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Regulation of Photosynthesis by Chloroplast Protein Phosphorylation [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1983 **302**, 113-125
doi: 10.1098/rstb.1983.0044

Phil. Trans. R. Soc. Lond. B **302**, 113–125 (1983) [113]
Printed in Great Britain

Regulation of photosynthesis by chloroplast protein phosphorylation

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Several polypeptides of the chloroplast photosynthetic membrane are reversibly phosphorylated *in vivo* and *in vitro*. The most conspicuous phosphoproteins belong to the light-harvesting chlorophyll *a*/*b* complex (LHC), which accounts for about half of the photons absorbed by the pigments of the photosynthetic membrane and can transfer excitation energy to either photosystem I or photosystem II. Phosphorylation of LHC increases (and dephosphorylation decreases) the proportion of excitation energy transferred to photosystem I at the expense of photosystem II. The LHC kinase is activated *in vivo* and *in vitro* by overexcitation of photosystem II and inactivated by overexcitation of photosystem I. The redox state of the plastoquinone pool governs the activity of the kinase and enables the photosynthetic membrane to detect and then correct any imbalance in the rate of excitation of the two photosystems. Reversible phosphorylation of LHC also enables the chloroplast to regulate the relative rates of cyclic and non-cyclic electron transport and thereby coordinates the rates of synthesis of ATP and NADPH with the demands of the Calvin cycle and other metabolic pathways operating within the organelle.

Introduction

Protein phosphorylation has been studied in plants for more than a decade. Early studies were concerned with phenomena that had parallels in animal cells, such as the phosphorylation of ribosomal and nuclear proteins (Trewavas 1976), but more recently considerable attention has been given to an example without parallel in animal cells: the phosphorylation of chloroplast proteins. The chloroplast is the site of photosynthesis in plants. A double envelope delimits the organelle and encloses the photosynthetic membranes (or thylakoids) and the soluble phase (or stroma). The thylakoids use the energy of sunlight to drive the formation of NADPH and ATP, which are then consumed in the stroma by such activities as the fixation of CO_2 into sugar phosphates and starch. When isolated intact chloroplasts from green plants are incubated in the light with [^{32}P]orthophosphate, radioisotope is incorporated not only into low molecular mass substances such as ATP but also into macromolecules such as RNA and protein (Bennett 1977). The major phosphoproteins are associated with the thylakoids: they range in molecular weight from 7 to 90 kDa, as judged by sodium dodecyl sulphate polyacrylamide gel electrophoresis (figure 1). The two most conspicuous thylakoid phosphoproteins have molecular masses of 23 and 25 kDa and have been identified as components of the light-harvesting chlorophyll *a*/*b* complex (Bennett 1979*a*; Bennett *et al.* 1981). Four other phosphoproteins (9, 33, 35 and 45 kDa) are thought to be components of photosystem II (Steinback *et al.* 1982; Owens & Ohad 1982). These six proteins are reversibly phosphoryllated *in vivo* and *in vitro*, and considerable progress has been made in understanding the regulation and physiological significance of their phosphorylation. In this article I shall review the effects of LHC phosphorylation on the structure and function of the photosynthetic membranes.

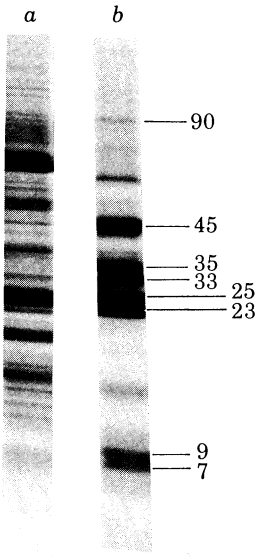

FIGURE 1. SDS-polyacrylamide gel electrophoresis of ^{32}P-labelled thylakoids of pea chloroplasts. (*a*) Gel stained with Coomassie Brilliant Blue. (*b*) Radioautogram of gel. Apparent molecular masses (kilodaltons) are indicated.

EXCITATION ENERGY TRANSFER BETWEEN CHLOROPHYLL–PROTEIN COMPLEXES

Until the early 1970s all the chlorophyll of the photosynthetic membranes of green plants was considered to be located in two types of sub-membrane particles known as photosystem I and photosystem II (PS I and PS II), but it is now recognized that there is a third major chlorophyll-containing particle: the light-harvesting chlorophyll *a*/*b* complex (LHC) (Thornber & Highkin 1974). In most plants, PS I, PS II and LHC account for approximately 30, 20 and 50%, respectively, of total chlorophyll (Waldron & Anderson 1979; Anderson 1982). The predominant pigment in each particle is Chl *a*. The particle with the highest content of Chl *b* is the LHC, which has a Chl *a*/Chl *b* ratio of 1.0–1.1 in most green plants. Each particle contains its own distinctive set of chlorophyll-binding polypeptides, supplemented in PS I and PS II with other polypeptides concerned with electron transport. PS I has at least one major chlorophyll-binding protein (about 68 kDa) together with several minor polypeptides (approximately 20, 21 and 22 kDa), which are probably also chlorophyll-binding proteins (Mullet *et al.* 1980; Bellemare *et al.* 1982). There are thought to be two chlorophyll-binding proteins in PS II, which, in spinach, have molecular masses of about 47 and 39 kDa (Larkum & Anderson 1982). The three polypeptides of barley LHC have apparent molecular masses of 24, 25 and 27 kDa (Bellemare *et al.* 1982).

PS I and PS II participate directly in photosynthetic electron transport. Each photosystem contains a distinctive reaction centre consisting of 1 or 2 photochemically active Chl *a* molecules; associated with the reaction centre are 100–150 antenna chlorophylls (mainly Chl *a*). The antenna chlorophylls absorb quanta and transfer excitation energy to the reaction centre, where electron transport is initiated. LHC is not photochemically active and possesses no reaction centre. LHC is believed to exist in the form of oligomers, with about 4–7 polypeptides per complex and about 6–13 chlorophylls per polypeptide (Haworth *et al.* 1982*b*). The oligomeric LHC units are probably 3–5 times more abundant than PS I or PS II. LHC absorbs strongly

in the 640–680 nm region, while PS I and PS II absorb strongly in the 660–710 nm and 660–690 nm regions. Strong absorption by LHC in the 640–650 nm region is due largely to the presence of Chl *b* in that complex, but the other differences in absorption among the three complexes reflect the fact that they are composed of different chlorophyll-binding proteins that endow Chl *a* with different electronic environments and spectral properties.

To judge by the absorption spectra, excitation energy should flow readily from LHC to both photosystems and from PS II to PS I but flow from PS I to the other complexes should be energetically unfavourable. However, the transfer of excitation energy depends not only on the spectral properties of the complexes but also on their proximity. A characteristic feature of the thylakoids of most green plants is the differentiation of the membranes into appressed and non-appressed regions (figure 2) (Anderson & Andersson 1982). PS II and LHC are concentrated in the appressed regions and are present in the non-appressed membranes to the extent of about 20%. PS I and ATP synthase are found almost exclusively in the non-appressed regions,

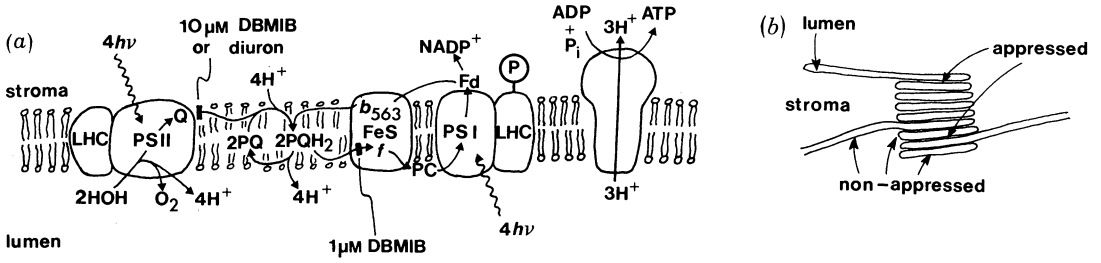

FIGURE 2. Schematic diagram showing the architecture of the thylakoid membrane. (*a*) Diagram of photosynthetic electron transport and proton translocation, showing only selected thylakoid components. The light-harvesting chlorophyll *a*/*b* complex (LHC) is depicted as being associated with photosystem II (PS II) when dephosphorylated and associated with photosystem I (PS I) when phosphorylated. Also included are the primary electron acceptor of PS II (Q), plastoquinone (PQ), plastoquinol (PQH_2), the complex containing cytochrome b_{563}, cytochrome *f* and the Rieske iron–sulphur protein, plastocyanin (PC), ferredoxin (Fd) and ATP synthase complex. The sites of inhibition of 1 and 10 μM DBMIB and of 10 μM diuron are indicated. (*b*) Diagram of thylakoid membranes, illustrating the differentiation of the stromal surface into appressed and non-appressed (exposed) regions.

while the cytochrome b_{563}/f complex is equally distributed between the two regions. One consequence of this lateral heterogeneity in the distribution of sub-membrane particles is that excitation energy transfer from LHC to PS II is favoured over transfer from either PS II or LHC to PS I.

PHOTOSYNTHETIC ELECTRON TRANSPORT

There are to principal pathways of electron transport in thylakoids: non-cyclic and cyclic electron transport. The non-cyclic pathway from water to $NADP^+$ is performed by an electron transport chain that includes both PS I and PS II operating in series (figure 2). Coupled with the flow of electrons is the translocation of protons into the lumen of the thylakoids (Trebst 1974). The transport of four electrons along the chain (the number involved in the evolution of one molecule of O_2) requires eight quanta (four for each photosystem) and generates two molecules of NADPH. At the same time, eight protons are deposited into the lumen, four at the O_2-evolving site of PS II and four as a result of the oxidation of plastoquinol (PQH_2) near the luminal surface of the membrane. Because in the steady state three protons are translocated by ATP synthase for each molecule of ATP formed (Portis & McCarty 1974), it follows that the

reduction of two molecules of $NADP^+$ by the non-cyclic electron transport chain is accompanied by the synthesis of 2.67 molecules of ATP. However, the fixation of one molecule of CO_2 into sugar phosphates actually requires two molecules of NADPH and three molecules of ATP. Thus, by non-cyclic electron transport alone, there is a deficit of 0.33 of an ATP molecule per molecule of CO_2 fixed.

Under most circumstances the additional ATP is probably supplied by the operation of the cyclic electron transport pathway around PS I (Hind *et al.* 1981). Whereas in the non-cyclic pathway electrons are passed from ferredoxin to $NADP^+$, in the cyclic pathway they are passed to cytochrome b_{563} and then back to PS I via intermediates that are also components of the non-cyclic pathway(plastoquinone (PQ), the Rieske Fe–S centre, cytochrome *f* and plastocyanin) (figure 2). For every electron that takes the cyclic pathway, one quantum is absorbed by PS I, one proton is deposited into the lumen and 0.33 of an ATP molecule is formed. Thus, in principle, the ATP and NADPH requirements for the fixation of one molecule of CO_2 into sugar phosphates can be adequately met by nine quanta: five quanta are required for the excitation of PS I and four quanta are required for the excitation of PS II.

Why a flexible light-harvesting apparatus is necessary

It was stated above that PS I, PS II and LHC account for about 30 %, 20 % and 50 %, respectively, of total chlorophyll in green plants. If about 80 % of LHC is in the appressed regions of the thylakoids, in association with PS II, then PS II would have available to it about 60 % of the light-harvesting capacity of the membrane, while PS I would have available to it only about 40 %. Is such an arrangement consistent with the aim of delivering about five quanta to PS I for every four quanta delivered to PS II? At first sight it might appear that such an arrangement would lead to the permanent overexcitation of PS II but in fact the rates of excitation of PS I and PS II do not depend solely on the distribution of the light-harvesting chlorophylls. They also depend on the spectral distribution of ambient light. Smith (1982) has recently reviewed the spectral qualities of different forms of natural illumination and although the article is centred around the phytochrome-mediated responses of plants, the spectral data are relevant also to photosynthesis.

As Smith (1982) points out, plants growing in the shade of other plants are exposed to light that has been filtered through photosynthetic pigments and is therefore markedly depleted in wavelengths below 700 nm. Since only PS I absorbs significantly above 700 nm, shaded plants can achieve a balanced distribution of excitation energy between the two photosystems only if the greatest possible fraction of any light below 700 nm is harvested by PS II through its association with the maximum possible fraction of LHC. It is partly for this reason that membrane appression is maximal in plants adapted to growth on the floor of a tropical rain forest (Anderson 1982). Such plants also contain especially high levels of light-harvesting chlorophylls.

Shading is, of course, a highly variable parameter in a crop plant, and even on the floor of a rain forest the intensity and the spectral quality of light are liable to vary as a result of sun flecks. How do plants respond to changes in the degree of shading? Do they possess a mechanism for detecting and then correcting any imbalance in the rates of excitation of the photosystems in response to changes in the spectral quality of ambient light?

That plants possess such a mechanism is most convincingly shown by the work of Bonaventura

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& Myers (1969) on the green alga *Chlorella*. In their experiments, photosynthetic oxygen evolution was measured in cultures exposed to 645 nm light, with or without excess 710 nm light; 645 nm light was absorbed preferentially by LHC, while 710 nm light was absorbed exclusively by PS I. The data show that *Chlorella* has the ability to direct excitation energy derived from absorbed 645 nm light to whichever photosystem is rate-limiting. The proportion of absorbed 645 nm light exciting PS I increases over a 5–10 min period under 645 nm light alone but decreases with similar kinetics under a combination of the two wavelengths. In each case, an approximate balance in the rates of excitation of the photosystems is attained.

Higher plants also possess the ability to alter the organization of their light-harvesting apparatus to adapt to changes in the spectral quality of ambient light (Chow *et al.* 1981). But how are such changes in the rates of excitation of the photosystems detected and then corrected? In subsequent sections I present evidence that it is the reversible phosphorylation of LHC that regulates the distribution of excitation energy between the two photosystems and that the degree of LHC phosphorylation is controlled in part by the redox state of the PQ pool.

The thylakoid protein kinase is regulated by the redox state of the plastoquinone pool

Thylakoid protein phosphorylation may be detected *in vivo* in leaves and algal cells and *in vitro* in isolated intact chloroplasts and washed thylakoids. In a direct comparison between intact chloroplasts supplied with [^{32}P]orthophosphate and washed thylakoids incubated with [γ-^{32}P]ATP, the same thylakoid polypeptides proved to be labelled to the same relative extents and with similar kinetics, establishing that the relevant protein kinase is located on the thylakoids themselves (Bennett 1979*b*, 1980). To obtain kinase activity in a suspension of thylakoids buffered at about pH 8.0, one may simply add 3–10 mM $MgCl_2$ and 100–300 μM ATP and illuminate the suspension. Activation of the kinase in the light is blocked by diuron (which inhibits electron transport between PS II and PQ) but is not inhibited by uncouplers. The kinase is not, however, strictly light-dependent, since it is activated in the dark with strong reducing agents such as reduced ferredoxin (Bennett 1979*b*) and dithionite (Allen *et al.* 1981).

The implication of the above results is that for the kinase to be activated, some component of the electron transport chain after the site of inhibition by diuron must become reduced. There are several lines of evidence that the crucial component is PQ:

(i) activation of the kinase in the light is not prevented by 1 μM DBMIB (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone), which inhibits the oxidation of PQH_2, but is prevented by 10 μM DBMIB, which, like diuron, inhibits the reduction of PQ by PS II (Allen *et al.* 1981);

(ii) the kinase is activated in the dark by duroquinol, which donates electrons directly to PQ (Allen & Horton 1981);

(iii) the ability of reduced ferredoxin to activate in darkness can be explained in terms of electron flow via cytochrome b_{563} to PQ (Hind *et al.* 1981);

(iv) a sequence of single turnover flashes that progressively reduces the PQ pool also progressively activates the kinase (Allen *et al.* 1981);

(v) redox titrations in darkness show that protein kinase activation has the same midpoint potential as PQ reduction (about 0 mV at pH 7.8), and, furthermore, the titration curve for kinase activation is that of a two-electron carrier, consistent with the involvement of PQ (Horton *et al.* 1981).

The sensitivity of the thylakoid protein kinase to the redox state of the PQ pool is highly significant. Since PQ lies between PS I and PS II in the electron transport chain, its redox state provides a sensitive measure of the relative rates of excitation of the two photosystems, provided that there is an adequate supply of electron acceptors for PS I.

Enzymology of LHC phosphorylation–dephosphorylation

The thylakoid protein kinase has a K_m for ATP of about 90 μM (Bennett *et al.* 1981). The enzyme is inhibited by ADP and 3′,5′-cyclic AMP but not by AMP (Baker *et al.* 1982; Markwell *et al.* 1982). The inhibitory effect of cyclic AMP occurs at concentrations (10 μM and over) that are almost certainly not physiological, but the inhibitory effects of ADP are sufficiently strong at physiological concentrations (100–1000 μM) to suggest that kinase activity will be markedly affected both *in vivo* and *in vitro*.

If thylakoid protein phosphorylation is to have a regulatory role in photosynthesis, the chloroplast would need to possess protein phosphatase activity. Bennett (1977, 1980, 1983) has demonstrated thylakoid protein dephosphorylation *in vivo* in barley leaves, and *in vitro* in isolated intact chloroplasts and washed thylakoids of peas, thereby establishing that the activity is membrane-bound. In each case, the LHC is the most rapidly dephosphorylated protein, followed by the 33–45 kDa polypeptides of PS II, while the 9 kDa polypeptide of PS II is dephosphorylated very slowly. The enzyme responsible for dephosphorylation has been only partly characterized but it has the properties expected of a phosphatase, including the fact that it is inhibited by almost 90 % by 10 mM NaF (Bennett 1980) and 10 mM sodium molybdate (Owens & Ohad 1982). The enzyme does not appear to be modulated by either ADP or ATP or by light. It is markedly stimulated by the presence of Mg^{2+}. The dephosphorylation of the 9 kDa protein requires 5–10 mM $MgCl_2$, whereas dephosphorylation of LHC occurs with as little as 10 μM $MgCl_2$ (Bennett 1980).

When isolated intact chloroplasts are incubated in the light with [^{32}P]orthophosphate, LHC becomes labelled with a half-time of about 2 min (Bennett 1977). Addition of the uncoupler carbonyl cyanide-*m*-chlorophenylhydrazone, to inhibit further synthesis of radioactive ATP, immediately halts the labelling of LHC and induces net dephosphorylation, with a half-time of about 8 min. Very similar kinetics for LHC phosphorylation and dephosphorylation are seen *in vitro* in washed thylakoids and *in vivo*. The phosphatase seems to be operative at all times. Thus, the level of phosphorylation of LHC is regulated principally through various controls on the kinase (including the $[PQH_2]/[PQ]$ and [ATP]/[ADP] ratios).

LHC phosphorylation regulates excitation energy transfer between chlorophyll–protein complexes

A clue to the physiological significance of LHC phosphorylation was provided by the discovery that LHC is phosphorylated on the surface-exposed N-terminal segment that is removed by trypsin (Bennett 1980). The segment is known to be important in the maintenance of membrane appression and lateral heterogeneity (Steinback *et al.* 1979; Carter & Staehelin 1980). When thylakoids are treated with trypsin under mild conditions, the molecular masses of the 23 and 25 kDa polypeptides are reduced by 1–2 kDa (Andersson *et al.* 1982*b*), any ^{32}P-label within the protein is lost, and membrane appression and lateral heterogeneity are abolished.

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PS I, PS II and LHC intermingle and there is increased excitation energy transfer from LHC and PS II to PS I.

Does phosphorylation of the surface-exposed segment affect the relation between LHC and the other complexes? Several lines of evidence indicate that LHC phosphorylation increases (and dephosphorylation decreases) excitation energy transfer to PS I at the expense of PS II. Firstly, addition of ATP to thylakoids under phosphorylating conditions induces a slow decline in chlorophyll fluorescence at 20° C with kinetics similar to those of LHC phosphorylation (Bennett *et al.* 1980; Horton & Black 1980; Telfer *et al.* 1983). Because PS II and LHC are more strongly fluorescent at 20 °C than PS I, such a decline in fluorescence is consistent with an increase in any process that competes with PS II/LHC fluorescence as a mechanism for utilizing or dissipating excitation energy. The transfer of excitation energy to PS I at the expense of PS II is certainly one such mechanism. Secondly, when thylakoids are incubated with ATP under phosphorylating conditions and are then frozen and illuminated at 77 K (a temperature at which PS I, PS II and LHC all fluoresce at characteristic wavelengths), chlorophyll

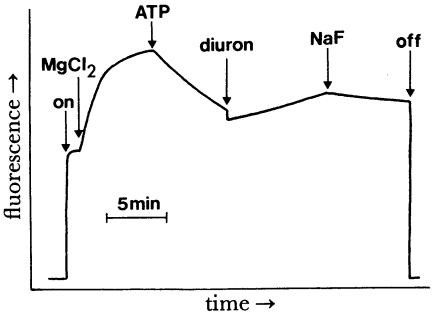

FIGURE 3. Chlorophyll fluorescence trace from pea thylakoids incubated at 20 °C in the presence of the uncoupler nigericin (1 μM) to demonstrate a decline in LHC phosphorylation-dependent chlorophyll fluorescence without interference from ATP-induced high-energy state quenching. The buffer was 10 mM Tricine–KOH, pH 8.0. After the light was switched on, the following were added as shown: $MgCl_2$ (5 mM), ATP (0.2 mM), diuron (10 μM), NaF (10 mM).

fluorescence emission spectra reveal that ATP increases the yield of fluorescence from PS I (Bennett *et al.* 1980; Allen *et al.* 1981; Horton & Black 1980, 1981, 1982; Steinback *et al.* 1982; Chow *et al.* 1981; Haworth *et al.* 1982*a*; Saito *et al.* 1983). By including an internal standard for fluorescence, Saito *et al.* (1983) were able to show that the increase in PS I fluorescence on phosphorylation represents an absolute increase in excitation energy transfer to PS I at the expense of PS II, not just a relative increase owing to a reduction in the fluorescence yield from PS II. Thirdly, ATP causes a decrease of approximately 15 % in the rate of electron transport through PS II (Steinback *et al.* 1982; Farchaus *et al.* 1982) and a corresponding increase in the rate of electron transport through PS I (Horton & Black 1982; Farchaus *et al.* 1982). These results establish that the effect of ATP on the distribution of excitation energy within the thylakoid is reflected in the rates of turnover of the two photosystems.

Figure 3 illustrates the use of chlorophyll fluorescence measurements at 20 °C as a probe for the effects of phosphorylation and dephosphorylation on the distribution of excitation energy within the thylakoid. At the beginning of the recording, the thylakoids are suspended in a medium containing an uncoupler (to prevent the ATP-dependent generation of a high-energy state within the membranes) and very low ionic strength (10 mM Tricine–KOH, pH 8.0). Under

these conditions, membrane appression and lateral heterogeneity are abolished and the PS I, PS II and LHC particles intermingle. When the light is turned on, chlorophyll fluorescence from PS II/LHC rises immediately to a steady intensity. On the addition of 5 mM $MgCl_2$, the yield of fluorescence increases slowly over a period of about 5 min. During this time membrane appression and lateral heterogeneity are restored, and the rise in fluorescence is attributed to the consequent reduction in the probability of excitation energy transfer from PS II/LHC to the non-fluorescent PS I (Staehelin & Arntzen 1979). (However, see Melis & Ow (1982) for a different explanation.) When 200 μM ATP is added, a slow decline in chlorophyll fluorescence yield occurs as long as phosphorylation is permitted, but when the kinase is inactivated by the addition of 10 μM diuron, the decline in chlorophyll fluorescence is reversed by a mechanism that is blocked by the phosphatase inhibitor, NaF (10 mM). This sort of result, taken with the finding that there is a linear relation between the extent of the ATP-dependent decline in chlorophyll fluorescence yield and the extent of LHC phosphorylation (Horton *et al.* 1981), provides strong evidence that the effects of ATP on the distribution of excitation energy within the thylakoid are mediated by protein phosphorylation. The main evidence that it is the LHC rather than some other thylakoid protein whose phosphorylation is important in mediating the effects of ATP is the close correspondence both *in vivo* and *in vitro* between the dephosphorylation of LHC and the observed rise in chlorophyll fluorescence from PS II; dephosphorylation of the other thylakoid phosphoproteins is too slow to account for the speed of the increase in fluorescence (Bennett 1983; Chow *et al.* 1981; Steinback *et al.* 1982).

Molecular mechanisms

How does the phosphorylation of LHC increase the proportion of excitation energy that is transferred to PS I at the expense of PS II? At present the mechanism is not fully understood, but it probably involves the dissociation of phosphorylated LHC from PS II in the appressed regions of the thylakoids and its association with PS I in the non-appressed regions.

There are several lines of evidence consistent with such a mechanism.

(i) Although non-appressed membranes contain comparatively little LHC, they are specifically enriched for phosphorylated LHC (Andersson *et al.* 1982*a*).

(ii) Phosphorylation reduces the degree of connectivity and the effective light-harvesting capacity of PS II units, and increases the effective light-harvesting capacity of PS I (Kyle *et al.* 1982; Haworth *et al.* 1982*a*).

(iii) Freeze–fracture electron microscopy reveals the presence of two classes of LHC particle within thylakoids: 14–16 nm particles, which cleave with the luminal (EF) leaflet of the bilayer and which contain both LHC and PS II, and 9 nm particles, which cleave with the stromal (PF) leaflet and are considered to represent free LHC (Simpson 1979; Olive *et al.* 1981). A study of non-phosphorylated, phosphorylated and dephosphorylated thylakoids reveal that there is a reversible movement of 9 nm particles from the appressed to the non-appressed regions on phosphorylation, without a comparable movement of 14–16 nm particles (Kyle *et al.* 1983).

Taken together these results suggest that it is the 9 nm LHC particles that provide the connectivity and much of the light-harvesting capacity of PS II and which, on phosphorylation dissociate from PS II and migrate to PS I. The particles are presumably forced to leave the confines of the appressed regions by the high negative surface-charge density caused by phosphorylation (Barber 1982). Phosphorylation also leads to a small but detectable decrease in the

extent of membrane stacking (Kyle *et al.* 1983; Biggins 1982). This effect is also likely to be due to an increase in negative surface charge density but perhaps it is phosphorylation of the less mobile 14–16 nm particles that is responsible. Since such particles contain PS II, their release from the appressed regions may account for reports of ATP-induced excitation energy transfer from PS II to PS I (Bennett *et al.* 1980; Haworth *et al.* 1982*a*). If the LHC particles are phosphorylated in the appressed regions and dephosphorylated in the non-appressed regions, it would be expected that the kinase is restricted to the former, with the phosphatase restricted to the latter. Such a distribution would minimize the tendency of the kinase and the phosphatase to operate in concert as an ATPase or futile cycle.

Physiological significance of thylakoid protein phosphorylation

A mechanism has now been proposed by which an imbalance in the rates of excitation of PS I and PS II may be detected and corrected (Horton & Black 1980; Allen *et al.* 1981). The imbalance is detected through the redox state of the PQ pool and then corrected through the

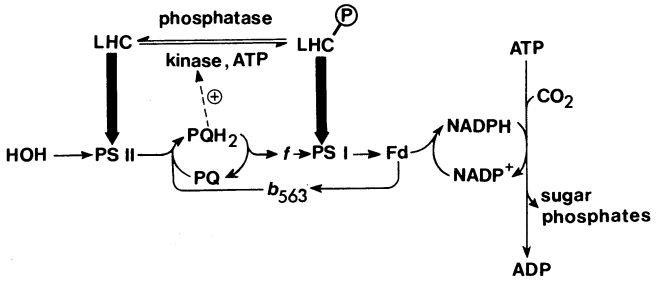

Figure 4. Model showing negative feedback control in the regulation of the distribution of excitation energy of the thylakoids by means of phosphorylation of the light-harvesting chlorophyll *a*/*b* complex (LHC), by a protein kinase which is activated by plastoquinol (PQH_2). The redox state of the PQ pool is affected by the rate of $NADP^+$ regeneration by CO_2 fixation.

phosphorylation or dephosphorylation of LHC (figure 4) (Allen *et al.* 1981). Overexcitation of PS II leads to the reduction of the PQ pool, activation of the protein kinase, and increased transfer of excitation energy from phosphorylated LHC to PS I at the expense of PS II. A major factor in the redirection of excitation energy is the migration of phosphorylated 9 nm LHC particles from the appressed regions of the thylakoids to the non-appressed regions (Kyle *et al.* 1983). Conversely, under certain circumstances, overexcitation of PS I leads to oxidation of the PQ pool, inactivation of the kinase, dephosphorylation of LHC by the phosphatase and the migration of the dephosphorylated LHC particles from PS I to PS II. In both examples, the mechanism enables a more balanced distribution of excitation energy to be obtained within a period of 5–10 min.

The mechanism may enable green plants to adapt to changes in the degree of shading produced by neighbouring foliage. Because shade is enriched for wavelengths absorbed only by by PS I, it will tend to overexcite PS I, promote dephosphorylation of LHC and lead to a more extensive interaction between PS II and LHC within the appressed regions. This will maximize the light-harvesting capacity of PS II and help to provide a more balanced distribution of excitation energy. However, when a shade-adapted plant emerges into diffuse or direct sunlight, which contains a more even spread of photon fluence rates across the 400–720 nm region

of the spectrum, the high concentration of LHC in the appressed regions will lead to the over-excitation of PS II. The resultant activation of LHC phosphorylation will lead to the migration of LHC out of the appressed regions and the attainment of a new balance in the distribution of excitation energy. Since changes in the degree of shading are due most frequently to the movement of the Sun across the sky, the phosphorylation–dephosphorylation mechanism should be sufficiently rapid to permit adaptation. However, complete adaptation to shade or bright sunlight will require additional structural changes that involve the regulation of the biosynthesis and turnover of chloroplast components and are outside the scope of this article. Indeed, it may be outside the genetic capabilities of most plant species to adapt completely to extremes of both light and shade (Boardman 1977).

The mechanism summarized in figure 4 does not strictly speaking provide a 1:1 distribution of excitation energy between PS I and PS II. What it does provide is a distribution that closely approximates to that required to satisfy simultaneously the demands within the chloroplast for ATP and reducing power (usually NADPH, but occasionally reduced ferredoxin). This is achieved by regulating the relative rates of cyclic and non-cyclic electron transport. To illustrate the point, let us consider a chloroplast that requires ATP and NADPH for the fixation of CO_2 into sugar phosphates but for no other process. As mentioned above, this requirement can be met by a distribution of excitation energy that is about 5:4 in favour of PS I. Let us suppose that the chloroplast has been fixing CO_2 under white light and that for some minutes the level of LHC phosphorylation has been such that the excitation energy has been distributed in a 5:4 ratio between PS I and PS II. Is the mechanism capable of restoring this ratio if the system is suddenly perturbed?

Consider a perturbation in which white light is suddenly supplemented with sufficient 645 nm light to provide a 1:1 distribution of excitation energy between the photosystems. The rate of turnover of PS II will increase without a corresponding increase in the rate of turnover of PS I. As a result, the PQ pool becomes reduced, PS II traps begin to close and much of the additional excitation energy in PS II is dissipated by chlorophyll fluorescence and other mechanisms. At the same time, the protein kinase becomes more highly activated. Because cyclic electron transport will persist under these conditions (Hind *et al.* 1981), the ATP/ADP ratio will remain sufficiently high to permit enhanced LHC phosphorylation. As additional phosphorylated LHC particles migrate from PS II to PS I, and the distribution of excitation energy between PS I and PS II approaches the critical ratio of 5:4, the PQ pool becomes progressively oxidized, the PS II traps open and a new position of equilibrium is attained in which the additional 645 nm light is distributed between the photosystems and drives both cyclic and non-cyclic electron transport and CO_2 fixation at higher rates.

How expensive is the mechanism in terms of ATP consumption? If the system is assumed to be at equilibrium for most of the time, with the rate of LHC phosphorylation equal to the rate of dephosphorylation, if dephosphorylation has a half-time of about 8 min (Bennett 1983), and if the average level of LHC phosphorylation is about 20 nmol mg^{-1} chlorophyll, then the rate of phosphorylation of LHC is about 75 nmol ATP consumed mg^{-1} chlorophyll h^{-1}, or less than 0.1 % of the maximum rate of ATP synthesis seen in isolated intact chloroplasts rapidly fixing CO_2 (about 300 μmol ATP mg^{-1} chlorophyll h^{-1}) (Hind *et al.* 1981). Thus, even under very low light intensities, this mechanism for regulating the relative rates of ATP and NADPH synthesis consumes negligible quantities of ATP.

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I gratefully acknowledge financial assistance in my research from the Science and Engineering Research Council.

References

Allen, J. F. & Horton, P. 1981 Chloroplast protein phosphorylation and chlorophyll fluorescence quenching. Activation by tetramethyl-*p*-hydroquinone, an electron donor to plastoquinone. *Biochim. biophys. Acta* **638**, 290–295.

Allen, J. F., Bennett, J., Steinback, K. E. & Arntzen, C. J. 1981 Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. *Nature, Lond.* **291**, 1–5.

Anderson, J. M. 1982 The significance of grana stacking in chlorophyll *b* containing chloroplasts. *Photobiochem. Photobiophys.* **3**, 225–241.

Anderson, J. M. & Andersson, B. 1982 The architecture of the photosynthetic membrane: lateral and transverse organization. *Trends biochem. Sci.* **7**, 288–292.

Andersson, B., Akerlund, H.-E., Jergil, B. & Larsson, C. 1982*a* Differential phosphorylation of the light-harvesting chlorophyll–protein complex in appressed and non-appressed regions of the thylakoid membrane. *FEBS Lett.* **149**, 181–185.

Andersson, B., Anderson, J. M. & Ryrie, I. J. 1982*b* Transbilayer organization of the chlorophyll-proteins of spinach thylakoids. *Eur. J. Biochem.* **123**, 465–472.

Baker, R. N., Markwell, J. P. & Thornber, J. P. 1982 Adenine nucleotide inhibition of phosphorylation of the light-harvesting chlorophyll *a*/*b*-protein complex. *Photobiochem. Photobiophys.* **4**, 211–217.

Barber, J. 1982 Influence of surface charges on thylakoid structure and function. *A. Rev. Pl. Physiol.* **33**, 261–295.

Bellemare, G., Bartlett, S. & Chua, N.-H. 1982 Biosynthesis of chlorophyll *a*/*b*-binding polypeptides in wild type and the chlorina f2 mutant of barley. *J. biol. Chem.* **257**, 7762–7767.

Bennett, J. 1977 Phosphorylation of chloroplast membrane polypeptides. *Nature, Lond.* **269**, 344–346.

Bennett, J. 1979*a* Chloroplast phosphoproteins. Phosphorylation of polypeptides of the light-harvesting chlorophyll protein complex. *Eur. J. Biochem.* **99**, 133–137.

Bennett, J. 1979*b* Chloroplast phosphoproteins. The protein kinase of thylakoid membranes is light-dependent. *FEBS Lett.* **103**, 342–344.

Bennett, J. 1980 Chloroplast phosphoproteins. Evidence for a thylakoid-bound phosphoprotein phosphatase. *Eur. J. Biochem.* **104**, 85–89.

Bennett, J. 1983 Regulation of photosynthesis by reversible phosphorylation of the light-harvesting chlorophyll *a*/*b* protein. *Biochem. J.* (In the press.)

Bennett, J., Markwell, J. P., Skrdla, M. P. & Thornber, J. P. 1981 Higher plant chlorophyll *a*/*b*–protein complexes: studies on the phosphorylated apo-proteins. *FEBS Lett.* **131**, 325–330.

Bennett, J., Steinback, K. E. & Arntzen, C. J. 1980 Chloroplast phosphoproteins: regulation of excitation energy transfer by phosphorylation of thylakoid membranes. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5253–5257.

Biggins, J. 1982 Thylakoid conformational changes accompanying membrane protein phosphorylation. *Biochim. biophys. Acta* **679**, 479–482.

Boardman, N. K. 1977 Comparative photosynthesis of sun and shade plants. *A. Rev. Pl. Physiol.* **28**, 355–377.

Bonaventura, C. & Myers, J. 1969 Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. *Biochim. biophys. Acta* **189**, 366–383.

Carter, D. P. & Staehelin, L. A. 1980 Proteolysis of chloroplast thylakoid membranes. II. Evidence for the involvement of the light-harvesting chlorophyll *a*/*b*–protein complex in thylakoid stacking and for effects of magnesium ions on photosystem II–light-harvesting complex aggregates in the absence of membrane stacking. *Archs Biochem. Biophys.* **200**, 374–386.

Chow, W. S., Telfer, A., Chapman, D. J. & Barber, J. 1981 State 1–state 2 transitions in leaves and its association with ATP induced chlorophyll fluorescence quenching. *Biochim. biophys. Acta* **638**, 60–68.

Farchaus, J. W., Widger, W. R., Cramer, W. A. & Dilley, R. A. 1982 Kinase-induced changes in electron transport rates of spinach chloroplasts. *Archs biochem. Biophys.* **217**, 362–367.

Haworth, P., Kyle, D. J. & Arntzen, C. J. 1982*a* Demonstration of the physiological role of membrane phosphorylation in chloroplasts, using the bipartite and tripartite models of photosynthesis. *Biochim. biophys. Acta* **680**, 343–351.

Haworth, P., Kyle, D. J., Horton, P. & Arntzen, C. J. 1982*b* Chloroplast membrane protein phosphorylation. *Photochem. Photobiol.* **36**, 743–748.

Hind, G., Crowther, D., Shahak, Y. & Slovacek, R. E. 1981. The function and mechanism of cyclic electron transport. In *Photosynthesis II. Electron transport and photophosphorylation* (ed. G. Akoyunoglou), pp. 87–97. Philadelphia, Pa: Balaban International Science Services.

Horton, P., Allen, J. F., Black, M. T. & Bennett, J. 1981 Regulation of phosphorylation of chloroplast membrane polypeptides by the redox state of plastoquinone. *FEBS Lett.* **125**, 193–196.

Horton, P. & Black, M. T. 1980 Activation of adenosine 5′-triphosphate induced quenching of chlorophyll fluorescence by reduced plastoquinone. The basis of state I–state II transitions in chloroplasts. *FEBS Lett.* **119**, 141–144.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Horton, P. & Black, M. T. 1981 Light-dependent quenching of chlorophyll fluorescence in pea chloroplasts induced by adenosine 5′-triphosphate. *Biochim. biophys. Acta* **635**, 53–62.
Horton, P. & Black, M. T. 1982 On the nature of the fluorescence decrease due to phosphorylation of chloroplast membrane proteins. *Biochim. biophys. Acta* **680**, 22–27.
Kyle, D. J., Haworth, P. & Arntzen, C. J. 1982 Thylakoid membrane protein phosphorylation leads to a decrease in connectivity between photosystem II reaction centres. *Biochim. biophys. Acta* **680**, 336–342.
Kyle, D. J., Staehelin, L. A. & Arntzen, C. J. 1983 Lateral mobility of the light-harvesting complex in chloroplast membranes controls excitation energy distribution in higher plants. *Archs Biochem. Biophys.* (In the press.)
Larkum, A. W. D. & Anderson, J. M. 1982 The reconstitution of a photosystem II protein complex, P-700–chlorophyll *a*–protein complex and light-harvesting chlorophyll *a*/*b*–protein. *Biochim. biophys. Acta* **679**, 410–421.
Markwell, J. P., Baker, N. R. & Thornber, J. P. 1982 Metabolic regulation of the thylakoid protein kinase. *FEBS Lett.* **142**, 171–174.
Melis, A. & Ow, R. A. 1982 Photoconversion kinetics of chloroplast photosystems I and II. Effect of Mg^{2+}. *Biochim. biophys. Acta* **682**, 1–10.
Mullet, J. E., Burke, J. J. & Arntzen, C. J. 1980 Chlorophyll proteins of photosystem I. *Pl. Physiol.* **65**, 814–822.
Olive, J., Wollman, F.-A., Bennoun, P. & Recouvreur, M. 1981 Ultrastructure of thylakoid membranes in *C. reinhardtii*: evidence for variations in the partition coefficient of the light-harvesting complex-containing particles upon membrane fracture. *Archs Biochem. Biophys.* **208**, 456–467.
Owens, G. C. & Ohad, I. 1982 Phosphorylation of *Chlamydomonas reinhardi* chloroplast membrane proteins in vivo and in vitro. *J. Cell Biol.* **93**, 712–718.
Portis, A. R. & McCarty, R. E. 1974 Effects of adenine nucleotides and of phosphorylation on H^+ uptake and the magnitude of the H^+ gradient in illuminated chloroplasts. *J. biol. Chem.* **249**, 6250–6254.
Saito, K., Williams, W. P., Allen, J. F. & Bennett, J. 1983 Comparison of ATP-induced and state I/state II related changes in excitation energy distribution in *Chlorella vulgaris*. *Biochim. biophys. Acta*. (In the press.)
Simpson, D. J. 1979 Freeze-fracture studies on barley thylakoid membranes. III Location of the light-harvesting chlorophyll–protein. *Carlsberg Res. Commun.* **44**, 305–336.
Smith, H. 1982 Light quality, photoperception and plant strategy. *A. Rev. Pl. Physiol.* **33**, 481–518.
Staehelin, L. A. & Arntzen, C. J. 1979 Effects of ions and gravity forces on the supramolecular organization and excitation energy distribution in chloroplast membranes. In *Chlorophyll organization and energy transfer in photosynthesis* (Ciba Foundation Symposium, New Series), pp. 147–175. Amsterdam: Excerpta Medica.
Steinback, K. E., Bose, S. & Kyle, D. J. 1982 Phosphorylation of the light-harvesting chlorophyll–protein regulates excitation energy distribution between photosystem II and photosystem I. *Archs Biochem. Biophys.* **216**, 356–361.
Steinback, K. E., Burke, J. J. & Arntzen, C. J. 1979 Evidence for the role of surface-exposed segments of the light-harvesting complex in cation-mediated control of chloroplast structure and function. *Archs Biochem. Biophys.* **195**, 546–557.
Telfer, A., Allen, J. F., Barber, J. & Bennett, J. 1982 Thylakoid protein phosphorylation during state 1-state 2 transitions in osmotically shocked pea chloroplasts. *Biochim. biophys. Acta* **722**, 176–181.
Thornber, J. P. & Highkin, H. R. 1974 Composition of the photosynthetic apparatus of normal barley leaves and a mutant lacking chlorophyll *b*. *Eur. J. Biochem.* **41**, 109–116.
Trebst, A. 1974 Energy conservation in photosynthetic electron transport of chloroplasts. *A. Rev. Pl. Physiol.* **25**, 423–458.
Trewavas, A. 1976 Post-translational modification of proteins by phosphorylation. *A. Rev. Pl. Physiol.* **27**, 349–374.
Waldron, J. C. & Anderson, J. M. 1979 Chlorophyll–protein complexes from thylakoids of a mutant barley lacking chlorophyll *b*. *Eur. J. Biochem.* **103**, 357–362.

Discussion

P. Cohen. Since we know that calmodulin is present in chloroplasts (H. W. Jarrett *et al.*, *J. biol. Chem.* **257**, 13795–13804 (1982)) and that it activates NAD kinase (J. M. Anderson & M. J. Cormier, *Biochem. biophys. Res. Commun.* **84**, 595–602 (1978)) as well as a protein kinase that phosphorylates plant quinate:NAD oxidoreductase (R. Ranjeva *et al.*, *Proc. natn. Acad. Sci. U.S.A.* (in the press)), has Dr Bennett investigated whether LHC kinase activity is affected by calcium ions or calmodulin, or by calmodulin antagonists such as the phenathiazine drugs?

J. Bennett. The only data that I know of concerning the effects of Ca^{2+} on the thylakoid protein kinase were published by Alfonzo *et al.* (1980), who showed that 3 mM Ca^{2+} is only 8% as

effective as 3 mM Mg^{2+} in satisfying the requirement of the kinase for a divalent metal cation cofactor. A Ca^{2+}-dependent protein kinase has been detected in extracts of dark-grown pea shoots by Hetherington & Trewavas (1982). Ca^{2+} activated in the 0.1–10 μM range. The kinase was thought to be membrane-bound. The membranes concerned sedimented at 90000 *g* but not at 12000 *g*. Because thylakoids should sediment at the latter value, this kinase is probably not related to the thylakoid protein kinase.

References

Alfonzo, R., Nelson, N. & Racker, E. 1980 *Pl. Physiol.* **65**, 730–734.
Hetherington, A. & Trewavas, A. 1982 *FEBS Lett.* **145**, 67–71.

D. E. Hanke (*Department of Botany, University of Cambridge, U.K.*). There is evidence that the chromoprotein phytochrome, which mediates many plant responses to light, is present in chloroplast envelope. Since one of its modes of action is known to be to induce increased passive fluxes of Ca^{2+} across membranes on absorbing red light, reversible by far-red light, the possibility that calcium might be involved in the activation of LHC kinase is certainly worth considering.

J. Bennett. There is no direct evidence on this point. I have looked for effects of external Ca^{2+} on kinase activity in isolated mature chloroplasts, but the results have been negative so far. It is possible that the membranes studied by Hetherington & Trewavas (1982) were etioplast envelopes. If phytochrome is bound to etioplast envelopes it may activate a protein kinase on the same membrane.

L. A. Pinna (*The University, Padua, Italy*). Are there data available either supporting or ruling out the possibility that the two protein kinases from chloroplasts recently described by Racker *et al.* might be involved in the phosphorylation of Dr Bennett's proteins?

J. Bennett. The two recent papers from Professor Racker's laboratory (Lin *et al.* 1982; Lucero *et al.* 1982) report the purification and properties of two kinases from thylakoids. The authors themselves express some doubt concerning the relation between these enzymes and the PQH_2-dependent enzyme.

References

Lin, Z.-F., Lucero, H. A. & Racker, E. 1982 *J. biol. Chem.* **257**, 12153–12156.
Lucero, H. A., Lin, Z.-F. & Racker, E. 1982 *J. biol. Chem.* **257**, 12157–12160.

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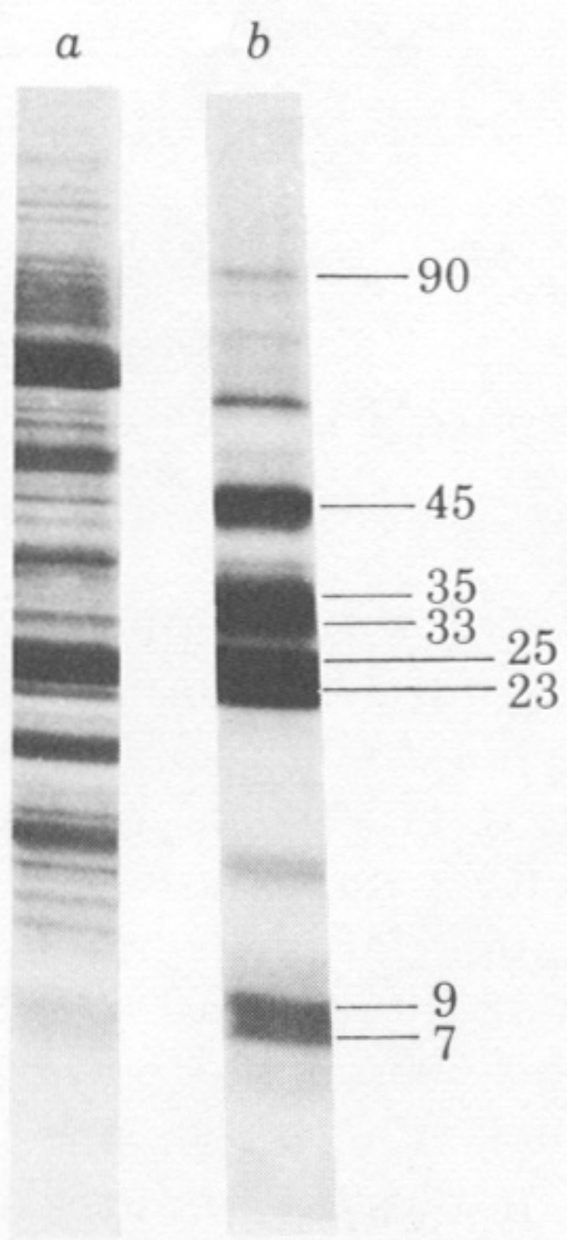

FIGURE 1. SDS-polyacrylamide gel electrophoresis of ^{32}P-labelled thylakoids of pea chloroplasts. (*a*) Gel stained with Coomassie Brilliant Blue. (*b*) Radioautogram of gel. Apparent molecular masses (kilodaltons) are indicated.

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