Biochimica et Biophysica Acta, 974 (1989) 267–273
Elsevier

BBA 42991

Thylakoid protein phosphorylation and associated Photosystem II fluorescence changes: a study with the ATP analogue adenosine-5-*O*-thiotriphosphate (ATP-γ-S)

Khalid Islam

Centro CNR di Biologia Cellulare e Molecolare delle Piante, Dipartimento di Biologia, Università di Milano, Milano (Italy)

(Received 14 November 1988)

Key words: ATP-γ-S; Fluorescence; Thylakoid; Chloroplast; Phosphorylation; Photosystem II; (Spinach leaf)

The effects of adenosine-5-*O*-thiotriphosphate (ATP-γ-S), an analog of ATP, on the phosphorylation of the thylakoid membrane proteins and Photosystem II fluorescence decline in isolated spinach thylakoids has been investigated. It is shown that ATP-γ-S is (a) an extremely inefficient substrate for the endogenous protein kinase(s), but (b) an efficient inhibitor of the ATP-induced phosphorylation. By contrast, ATP-γ-S appears to behave in a similar manner compared with ATP with respect to the Photosystem II fluorescence decline in that (a) the time-course of the decline is similar for both nucleotides; (b) similar changes in the fluorescence parameters F_v/F_0, at two different screening cation concentrations, occur in the presence of the two nucleotides; and (c) digitonin fractionation exhibits an altered distribution of LHC II and chlorophyll *a*/*b* ratios in the granal and stromal fractions of thylakoids incubated in the presence of the two nucleotides when compared with the control. The data are discussed with respect to the correlations between LHC II phosphorylation and the ATP-induced Photosystem II fluorescence decline.

Introduction

The phosphorylation/dephosphorylation of the light-harvesting chlorophyll *a*/*b* binding protein complex (LHC II) polypeptides has been proposed to constitute the molecular mechanism for the controlled redistribution of excitation energy between the two photosystems [1]. The energy redistribution has been variously proposed to occur either by changes in the optical cross-section of the photosystems or by energy 'spillover' from PS II to PS I due to electrostatic interactions (for reviews, see Refs. 2–8). However, these changes are generally thought to occur as a consequence of the negative charge introduced by the phosphate incorporated in the surface-exposed segment of LHC II [8–10]. Earlier studies had indicated that cation and consequently charge interactions may be responsible for the energy redistribution [11–13], although the influx or efflux of large quantities of cations in the thylakoids has not been demonstrated.

In view of the earlier models it is not hard to imagine that such charge interactions could be brought about by the phosphorylation of surface residues in the already highly charged region of the exposed protein [14–16]. Indeed, Barber and co-workers [7,8,17–19] have proposed models for such hypotheses based on calculations of the amount of charge introduced as a consequence of the modification and those required to lead to membrane disappression.

Perfect or close correlations have been reported between the rate and extent of phosphorylation and dephosphorylation of the LHC II protein and the ATP-induced fluorescence changes in Photosystem II [20–22]. Such correlations imply a causative relationship between the energy changes and the phosphorylation. In contrast, other studies indicate that there is little or no direct correlation between the rate and extent of phosphorylation of the LHC II protein and the energy changes [23–28], questioning a causative relationship. Similarly, inhibition studies such as the use of ADP analogs (FSBA) support a causative relationship [29] while those such as the use of zinc ions question such a relationship [22]. The use of GTP [30] would also tend to suggest that a direct causative relationship between LHC II phosphorylation and the fluorescence decline does not exist.

ATP-γ-S an analog of ATP (where an oxygen on the γ-phosphate is substituted by a sulphur) has been utilised in several studies to effect protein phosphorylation

Abbreviations: PS, Photosystem; LHC II, light-harvesting chlorophyll *a*/*b* binding protein complex II. Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; SDS, sodium dodecyl sulphate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Correspondence: K. Islam, (present address:) Merrell-Dow Research Institute, Lepetit Research Center, Via R. Lepetit 34, 21040 Gerenzano (Varese), Italy.

[31–34]. This analog is hydrolysed very slowly by ATPases and the phosphorylated protein is resistant to dephosphorylation [35–37]. Furthermore, the newly phosphorylated proteins can be easily separated from other proteins by utilising affi-gel affinity chromatography [37,38]. Thus this analog can be used to try and distinguish between an ATP effect per se or effects due to ATP-dependent phosphorylation [31], and, if utilised by the protein kinase, it can then be used for the separation of the phosphorylated peptides such as to allow their characterisation.

In view of the contrasting results on the correlations between LHC II phosphorylation and the associated changes in Photosystem II fluorescence this analog may provide useful information. In this paper the effect of ATP-γ-S on the phosphorylation and fluorescence decline of Photosystem II has been investigated. It is shown that the ATP-γ-S binds efficiently to the protein kinase but is utilised inefficiently as a substrate. The energy transfer by 'detachment' or 'spillover' mechanisms has also been examined by digitonin fractionation and changes in the fluorescence parameters.

Materials and Methods

Isolated thylakoids were prepared from freshly harvested spinach leaves as described previously [24–28]. Phosphorylation of the thylakoids, after the chemical reduction of the protein kinase(s), was essentially as described previously [24–28]. At various times aliquots were removed and the reaction terminated by the addition of ice-cold 80% acetone, for SDS-PAGE analysis, or by the addition of 100–200-fold excess of ice-cold phosphorylation medium.

The delipidated proteins were fractionated on 15% acrylamide gels by SDS-PAGE, stained with Coomassie brilliant blue, dried and autoradiographed using either Kodak X-Omat or Hyperfilm [24]. The phosphate incorporation into the LHC II and the total protein was determined by excision of the protein bands from the gel and counting in a liquid scintillation counter. For the competition studies ATP-γ-S was added 1 min prior to being challenged by [γ-^{32}P]ATP.

Fluorescence measurements were carried out in a home-built apparatus at a chlorophyll concentration of 2–4 μg/ml at 20 °C. F_0 was determined prior to the addition of 25 μM DCMU to determine the maximal fluorescence [24–28]. The excitation light was filtered through a Corning 4-96 filter and the fluorescence was measured at 691 nm (Balzers B-40 interference filter). Fluorescence quenching is expressed as:

$$\frac{F_m - \mathrm{NTP} - (F_m + \mathrm{NTP})}{F_m - \mathrm{NTP}}$$

where NTP was either ATP or ATP-γ-S.

Digitonin fractionation was carried out as described previously [25]. Isolated thylakoids incubated either in the absence (control) or in the presence of either ATP or ATP-γ-S for 10 min and left to stand on ice for 30 min prior to the addition of Digitonin to a final concentration of 0.5% and left on ice for a further 30 min with gentle agitation. The reaction was terminated by the addition of 10 volumes of ice-cold phosphorylation medium and the heavy fraction (10 000 × g, 30 min) and the light fraction (144 000 × g, 90 min) were collected by centrifugation.

ATP-γ-S was purchased from Boehringer, other biochemicals were from the Sigma Chemical Co., and all other reagents were of Analar grade. High specific activity [γ-^{32}P]ATP (3000 Ci/mmol) and [γ-^{35}S]ATP-γ-S (greater than 600 Ci/mmol) were purchased from the Radiochemical center, Amersham.

Results

In order to determine if ATP-γ-S could act as a substrate for the protein kinases, isolated thylakoids were incubated either in the presence of 1 mM ATP-γ-S containing labelled γ-^{35}S or 1 mM ATP containing labelled γ-^{32}P, after the chemical activation of the protein kinases. After fractionation of the proteins by SDS-PAGE the labelled proteins were visualised by autoradiography (Fig. 1). While extensive phosphorylation of several proteins is observed in the presence of ^{32}P there appears to be little phosphorylation of the polypeptides by ATP-γ-S even after 2 h of incubation.

The gel was further sliced into 0.5 cm slices and, after counting in the scintillation counter, it was observed that the incorporation ^{35}S/^{32}P was only about 1% for the LHC II protein (approx. 0.029 and 0.133 pmol phosphate were incorporated into the LHC II polypeptides after 5 and 120 min of incubation, respectively, in the presence of ATP-γ-S compared with 3.5 and 9.7 pmol phosphate incorporated in the presence of ATP) but about 3–4% for total protein. Some of the proteins exhibit a slightly higher incorporation of ^{35}S on the basis of the ratio of incorporation with ^{35}S/^{32}P; these differences in the incorporation levels may reflect the utilisation of different protein kinases [30].

A time-course of phosphorylation of the LHC II polypeptides was also undertaken. Isolated thylakoids were either incubated in the presence of 1 mM ATP-γ-S or with 1 mM ATP and after various times of incubation the incorporation into the LHC II polypeptides was determined (Fig. 2). While rapid phosphorylation occurs in the presence of ATP there is very inefficient phosphorylation in the case of ATP-γ-S. Indeed the rate of phosphorylation with ATP-γ-S is about 100-fold slower compared with ATP.

ATP-γ-S appears to be an inefficient substrate for the protein kinases. This may be due to inefficient

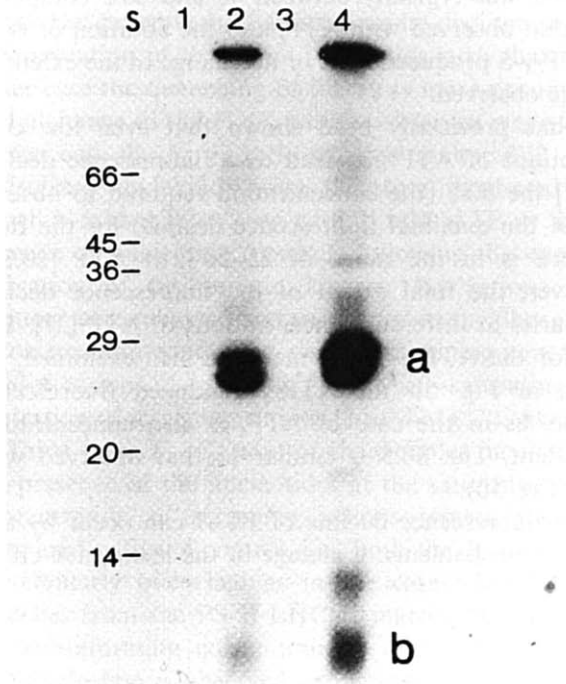

Fig. 1. An autoradiograph of thylakoid proteins labelled in the presence of either [γ-^{32}P]ATP (2,4) and [γ-^{35}S]ATP-γ-S (1,3). Isolated thylakoids were incubated in the presence of either 1 mM ATP or 1 mM ATP-γ-S containing the appropriate labelled nucleotide (see Materials and Methods) for either 5 (1,2) or 120 min (3,4). The reaction was then terminated and the proteins visualised by autoradiography, following fractionation by SDS-PAGE on 15% acrylamide gels. The position of the LHC II polypeptides (a) and the 9–11 kDa protein (b) are indicated. S refers to the molecular mass standards indicated and expressed in kDa.

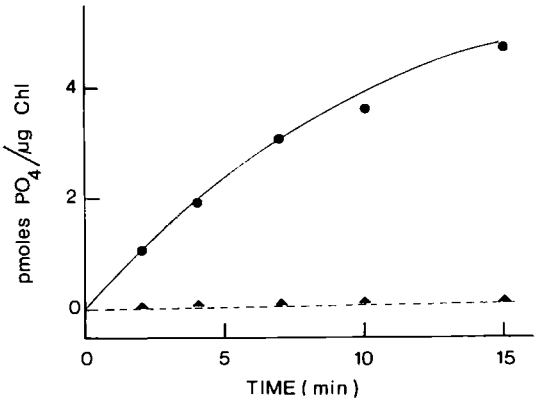

Fig. 2. Time-course of phosphorylation of the LHC II polypeptides in the presence of either ATP (●———●) or ATP-γ-S (▲- - - - -▲). Isolated thylakoids were incubated in the presence of either 1 mM ATP or 1 mM ATP-γ-S containing the appropriate labelled nucleotide. After various times aliquots were removed and following fractionation by SDS-PAGE the specific incorporation into the LHC II polypeptides was determined (see Materials and Methods).

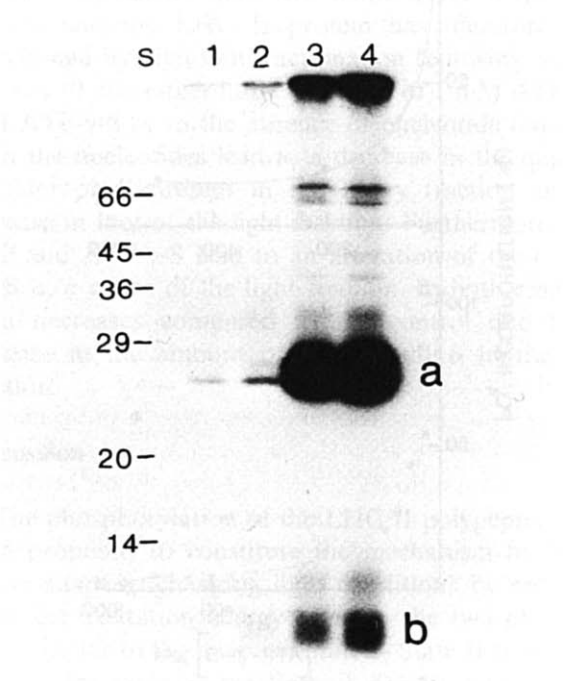

Fig. 3. An autoradiograph of phosphorylated thylakoid proteins incubated in the presence of either unlabelled ATP (3,4) or unlabelled ATP-γ-S (1,2) and challenged with [γ-^{32}P]ATP. Isolated thylakoids incubated in the presence of either ATP or ATP-γ-S and challenged with γ-^{32}P-labelled ATP for either 5 (1,3) or 120 min (2,4). The reaction was terminated and following fractionation by SDS-PAGE, the labelled polypeptides were visualised by autoradiography (see Materials and Methods). The positions of the LHC II polypeptides (a) and the 9–11 kDa protein (b) are indicated. S refers to the molecular-mass markers indicated and expressed in kDa.

recognition of this substrate by the protein kinase or due to the inefficient transfer of the phosphate group. One way to test whether the inefficiency of utilisation of ATP-γ-S is due to inefficient recognition is by determining if this substrate can compete with ATP. Clearly if it efficiently inhibits the phosphorylation by ATP then the low levels of phosphorylation by this substrate may reflect inefficient transfer of phosphate or alternatively if it does not inhibit phosphorylation by ATP then it is presumably recognised very poorly by the kinase.

Isolated thylakoids were therefore incubated in the presence of various concentrations of unlabelled ATP-γ-S and then challenged with ^{32}P labelled ATP. An autoradiograph of the dried gel showed that there is an marked inhibition of phosphorylation of the different polypeptides in the presence of ATP-γ-S (Fig. 3).

The amount of label co-migrating with the LHC II polypeptides (Fig. 4b) and the label incorporated into

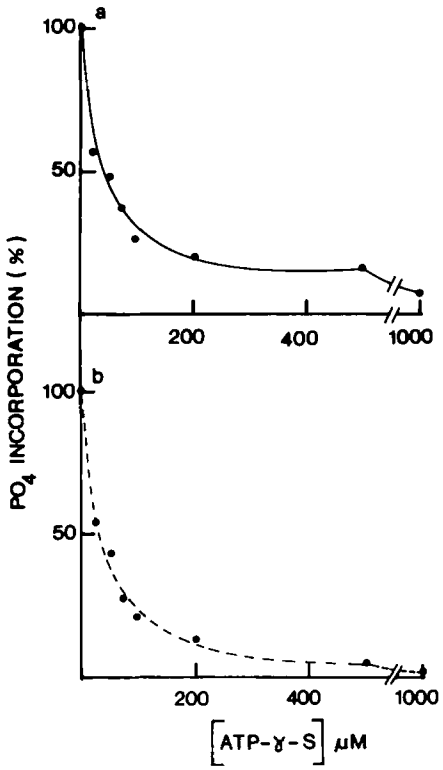

Fig. 4. Inhibition of the ATP-induced phosphorylation by ATP-γ-S into (a) total protein and (b) into the LHC II polypeptides. Isolated thylakoids were incubated in the presence of various concentrations of unlabelled ATP-γ-S for 1 min prior to being challenged with 100 μM ATP containing [γ-^{32}P]ATP. After a further 10 min of incubation the reaction was terminated and the incorporation into the total protein and into the LHC II polypeptides determined, following SDS-PAGE, by excision of the bands and liquid scintillation. The incorporation is expressed as a percentage of the phosphate incorporated into the control sample incubated under identical conditions except that of ATP-γ-S was omitted.

the total protein (Fig. 4a) was determined after excision of the protein bands from the gel. As can be seen ATP-γ-S is an efficient inhibitor of the ATP-induced phosphorylation, indicating that ATP-γ-S is recognised efficiently by the kinase. It is therefore more probable that it is the transfer of the phosphate which limits the reaction rate with this analogue.

It is clearly of interest to determine if ATP-γ-S leads to a fluorescence decline of PS II. Neither ATP nor ATP-γ-S induces a fluorescence decline if the plastoquinone pool is not reduced (i.e., in the absence of light or chemical activation of the kinases). The time-courses of the ATP-induced and the ATP-γ-S-induced fluorescence decline were also examined (Fig. 5) under the same conditions as utilised for the phosphorylation. The time-course of the ATP-induced decline is that typically observed under our experimental conditions [24–28,30]: in that a more or less steady plateau is observed between 5 and 15 min. ATP-γ-S also exhibits a similar time-course and the $t_{1/2}$ for the two nucleotides is between 2 and 3 min [20,24–28] with little further increase observed between 5 and 15 min. In different experiments the final fluorescence decline observed with ATP-γ-S was typically between 60 and 80% compared with that observed with ATP, and the addition of ATP to ATP-γ-S produced little or no change in the extent of decline observed.

It has previously been shown that even low concentrations of ATP can lead to a fluorescence decline [20,27] the S0.5 (the concentration required to observe 50% of the maximal fluorescence decline) for the fluorescence is in the range of 25–50 μM ATP [20,27]. However, the final extent of the fluorescence decline also varies at different concentrations of ATP [27]. The S0.5 for the ATP-γ-S was therefore also examined. As shown in Fig. 5b the ATP-γ-S-induced fluorescence decline, as in the case of ATP, is also concentration dependent. The S0.5 is similar to that observed with ATP (Fig. 5b).

The fluorescence decline of PS II can occur by two possible mechanisms: a change in the absorptive cross-

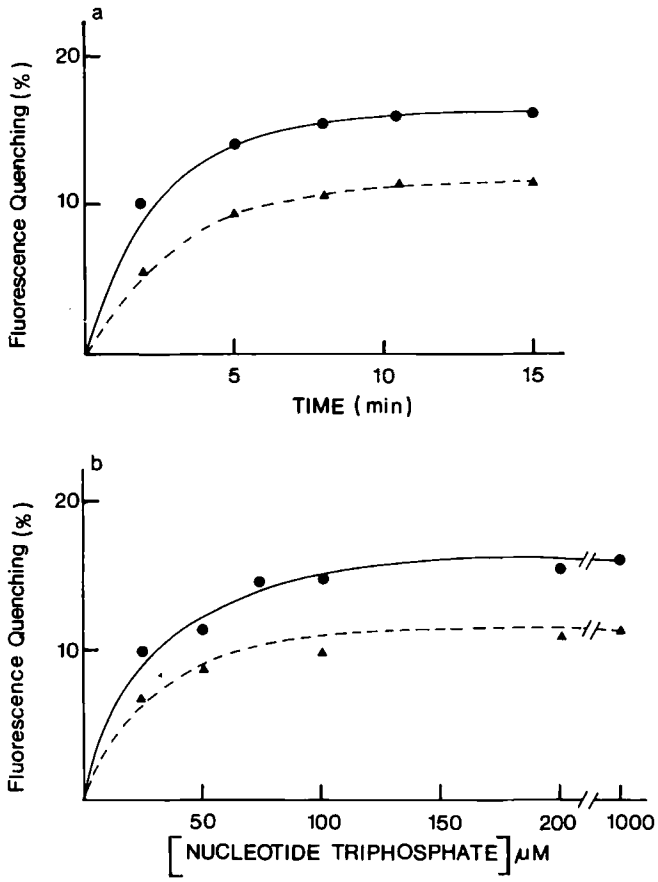

Fig. 5. ATP (●———●) or ATP-γ-S (▲- - - - -▲) induced Photosystem II fluorescence decline (a) time-course and (b) as a function of nucleotide concentration. (a) Isolated thylakoids were incubated with either 1 mM ATP or 1 mM ATP-γ-S or in the absence of nucleotide and aliquots were removed at the indicated times and diluted 200-fold with cold phosphorylation medium. After 30 min the fluorescence decline was determined as described in the Materials and Methods. (b) Same as in (a), except the concentration of the nucleotides was varied as indicated.

section, e.g., detachment of LHC II from the PS II LHC II matrix or by 'spillover' interactions such that the PS II-LHC II is directly quenched by interaction with PS I. These two possible mechanisms can be distinguished by determination of the F_v and F_0 ratios such that in the latter case the quenching of the F_v is more pronounced and a change in the F_v/F_0 ratio is observed while in the former case the F_v/F_0 ratio remains unaltered [2–8].

Isolated thylakoids were therefore incubated with either 1 mM ATP-γ-S or with 1 mM ATP or in the absence of nucleotide (control) following the chemical activation of the protein kinases. At various times aliquots were removed and suspended in the phosphorylation medium containing either a screening concentration of cations (5 mM Mg^{2+}) or a sub-saturating concentration of screening cations (1 mM Mg^{2+}). As shown in Table I the F_v/F_0 ratio of the samples incubated in the presence of the nucleotides at the saturating cation concentration of screening cations remain unaltered compared with the control, i.e., both the F_v and the F_0 are similarly quenched as in the case when LHC II detaches from the PS II-LHC II matrix. However, under subsaturating concentrations of screening cations the F_m decline is observed to increase by about 2-fold for both the nucleotides compared with that observed at saturating concentrations and the F_v/F_0 ratios are altered compared with the control. Thus it appears that at the sub-saturating concentration of the cations there is much greater quenching of the F_v compared to F_0 a situation similar to that encountered in the case of 'spillover' interactions [2–8]. These data would therefore indicate that under the normal phosphorylating conditions the fluorescence decline is most presumably a consequence of LHC II detachment leading to a change in the absorptive cross-section of PS II [2–8].

Digitonin and phase-partition fractionation techniques [2–8] have shown that following phosphorylation the fraction of LHC II present in the stroma increases in comparison with control thylakoids. Such techniques have generally supported the concept that phosphorylated LHC II detaches from the PS II-LHC II matrix and migrates to the stroma to transfer energy to PS I. Both digitonin fractionation and the Yeda press yield qualitatively similar data. The distribution of the chlorophyll and the LHC II protein has therefore been determined by digitonin fractionation following incubation for 10 min either in the presence of 1 mM ATP or 1 mM ATP-γ-S or in the absence of nucleotide (control). Both the nucleotides lead to a decrease in the quantity of chlorophyll present in the heavy fraction and an increase in that of the light fraction. Furthermore, both ATP and ATP-γ-S lead to an alteration of the chlorophyll *a/b* ratios of the light fraction. In both cases the ratio decreases compared to the control due to an increase in the amount of chlorophyll *b* in the light fraction.

Discussion

The phosphorylation of the LHC II polypeptides has been proposed to constitute the mechanism by which plants adapt to changing light conditions by redistributing the excitation energy between the two photosystems; similar to the adaptive State I–State II transitions in vivo (for reviews, see Refs. 2–8). An ATP-induced Photosystem II fluorescence decline in vitro is observed only under conditions that the plastoquinone pool is reduced, and these conditions lead to the activation of the protein kinase(s) and LHC II protein phosphorylation. Evidence for both a close corelation between the LHC II phosphorylation and the fluorescence decline [20–22,29] or lack of such correlation [23–28] have been reported.

ATP-γ-S, an ATP analog, has been used to distinguish between ATP or phosphorylation-induced effects in rat brain synaptosomes [31]. This analog has also been shown to act as a substrate for several protein kinase activities [31–34]. However, unlike ATP, ATP-γ-S appears to be an inefficient substrate for the chloroplast membrane associated protein kinases (Figs. 1–3) although it appears to bind extremely efficiently to te kinase (Fig. 4). In contrast, ATP-γ-S brings about fluo-

TABLE I

ATP- and ATP-γ-S induced Photosystem II fluorescence changes at two different concentrations of screening cations

Isolated spinach thylakoids were incubated either in the absence (control) or in the presence of 1 mM ATP or 1 mM ATP-γ-S for 10 min. The reaction was terminated by the addition of 200-fold excess of ice-cold phosphorylation medium containing either 5 mM or 1 mM $MgCl_2$. The fluorescence parameters under these two cation regimes were determined (see Materials and Methods).

Sample	5 mM $MgCl_2$			1 mM $MgCl_2$		
	F_m decline (%)	F_v/F_0	F_v/F_0 ratio (treated/control)	F_m decline (%)	F_v/F_0	F_v/F_0 (treated/control)
Control	–	4.47	–	–	3.72	–
ATP	21	4.50	1.01	35.6	3.00	0.81
ATP-γ-S	15	4.44	0.99	24.5	2.94	0.79

TABLE II

Digitonin fractionation of thylakoids incubated in the presence of ATP or ATP-γ-S or in the absence of added nucleotide

Isolated thylakoids, after chemical activation of the protein kinases, were incubated in the absence (control) or in the presence of 1 mM ATP or 1 mM ATP-γ-S. After 10 min the thylakoids were subjected to digitonin treatment and the light and heavy fractions separated by the centrifugation (see Materials and Methods) and the chlorophyll content and the chlorophyll *a/b* ratio determined [25].

Sample	F_m decline (%)	Heavy fraction % Chl content	Light fraction % Chl content	Light fraction Chl *a/b* ratio
Control	–	79	21	4.26
ATP	16	66	34	2.98
ATP-γ-S	12	70	30	3.34

rescence changes which appear to be similar to those observed with ATP: (a) Both ATP and ATP-γ-S lead to a fluorescence decline if the plastoquinone pool is reduced (Fig. 5), but not under non-reducing conditions. (b) The time-course for the fluorescence decline for both nucleotides is very similar exhibiting a half-time of about 2–3 min and a stable plateau between 5 and 15 min where little or no fluorescence decline is observed (Fig. 5). (c) The S0.5 is similar for both nucleotides (Fig. 5) and the marked inhibition of the ATP-induced phosphorylation by low concentrations of ATP-γ-S (Fig. 4) would suggest a K_m for the LHC II kinase which is similar to that with ATP [30]. (d) In the presence of saturating concentrations of screening cations neither nucleotide leads to a change in the F_v/F_0 ratio, but both nucleotides lead to a decrease in the F_v/F_0 ratio in the presence of sub-saturating concentrations of screening cations (Table I). (e) Digitonin fractionation shows an increase in the chlorophyll *b* content in the stromal 'light' fraction and a decrease in the granal 'heavy fraction' (Table II).

These data would therefore suggest that it is unlikely that the ATP- and the ATP-γ-S-induced fluorescence decline are brought about by two very different mechanisms.

We have previously shown that there is little or no direct correlation between the rate and level of LHC II phosphorylation and the fluorescence decline [24–28] by studying the relative kinetics of these two phenomena. Furthermore, the extent of the ATP-induced fluorescence decline appears to be dependent on the ATP concentration rather than on phosphorylation [27]. In addition, while GTP-induced phosphorylation fails to lead to a fluorescence decline [30], ATP-γ-S induces a fluorescence decline but fails to exhibit any significant phosphorylation of the LHC II polypeptides.

Interestingly, ATP-γ-S leads to changes in the F_v/F_0 ratios in the sub-saturating cation regime, suggesting that charge interactions may indeed be involved. These data could be interpreted to suggest that the charge may not be identical to the charge introduced as a consequence of phosphorylation of the surface-exposed segments of the LHC II molecules.

The earlier suggestions coupling the phosphorylation of the LHC II polypeptides and the fluorescence decline rely on two important observations: (a) only under conditions when the plastoquinone pool is reduced is a fluorescence decline observed; (b) under the same conditions membrane-associated protein kinases are activated consenting the phosphorylation of the LHC II and other thylakoid proteins (the thylakoid proteins appear to be phosphorylated by several different protein kinases [2,30]). However, it should be pointed out that the molecular mechanism by which the reduction of the plastoquinone pool leads to the activation of the protein kinases still remains unknown. It is therefore possible that electron-transport dependent conformational changes reveal the phosphorylation sites of all the substrates or alternatively changes in the different protein kinases.

Horton et al. [21] have shown that by altering the redox-state of the plastoquinone pool that the fluorescence decline and the phosphorylation of the LHC II polypeptides are perfectly correlated. However, it remains unclear whether such a relationship also applies under other conditions, for example, when the plastoquinone pool is fully reduced the extent of phosphorylation of the LHC II polypeptides is not directly correlated with the fluorescence decline [24–28]. Similarly, the studies with ATP-γ-S and GTP [30] would also suggest a lack of such direct correlation, although the plastoquinone pool needs to be reduced also in the case of the GTP-induced phosphorylation [30] as well as in the case of the ATP-γ-S induced fluorescence decline. Clearly, the data of Horton et al. [21] suggests that both the phosphorylation of the thylakoid proteins and the fluorescence decline depend in some way on the redox-state of the plastoquinone pool, and therefore not in disagreement with our observations [24–28,30].

The adenylate charge has also been shown to regulate the protein kinase activities [39]. Clearly, this is an important observation as regards the ATP-generating role of the chloroplasts. In this context it should be noted that the guanine nucleotide GTP acts as a substrate for phosphorylation but does not lead to a fluorescence decline [30] or protect against the action of sulphydryl directed reagents [40]. While ATP and ATP-γ-S both lead to a fluorescence decline, and the protective effect of ATP and ADP against the sulphydryl-directed reagents [40] could be interpreted to indicate a second binding site which is specific for the adenine nucleotides [30]. The regulation of the fluorescence decline may be coupled to the adenylate charge by some mechanism requiring one or both of these adenine sites.

Finally Steinback et al. [22] have shown that dephosphorylation of the LHC II polypeptides leads to an increase in the PS II fluorescence and that this change is inhibited by NaF. Our data has attempted to analyse only the phosphorylation and its relationship with the fluorescence decline. However, ATP-γ-S results only in minimal phosphorylation of LHC II but an almost complete fluorescence decline, while GTP results in phosphorylation but no fluorescence decline. In view of these and our earlier data, it is suggested that the role of dephosphorylation also needs to be examined more carefully. For example, it may be that dephosphorylation is a pre-requisite for the re-entry of the LHC II molecules, or alternatively, that the phosphorylated polypeptides, but not the non-phosphorylated polypeptides, may interact with PS I. Clearly the environment of the LHC II molecule in the stromal or partition zones is quite different to that in the grana where it is associated with PS II. It is also important to recognise that only a fraction of the 'migrating' LHC II molecules are phosphorylated (for references, see Ref. 28) and that both the 'migrating' and 'non-mobile' sub-populations of LHC II are phosphorylated. While there is little or no data regarding the kinetics of dephosphorylation of the different polypeptides and the different sub-populations of LHC II.

In recent years it has become clear that the phosphorylation of LHC II polypeptides is much more complex than thought earlier. However, this complexity of LHC II phosphorylation has not been fully collocated to the relationship between phosphorylation and the fluorescence decline. Contrasting data on the correlation of LHC II phosphorylation and the fluorescence decline would suggest that the models for correlation need to be examined in much more detail and must take into account the complexity of the two phenomena.

Acknowledgements

I would like to thank Dr. Giorgio Forti (Centro CNR) and Dr. Roy G. Burns (Imperial College) for helpful discussions. I would also like to thank Mr. Tullio Arnaboldi for generously supplying the spinach. This work was supported by a long-term fellowship from EMBO.

References

1 Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) Nature (London) 291, 25–29.
2 Bennett, J. (1983) Biochem. J. 212, 1–13.
3 Anderson, J.M. (1986) Annu. Rev. Plant Physiol. 37, 93–106.
4 Murphy, D.J. (1986) Biochim. Biophys. Acta 864, 33–94.
5 Horton, P. (1983) FEBS Lett. 152, 47–52.
6 Staehlin, L.A. and Arntzen, C.J. (1983) J. Cell. Biol. 97, 1327–1337.
7 Barber, J. (1983) Photobiochem. Photobiophys. 5, 181–190.
8 Barber, J. (1986) in Encyclopedia of Plant Physiology 19, Photosynthesis III (Staehelin, L.A. and Arntzen, C.J., eds.), pp. 653–665, Springer-Verlag, Berlin.
9 Mullett, J. (1983) J. Biol. Chem. 258, 9941–9948.
10 Michel, H.P. and Bennett, J. (1987) FEBS Lett. 212, 103–108.
11 Barber, J. (1980) FEBS Lett. 118, 1–10.
12 Barber, J. (1976) in Intact Chloroplast, Vol. II (Barber, J., ed.), Topics in Photosynthesis, pp. 283–304, Elsevier, Amsterdam.
13 Murata, N. (1971) Biochim. Biophys. Acta 226, 422–432.
14 Karlin-Neumann, G.A., Kohorn, B.D., Thornber, J.P. and Towbin, E.M. (1985) J. Mol. Appl. Genet. 3, 45–61.
15 Cahmore, A.R. (1984) Proc. Natl. Acad. Sci. USA 81, 2960–2864.
16 Dansmuir, P. (1986) Nucl. Acids Res. 13, 2503–2518.
17 Chow, W.S., Telfer, A., Chapman, D. and Barber, J. (1981) Biochim. Biophys. Acta 638, 60–68.
18 Telfer, A., Hodges, M. and Barber, J. (1983) Biochim. Biophys. Acta 724, 167–175.
19 Telfer, A., Hodges, M., Millner, P.A. and Barber, J. (1984) Biochim. Biophys. Acta 766, 554–562.
20 Black, M.T., Foyer, C. and Horton, P. (1984) Biochim. Biophys. Acta 767, 557–562.
21 Horton, P., Allen, J.F., Black, M.T. and Bennett, J. (1981) FEBS Lett. 125, 193–196.
22 Steinback, K.E., Rose, S. and Kyle, D.J. (1982) Arch. Biochem. Biophys. 216, 356–361.
23 Markwell, J.P., Baker, N.R., Bradbury, M. and Thornber, J.P. (1984) Plant Physiol. 74, 348–354.
24 Islam, K. and Jennings, R.C. (1985) Biochim. Biophys. Acta 810, 158–163.
25 Jennings, R.C., Islam, K. and Zucchelli, G. (1986) Biochim. Biophys. Acta 850, 483–489.
26 Zucchelli, G., Islam, K. and Jennings, R.C. (1987) in Progress in Photosynthesis Research, (Biggins, J., ed.), Vol. II, pp. 725–728, Martinus-Nijhoff Publishers, Dordrecht.
27 Islam, K. (1987) Biochem. Soc. Trans. 16, 46–47.
28 Islam, K. (1987) Biochim. Biophys. Acta 893, 333–341.
29 Frachaus, J., Dilley, R.A. and Cramer, W.A. (1985) Biochim. Biophys. Acta 809, 17–26.
30 Islam, K. (1989) Biochim. Biophys. Acta 974, 261–266 (preceding article).
31 Hauptmann, M., Wilson, D.F. and Erecinska, M. (1985) Biochem. Pharmacology 34, 1247–1254.
32 Sherry, J.M.F., Gorecka, A., Aksoy,. M.O., Dabrowska, R. and Hartshorne, D.J. (1978) Biochemistry 17, 4411–4418.
33 Gratecos, D. and Fischer, E.H. (1974) Biochem. Biophys. Res. Commun. 58, 960–967.
34 Morgan, M., Perry, S.V. and Ottaway, J. (1976) Biochem. J. 157, 687–697.
35 Cassidy, P., Hoar, E. and Kerrick, W.G.L. (1979) J. Biol. Chem. 254, 11148–11153.
36 Beltz, W.R. and O'Brien, K.J. (1981) Fed. Proc. 40, 1849.
37 Sun, I.Y.C., Johnson, E.M. and Allfrey, V.G. (1980) J. Biol. CHem. 255, 742–747.
38 Allfrey, V.G., Sterner, R. and Sun, I.Y.C. (1983) in Posttranslational Covalent Modifications of Proteins (Connor, J.B., ed.), pp. 181–203, Academic Press, New York.
39 Markwell, J.P., Baker, N.R. and Thornber, J.P. (1982) FEBS Lett. 142, 171–174.
40 Millner, P.A., Widger, W.R., Abbott, M.S., Cramer, W.A. and Dilley, R.A. (1982) J. Biol. Chem. 257, 1736–1742.