description of the model (fig.4), we further propose that the LHP complex that is involved in regulation of PS II function has the 9 kDa phosphoprotein [47-50] as one of its components. Similarities in amino acid composition of the pea thylakoid 9 kDa phosphoprotein and LHC-II [47] are consistent with our proposal that the 9 kDa phosphoprotein is a chlorophyll-binding protein. The 9 kDa and 25 kDa polypeptides are known to be phosphorylated simultaneously during state 2 transitions [14] and when their protein kinase is redox-activated [1,13]. Unlike LHC-II, the 9 kDa polypeptide does not migrate laterally but remains with PS II in appressed membranes even when phosphorylated [51]. This may explain its slower dephosphorylation than that of LHC-II [49]. The participation of the 9 kDa polypeptide in state 1-state 2 effects is perfectly consistent with its slower dephosphorylation if we assume that both the 9 kDa polypeptide and LHC-II must be phosphorylated during state 2: the state 1 transition will then have the kinetics of dephosphorylation of the more rapidly dephosphorylating component. Equally, if the 9 kDa phosphoprotein is a component of LHP then its reversible phosphorylation will have no effect on excitation energy transfer where phosphorylation of LHC-II is completely inhibited, and the absence of such an effect is by no means evidence against the involvement of the 9 kDa component in this process [52].

We conclude that thylakoid stacking and lateral heterogeneity are neither necessary nor sufficient for regulation of excitation energy distribution by a mechanism involving protein phosphorylation, and that the well-studied regulatory mechanism found in green plants is merely a particular case of a more general and fundamental phenomenon.

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REFERENCES

- [1] Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) *Nature* 291, 25-29.
- [2] Bennett, J., Steinback, K.E. and Arntzen, C.J. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5253-5257.
- [3] Allen, J.F. (1983) *Trends Biochem. Sci.* 8, 369-373.
- [4] Barber, J. (1982) *Annu. Rev. Plant Physiol.* 33, 261-295.
- [5] Bennett, J. (1983) *Biochem. J.* 212, 1-13.
- [6] Howarth, P., Kyle, D.J., Horton, P. and Arntzen, C.J. (1982) *Photochem. Photobiol.* 36, 743-748.
- [7] Horton, P. (1983) *FEBS Lett.* 152, 47-52.
- [8] Horton, P. and Black, M.T. (1980) *FEBS Lett.* 119, 141-144.
- [9] Kyle, D.J., Kuang, T.-Y., Watson, J.L. and Arntzen, C.J. (1984) *Biochim. Biophys. Acta* 765, 89-96.
- [10] Telfer, A., Bottin, H., Barber, J. and Mathis, P. (1984) *Biochim. Biophys. Acta* 764, 324-330.
- [11] Barber, J. (1983) *Photobiochem. Photobiophys.* 5, 181-190.
- [12] Staehelin, L.A. and Arntzen, C.J. (1983) *J. Cell Biol.* 97, 1327-1337.
- [13] Horton, P., Allen, J.F., Black, M.T. and Bennett, J. (1981) *FEBS Lett.* 125, 193-196.
- [14] Telfer, A., Allen, J.F., Barber, J. and Bennett, J. (1983) *Biochim. Biophys. Acta* 722, 176-181.
- [15] Malkin, S., Telfer, A. and Barber, J. (1986) *Biochim. Biophys. Acta* 848, 48-57.
- [16] Stanier, R.Y. and Cohen-Bazire, G. (1977) *Annu. Rev. Microbiol.* 31, 225-274.
- [17] Gantt, E. (1981) *Annu. Rev. Plant Physiol.* 32, 327-347.
- [18] Glazer, A.N. (1984) *Biochim. Biophys. Acta* 768, 29-51.
- [19] Tandeau de Marsac, N. (1983) *Bull. Inst. Pasteur* 81, 201-254.
- [20] Yamanaka, G., Lundell, D.J. and Glazer, A.N. (1982) *J. Biol. Chem.* 257, 4077-4086.

- [21] Larkum, A.W.D. and Barrett, J. (1983) *Adv. Bot. Res.* 10, 1-219.
- [22] Glazer, A.N. (1985) *Annu. Rev. Biophys. Biophys. Chem.* 14, 47-77.
- [23] Fork, D.C. and Satoh, K. (1983) *Photochem. Photobiol.* 37, 421-427.
- [24] Murata, N. (1969) *Biochim. Biophys. Acta* 172, 242-251.
- [25] Ried, A. and Reinhardt, B. (1977) *Biochim. Biophys. Acta* 460, 25-35.
- [26] Ley, A.C. and Butler, W.L. (1980) *Plant Physiol.* 65, 714-722.
- [27] Bruce, D., Biggins, J., Steiner, T. and Thewalt, M. (1985) *Biochim. Biophys. Acta* 806, 237-246.
- [28] Miller, K.R. and Lyon, M.K. (1985) *Trends Biochem. Sci.* 10, 219-222.
- [29] Biggins, J., Campbell, C.L. and Bruce, D. (1984) *Biochim. Biophys. Acta* 767, 138-144.
- [30] Biggins, J. and Bruce, D. (1985) *Biochim. Biophys. Acta* 806, 230-236.
- [31] Satoh, K. and Fork, D.C. (1983) *Biochim. Biophys. Acta* 722, 190-196.
- [32] Schuster, G., Owens, G.C., Cohen, Y. and Ohad, I. (1984) *Biochim. Biophys. Acta* 767, 596-605.
- [33] Sanders, C.E., Holmes, N.G. and Allen, J.F. (1986) *Biochem. Soc. Trans.* 14, 66-67.
- [34] Allen, J.F., Sanders, C.E. and Holmes, N.G. (1985) *FEBS Lett.* 193, 271-275.
- [35] Holuigue, L., Lucero, H.A. and Vallejos, R.H. (1985) *FEBS Lett.* 181, 103-108.
- [36] Loach, P.A., Parkes, P.S. and Bustamante, P. (1984) in: *Advances in Photosynthesis Research* (Sybesma, C. ed.) vol.2, pp.189-197, Nijhoff/Junk, The Hague.
- [37] Holmes, N.G. and Allen, J.F. (1986) *FEBS Lett.* 200, 144-148.
- [38] Holmes, N.G., Sanders, C.E. and Allen, J.F. (1986) *Biochem. Soc. Trans.* 14, 67-68.
- [39] Cogdell, R.J. and Thornber, J.P. (1980) *FEBS Lett.* 122, 1-8.
- [40] Varga, A.R. and Staehelin, L.A. (1985) *J. Bacteriol.* 161, 921-927.
- [41] Varga, A.R. and Staehelin, L.A. (1985) *Arch. Microbiol.* 141, 290-296.
- [42] Monger, T.G. and Parson, W.W. (1977) *Biochim. Biophys. Acta* 460, 393-407.
- [43] Cogdell, R.J., Lindsay, J.G., Valentine, J. and Durant, I. (1982) *FEBS Lett.* 150, 151-154.
- [44] Glazer, A.N. (1983) *Annu. Rev. Biochem.* 52, 125-157.
- [45] Manodori, A. and Melis, A. (1985) *FEBS Lett.* 181, 79-82.
- [46] Kyle, D.J., Haworth, P. and Arntzen, C.J. (1982) *Biochim. Biophys. Acta* 680, 336-342.
- [47] Allen, J.F. and Findlay, J.B.C. (1986) *FEBS Lett.*, submitted.
- [48] Widger, W.R., Farchaus, J.W., Cramer, W.A. and Dilley, R.A. (1984) *Arch. Biochem. Biophys.* 233, 72-79.
- [49] Black, M.T. and Horton, P. (1985) *Biochim. Biophys. Acta* 767, 568-573.
- [50] Farchaus, J. and Dilley, R.A. (1986) *Arch. Biochem. Biophys.* 244, 94-101.
- [51] Larsson, U.K., Jergil, B. and Andersson, B. (1983) *Eur. J. Biochem.* 136, 25-29.
- [52] Farchaus, J., Dilley, R.A. and Cramer, W.A. (1985) *Biochim. Biophys. Acta* 809, 17-26.