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The rate and extent of phosphorylation of the two light-harvesting chlorophyll *a/b* binding protein complex (LHC-II) polypeptides in isolated spinach thylakoids

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The level of phosphorylation of the 24 kDa and the 25 kDa light-harvesting chlorophyll *a/b* binding protein complex (LHC) II polypeptides in isolated spinach thylakoids has been determined by quantitative SDS-polyacrylamide gel electrophoresis. The time-course of phosphorylation, after correction for the molar abundance of these two polypeptides, shows that (a) the rate of phosphorylation of the 24 kDa polypeptide is at least 3-fold faster compared with the 25 kDa polypeptide, (b) the final extent of phosphorylation for both the polypeptides is very similar, and (c) the final extent of phosphorylation is typically between 0.15 and 0.25 mol phosphate per mol polypeptide. The low extent of phosphorylation is not simply a consequence of inactivation of the kinase and/or LHC II substrate or ATP depletion. These observations suggest that there are at least three different sub-populations of LHC II in isolated thylakoids: (i) phosphorylated 'mobile', (ii) phosphorylated 'PS II-coupled' and (iii) non-phosphorylated. Furthermore, the reported differences in the specific activity of phosphorylation for the 'mobile' and the 'PS II-coupled' LHC II sub-populations (Kyle, D.J. et al. (1984) Biochim. Biophys. Acta 765, 89–96) are no longer observed following correction for the non-phosphorylated LHC-II population.

Introduction

The light-harvesting chlorophyll *a/b* binding protein complex (LHC-II) is believed to occur in all eukaryotes containing chlorophyll *b* including green algae and *Euglena*. LHC-II is generally thought to function as a light-harvesting antennae for Photosystem II and in the photosynthetic membranes of higher plants it is generally located in the grana with PS II (see reviews Refs. 1–4).

The composition of the LHC-II is known to vary with respect to the number and molecular weights of the constituent polypeptides when examined by non-denaturating gel electrophoresis or by denaturing SDS-polyacrylamide gel electrophoresis [5–9]. Most green plants contain two or more different LHC-II polypeptides which all appear to be structurally closely related as judged by their amino-acid composition, partial proteolytic digestion, CNBr cleavage and immunological

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

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cross-reactivity using either polyclonal or monoclonal antibodies [9–13].

LHC-II polypeptides have been shown to be phosphorylated in vivo and in vitro by protein kinase(s), which require reduced plastoquinone for activation [14]. This phosphorylation by the kinase(s) and dephosphorylation by intrinsic phosphatase(s) has been proposed to redistribute energy between PS II and PS I [15]. The phosphorylation results in a decrease in the room-temperature PS II fluorescence emission of between 15–25% [1–4]. It is generally believed that following phosphorylation a fraction of the LHC-II becomes detached from the PS II-LHC-II matrix and subsequently transfers energy to PS I [1–4,16–9]. A close correlation has been reported between the ATP-induced fluorescence decline and the rate and level of LHC-II phosphorylation [14,20], though we have recently reported a lack of direct correlation with the latter [21,22].

In pea up to four LHC-II polypeptides can be present, but only two of these are phosphorylated [13,23,24]. Tryptic digestion of pea thylakoids showed that the 27 kDa LHC-II polypeptide is phosphorylated at a threonine residue on the surface exposed trypsin-cleavable fragment [23]. By contrast in spinach only two LHC-II polypeptides have been resolved by SDS-polyacrylamide gel electrophoresis and both can be phosphorylated [5,7,19,21,22]. The relative molar abundance of the two polypeptides and their kinetics of phosphorylation are significantly different [7,21,22].

In this paper quantitative SDS-polyacrylamide gel electrophoresis has been used to determine the level of phosphorylation of the two LHC-II polypeptides. The results indicate that while the rate of phosphorylation of the two polypeptides are significantly different the final extent of phosphorylation of both the polypeptides is very similar. However, typically sub-stoichiometric levels of phosphorylation are observed. The data are discussed with respect to the observed differences in the rate of phosphorylation and the reported differences in the specific activities of phosphorylation of the migrating compared with the PS II-coupled LHC-II populations in thylakoids. Abstracts of this work have been published previously [25,26].

Materials and Methods

Freshly harvested spinach leaves were kept in the dark for up to 2 h at which time the leaves were homogenised and thylakoids isolated as described previously [21]. Purified LHC-II was prepared essentially according to Ref. 5.

Acetone-precipitated thylakoids or purified LHC-II were suspended in a solution consisting of 90 mm sodium carbonate/10% 2-mercaptoethanol. Following suspension of the proteins an equal volume of 5% LDS/30 mM sucrose/1% bromophenol blue was added and the samples heated at 70 °C for 5 min and fractionated by SDS-polyacrylamide gel electrophoresis on rod gels. The gels were stained overnight with 0.025%. Coomassie R-250/25% propan-1-ol/10% acetic acid and destained by three changes in 10% acetic acid. The gels, following staining and destaining, were scanned at 595 nm using a Perkin-Elmer lambda 3 or a Beckman DU-8 spectrophotometer and the integrated peak areas determined [27]. Slab-gels were stained with Silver according to the protocol of Bio-Rad and scanned using an LKB 2202 ultroscan laser densitometer.

For Ferguson analysis [28] the gels were prepared in the same manner except that the concentration of the acrylamide was varied, though the ratio of acrylamide to bisacrylamide was maintained constant (37.5 : 1).

Phosphorylation of the thylakoids was initiated, in a reaction medium containing 30 mM Tricine (pH 8.0)/10 mM NaCl/5 mM $MgCl_2$/10 mM NaF/0.1 M sucrose in the presence of the NADPH-ferrodoxin reducing system as described previously [21,22], by addition of 1 mM Mg-ATP. Control thylakoids were similarly treated, except that ATP was omitted from the assay. The phosphorylated and control thylakoids were kept on ice for 30 min before determination of maximal fluorescence, induced by addition of 25 μM DCMU [21,22].

Separation of ATP from ADP was achieved on PEI plates developed in 1 M potassium phosphate (pH 3.4). Aliquots after various times of incubation were removed and the reaction terminated by the addition of an equal volume of 10% ice-cold trichloroacetic acid. Following the removal of the precipitated proteins and addition of unlabelled

ATP, 2 μl aliquots of the supernatant were spotted onto the PEI plates and developed for 2–3 h. The positions of the nucleotides were localised by ultraviolet or autoradiography and the counts comigrating with the ATP spot and the total counts spotted onto the plate determined by liquid scintillation.

Protein concentration was determined by the procedure of Hartree [29] using bovine serum albumin as the calibration standard and chlorophyll content by the procedure of Arnon [30].

All biochemicals were purchased from Sigma, reagents for SDS-polyacrylamide gel electrophoresis and silver stain from Bio-Rad. All other reagents were of Analar grade. High specific activity [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from Amersham International, Amersham. Polygram cell 300 PEI plates were purchased from Macherey-Nagel.

Results

High resolution denaturing SDS-polyacrylamide gel electrophoresis was used to examine the polypeptide composition of LHC-II purified by the procedure of Ryrie et al. [5]. The LHC-II (chlorophyll a/b ratio of 1.06) was fractionated on 15% acrylamide gels by SDS-polyacrylamide gel electrophoresis and stained with Coomassie R-250 showed that only two LHC-II polypeptides were present (Fig. 1a), constituting more than 95% of the total protein, in agreement with the results of Ryrie et al. [5]. When isolated thylakoids were similarly fractionated and stained, the same two polypeptides were present and were well separated by high-resolution SDS-polyacrylamide gel electrophoresis (Fig. 1b). However, the two polypeptides appear to be present in different amounts both in purified complexes and isolated thylakoids.

The relative intensity of staining with Coomassie could reflect differences in the stain-binding characteristics rather than differences in the amounts of the two polypeptides present. Silver staining, which differs significantly from Coomassie with respect to protein binding characteristics, was also used to visualise the LHC-II polypeptides following fractionation of purified LHC on 15% acrylamide slab-gel by SDS-polyacrylamide gel electrophoresis. The pattern of staining of the two

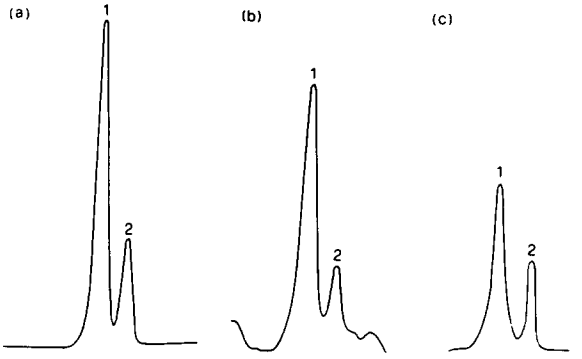

Fig. 1. Densitometric profiles of the LHC-II polypeptides fractionated by high-resolution SDS-polyacrylamide gel electrophoresis and stained with Coomassie R-250 (a,b) or silver (c). Purified LHC-II (a,c) or isolated thylakoids (b) were fractionated by SDS-polyacrylamide gel electrophoresis on 15% acrylamide gels, and after staining and destaining were scanned by laser densitometry. Only two LHC-II polypeptides were present and exhibited apparent molecular weights of 24830 (1) and 23730 (2), termed 25 kDa and 24 kDa, as determined from Ferguson plots. The molar abundance of these two LHC-II polypeptides calculated from the integrated peak areas (see Materials & Methods) are 1:3 and 1:2.6 for the 24:25 kDa by Coomassie and silver staining, respectively.

polypeptides with silver was similar to that observed with Coomassie (cf. Fig. 1a and c), strongly suggesting that the relative intensity of Coomassie staining reflects the amounts of the two polypeptides rather than differences in stain-binding characteristics.

Apparent molecular weights of 24830 and 23730, termed 25 kDa and 24 kDa respectively, were determined for the two LHC-II polypeptides by Ferguson plots [28]. A relative stoichiometry of 1:3 for the 24:25 kDa LHC-II polypeptides was observed with Coomassie staining [27].

Incubation of isolated thylakoids in the presence of [γ-^{32}P]ATP following chemical activation of the protein kinase(s), results in rapid incorporation of label into the two LHC-II polypeptides. A typical time-course of phosphorylation of the two-LHC-II polypeptides is shown in Fig. 2. The moles of LHC-II protein present were determined by quantitative SDS-polyacrylamide gel electrophoresis [27] and the specific phosphate incorporation determined by excision of the polypeptide bands following SDS-polyacrylamide gel electrophoresis and liquid scintillation [21]. The initial rate of phosphorylation of the two polypeptides differed

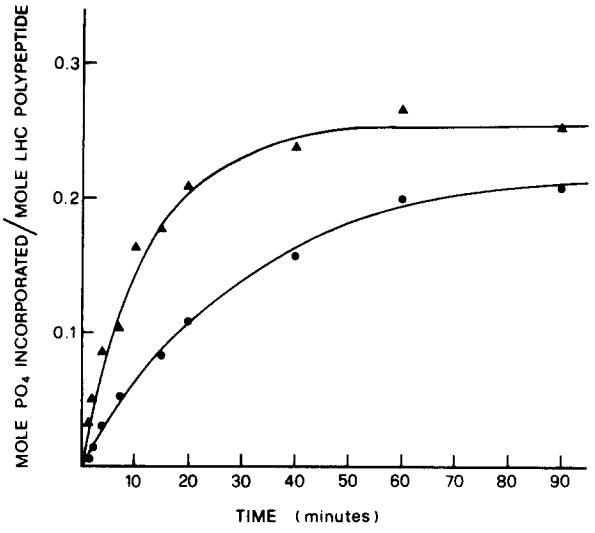

Fig. 2. Time-course of phosphorylation of the 24 kDA (▲———▲) and the 25 kDa (●———●) LHC-II polypeptides. Isolated thylakoids (400 μg Chl/ml) were incubated at 22° C in the presence of the reducing system (see Materials and Methods) and the phosphorylation initiated by the addition of 1 mM Mg-ATP containing [γ-^{32}P]ATP. At the indicated times aliquots were removed and the reaction terminated by the addition of ice-cold 80% acetone. The precipitated proteins were analysed by SDS-polyacrylamide gel electrophoresis and after Coomassie staining were scanned at 595 nm. The protein present (moles) on the gel was determined by quantitative SDS-polyacrylamide gel electrophoresis and the co-migrating γ-^{32}P by liquid scintillation.

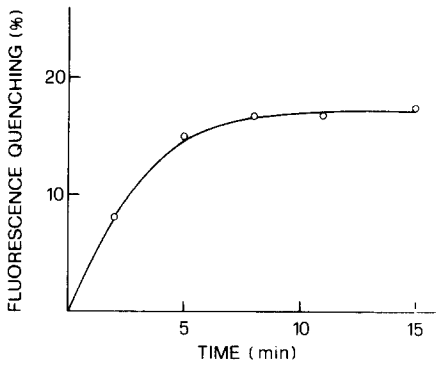

Fig. 3. Time-course of the ATP-induced Photosystem II fluorescence decline. Isolated thylakoids were treated as described above (see Fig. 2) and at the indicated times aliquots were removed and diluted 100–200-fold with ice-cold reaction medium. After equilibration on ice for 30 min the maximal fluorescence decline was determined following the addition of 25 μM DCMU. Fluorescence quenching is expressed as $(F_{max}(-ATP) - F_{max}(+ATP)/F_{max}(-ATP)$, where all F_{max} values were determined at each time-point.

significantly in that the 24 kDa polypeptide was phosphorylated about 3-fold faster compared with the 25 kDa polypeptide. However, both polypeptides show little further incorporation after 90 min of phosphorylation. The maximal extent of phosphorylation in this preparation did not differ significantly for the two polypeptides (0.22 mol PO_4/mol 25 kDa vs. 0.25 mol PO_4/mol 24 kDa polypeptide).

The time-course of the ATP-induced fluorescence decline was also determined (Fig. 3). By contrast to the time-course of phosphorylation (Fig. 2) the fluorescence decline, due to the changes in the absorptive cross-section of PS II [21,22], is more or less completed after the initial 5 min. The half-time for the ATP-induced fluorescence decline was typically 2–3 min (Fig. 3), while the half-time for phosphorylation was typically 8 and 20 min for the 24 kDa and 25 kDa polypeptides, respectively (Fig. 2).

The level of phosphorylation of LHC-II polypeptides appears to vary between different preparations [21]. Data from four different preparations are presented in Table I. While the final stoichiometries (0.16–0.25 mol PO_4/mol LHC) clearly varied between different preparations, the picomoles PO_4 incorporated per μg Chl into the 25 kDa compared with the 24 kDa polypeptides showed little change and yielded a ratio of 2.73. Indeed, the final stoichiometry of phosphorylation after correction for the molar abundance of the two polypeptides was close to unity (0.91). Similar analysis of the initial rate of phosphorylation though showed that the rate of phosphorylation of the 24 kDa was always about 3-times faster compared with the 25 kDa (Table II). Incubation of pre-phosphorylated thylakoids with trypsin resulted in the loss of labelled phosphate from both polypeptides suggesting that the phosphorylation sites are located on surface-exposed segments of both polypeptides (unpublished observation).

Both the polypeptides though show substoichiometric phosphorylation. However, dephosphorylation by phosphoprotein phosphatases can be excluded as (a) the phosphorylation medium contained 10 mM NaF which inhibits the phosphatase activities [1–4] and (b) unlike pea thylakoids there is little or no active phosphatase

TABLE I

FINAL EXTENT OF PHOSPHORYLATION OF THE 25 kDa AND 24 kDa LHC-II POLYPEPTIDES IN DIFFERENT PREPARATIONS

Isolated thylakoids (400 μgs Chl/ml) were phosphorylated for between 90 and 120 min to achieve maximal phosphorylation (see Fig. 2). The protein was fractionated by SDS-polyacrylamide gel electrophoresis and the amount of protein and the co-migrating counts determined by quantitative SDS-polyacrylamide gel electrophoresis and liquid scintillation. The amount of the LHC-II protein present on the gel varied in the different preparations.

Experiment	pmol PO_4/μg Chl			mol PO_4/mol protein		
	25 kDa	24 kDa	25/24 kDa	25 kDa	24 kDa	25/24 kDa
1	3.22	1.02	3.1	0.16	0.15	1.06
2	9.01	3.50	2.6	0.22	0.25	0.88
3	5.70	2.13	2.7	0.17	0.19	0.89
4	4.20	1.70	2.5	0.14	0.16	0.87
			2.7			0.92

present in spinach thylakoids under these phosphorylating conditions (Ref. 31; see also Islam, K., unpublished data). However, inactivation of either the LHC-II substrates and/or kinase during the incubation period or exhaustion of the ATP could though result in the observed sub-stoichiometric phosphorylation.

To investigate the possibility that the substrates or the kinases are inactivated during the incubation period a pulse-label experiment was also performed using the same chloroplast preparation as in Fig. 2. Isolated thylakoids were incubated at 22°C, following the chemical activation of the kinases, and at various times aliquots were removed and challenged for 2 min with labelled ATP. The reaction was terminated by the addition of ice-cold acetone and the precipitated protein fractionated by SDS-polyacrylamide gel electrophoresis. The initial rate of incorporation of label into each of the two LHC-II polypeptides was determined (Fig. 4). At the end of the incubation period a decrease of only 25–35% in the initial rate of phosphorylation was observed. Therefore

TABLE II

INITIAL RATE OF PHOSPHORYLATION OF THE 25 kDa AND 24 kDa LHC-II POLYPEPTIDES IN DIFFERENT PREPARATIONS

The experiments refer to the same preparations as in Table I. The initial rate was determined from the first 2-min period such that errors due to non-linearity observed at later times could be minimised (see Fig. 2). The 24 kDa polypeptide was phosphorylated at least 3-fold faster compared with the 25 kDa polypeptides

Experi-	pmol PO_4/min per μg Chl			mol PO_4/min per mol protein		
	25 kDa	24 kDa	25/24 kDa	25 kDa	24 kDa	25/24 kDa
1	0.106	0.117	0.91	0.0053	0.0176	0.30
2	0.311	0.340	0.91	0.0075	0.0247	0.30
3	0.232	0.224	1.04	0.0069	0.0201	0.34
4	0.184	0.215	0.86	0.0058	0.205	0.28
			0.93			0.31

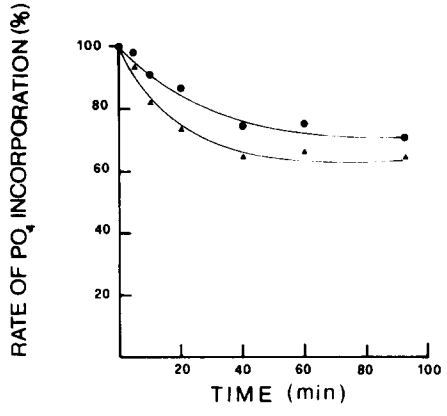

Fig. 4. Initial rate of phosphorylation of the LHC-II polypeptides as a function of the incubation time at 22°C. The pulse-label experiment was performed as described (see Results). The initial rate of phosphorylation of the 24 kDa (▲———▲) and 25 kDa (●———●) polypeptides was determined following fractionation by SDS-polyacrylamide gel electrophoresis and is expressed as a percentage of the initial kinase activity determined at zero-time.

more than 65% (mean of three different preparations) of the initial kinase activity remains at the end of the incubation period assuming no inactivation of the substrates.

The amount of ATP remaining after different times of incubation was also determined following separation of inorganic phosphate from the ATP by thin-layer chromatography. The rate of ATP hydrolysis remained more or less constant throughout the entire incubation period (Fig. 5). The ATPase activity was typically 0.011 nmol/min per μg Chl in these preparations. Significant concentrations of ATP (more than 500 μM) are therefore present at the end of the incubation period. Since the K_m ATP of the protein kinase phosphorylating the LHC-II polypeptides is about 40–60 μM (Refs. 20 and 32, see also Islam, K., unpublished results) the observed ATP depletion cannot account for the low extent of phosphorylation.

However, ADP has been reported to inhibit phosphorylation though significant inhibition is only observed when the ATP/ADP ratio is close to 0.25 [33]. The ATP/ADP ratio approaches unity only towards the end of the incubation period when little further phosphorylation is observed (Fig. 5). It therefore appears unlikely that the sub-stoichiometric phosphorylation is a consequence of ADP generation during this time period. This has been confirmed by the observation that the addition of an ATP-regenerating system, and correction for the ATP-specific activity, does not alter the stoichiometry of phosphorylation observed in its absence (Islam, K., unpublished observations).

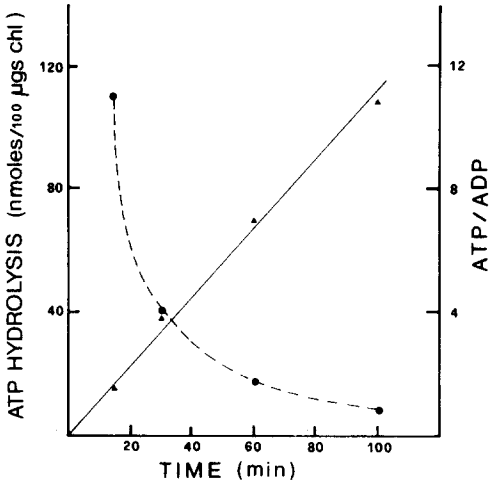

Fig. 5. ATP hydrolysis (▲———▲) and the ATP/ADP ratio (●— — —●) as a function of incubation time at 22° C. The amount of ATP remaining after various times of incubation under the phosphorylating conditions (see Figs. 2 and 4) was determined following the separation of the inorganic phosphate by thin-layer chromatography. Increasing amounts of ATP were hydrolysed with increasing time and resulted in a decrease in the ATP/ADP ratio.

Discussion

It has been proposed phosphorylation/dephosphorylation of the most conspicuous LHC-II polypeptides and its regulation by the redox-state of plastoquinone may control the energy distribution between the two photosystems [15]. However, only recent studies have focussed on the kinetics of phosphorylation and identification of the different LHC-II polypeptides (see introduction).

In spinach only two LHC-II polypeptides are present and both can be phosphorylated (Figs. 1 and 2 [5,7,19,21,22]). The 25 kDa (M_r 24830) and the 24 kDa (M_r 23730) have a relative stoichiometry of 1 mol 24 kDa/3 mol 25 kDa both in purified LHC-II and isolated thylakoids by quantitative SDS-polyacrylamide gel electrophoresis [27]. Similar differences in the amounts of the two polypeptides have also been reported by Larsson and Andersson [7]. Significant differences in the Coomassie stain binding characteristics of the two LHC-II polypeptides are highly unlikely both on the basis of the different staining procedures (Fig. 1) and the striking similarity of the two polypeptides as judged by various methods [9–13].

One advantage of the quantitative SDS-polyacrylamide gel electrophoresis is that it allows the determination of phosphate incorporation with respect to amount of protein present on the gel. The time-course of phosphorylation of the LHC-II polypeptides (Fig. 2 and Table II) shows that the rate of phosphorylation of the 24 kDa is about 3-times faster compared with the 25 kDa polypeptide. These data are therefore in good agreement with those of Larsson and Andersson [7] who noted a similar 3-fold difference in the rates of phosphorylation of the two LHC-II polypeptides.

The observed differences in the initial rate of phosphorylation of the two LHC-II polypeptides may reflect differences in either (a) conformation or structural localisation of the two polypeptides leading to an enhanced site recognition of the 24 kDa polypeptide by the kinase compared with the 25 kDa polypeptide or (b) a greater number of phosphorylation sites per polypeptide for the 24 kDa compared with the 25 kDa polypeptide or (c) a greater number of the total 24 kDa polypeptide chains phosphorylated compared with the 25 kDa polypeptide.

The final extent of phosphorylation of the two LHC-II polypeptides is very similar (Fig. 2 and Table I) although the half-time for phosphorylation of the 24 kDa is significantly shorter compared with the 25 kDa polypeptide. These observations suggest that a difference in either the number of phosphorylation sites/ polypeptide chain or in the total number of phosphorylated polypeptide chains for the 24 kDa and the 25 kDa polypeptides is unlikely. The observations are most consistent with the possibility that the conformation or the structural localisation of the 24 kDa results in enhanced recognition of this polypeptide by the kinase compared with the 25 kDa polypeptide. Recent evidence based on monoclonal antibodies [34] indicating a possible difference in conformation or protein sequence [35–38] of the LHC-II polypeptides in that part of the molecule which contains the phosphorylation site would be in agreement with such an interpretation.

However, typically sub-stoichiometric phosphorylation of both the polypeptides is observed (Table I). The possibility that the sub-stoichiometric phosphorylation is a consequence of inactivation of the kinase or substrate can though be excluded, as pulse-label experiments suggest that both the kinase and the substrates are relatively stable (Fig. 4). The alternative possibility that insufficient ATP was present can also be excluded (Fig. 5) as can the possibility that the phosphorylation is inhibited by the ADP generated during this period. The reasons for the low extent of phosphorylation remain unclear and may be a consequence of the structural characteristics of the appressed zones such that only the peripherally located LHC-II is available for phosphorylation [39]. It could be argued that the low extent of phosphorylation could be a consequence of incomplete dephosphorylation. However, this possibility is highly unlikely, though not totally excluded, since recent results in our laboratory suggest that a thylakoid-bound phosphatase activity, capable of dephosphorylating LHC-II in vitro within 60 min and inhibited by 10 mM NaF, is present in isolated spinach thylakoids (Forti, G. and Resta, C., personal communication).

No previous attempt has been made to determine the stoichiometry of phosphorylation of each of the different LHC-II polypeptides. However, one previous report has attempted to determine the extent of phosphorylation of the total LHC-II protein [40] and suggested that up to 80–90% of the LHC-II present in the thylakoids can be phosphorylated. However, since the phosphate incorporation into the total thylakoids was determined there is the possibility of overestimation as no correction was applied for the incorporation of phosphate by other proteins and/or entrapment of free or non-covalently bound ATP. Furthermore, these authors have utilised a value of 11–13 chlorophyll molecules bound to LHC-II although there is controversy about the protein/ pigment ratio with different groups reporting between 13–14 chlorophylls [8] or 6–7 chlorophylls [5,41,42] bound per mol LHC-II. Clearly if the protein/pigment ratio is closer to the latter value then the level of phosphorylation observed by these authors will be close to that observed in our preparations (the incorporation/μg Chl in these preparations are within the ranges described in the literature).

Previous studies have shown that the phosphorylated LHC-II consists of two populations: one that is 'mobile', while the other remains associated with PS II, the 'non-mobile' [7,18,19,21,22]. Fractionation studies have shown that up to 80–90% of the total LHC-II is found in the grana compared with 10–20% in the stroma [18,19]. By contrast, a roughly equal distribution of the labelled phosphate is detected in the stroma and the grana [18,19,22]. On the basis of these results the specific activity of phosphorylation of the 'mobile' population has been calculated to be between 4- and 10-fold higher compared with the 'non-mobile' population [18,19]. However, such calculations of differences in the specific activities

assume that all the LHC-II present in isolated thylakoids is phosphorylated.

We have previously shown that about 46% of the total phosphate incorporated into the LHC-II polypeptides in the unfractionated thylakoids remains associated with the grana after digitonin fractionation (see Table II in Ref. 22). These data would therefore be apparently in agreement with those previously reported [18,19], suggesting a difference in the specific activity of phosphorylation between the 'mobile' and the 'PS II-coupled' LHC-II sub-populations. However, the final extent of phosphorylation in this preparation was about 0.23 mol phosphate/mol LHC-II polypeptides and correction for the non-phosphorylated population suggests that there is little or no difference in the specific activity of phosphorylation between the two phosphorylated sub-populations. Further support for such an interpretation derives from the observations that the changes in the absorptive cross-sections of the photosystems are more or less completed in the first 5 min (Fig. 3 [21,22]) and that there is more than a 2-fold increase in the phosphate incorporated into the LHC-II polypeptides between 5 and 15 min (Fig. 2 [22]), but no alteration in the fraction of the label which remains associated with the grana at these two time-points (Table II in Ref. 22). Clearly, neither the 'mobile' nor the 'non-mobile' sub-populations is totally phosphorylated in the first 5 min and both the populations must be phosphorylated in a similar manner between 5 an 15 min for the distribution of the label between the grana and the stroma to remain unchanged.

It is therefore suggested that there are three distinct LHC-II populations in isolated thylakoids (a) the phosphorylated 'mobile' (b) the phosphorylated 'non-mobile' and (c) the non-phosphorylated. Only 20–30% of the total LHC-II present in the thylakoids is fully phosphorylated, while 70–80% cannot be phosphorylated. Furthermore, it is suggested that the two phosphorylatable sub-populations exhibit no differences in the specific activity of phosphorylation in our preparations, although whether the populations exhibit the same behavior in pea remains to be ascertained.

In addition the fluorescence decline is more or less completed when only some 5% of the 24 kDa and 3% of the 25 kDa polypeptide chains are phosphorylated (Figs. 2 and 3). The level of phosphorylation of the LHC-II polypeptide constituting the phosphorylated populations is therefore only about 0.25 and 0.12 mol phosphate/mol protein for the 24 kDa and 25 kDa polypeptides, respectively. It is generally thought that each LHC-II complex is composed of between 3–6 polypeptides [43,44] and the observed phosphorylation levels would be just sufficient to result in each complex containing at least one phosphorylated polypeptide.

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