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Use of Zinc Ions To Study Thylakoid Protein Phosphorylation and the State 1-State 2 Transition *In Vitro*[1]

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ABSTRACT

At ATP concentrations less than 0.2 millimolar, zinc ions cause a marked stimulation of endogenous protein phosphorylation in thylakoid membranes isolated from tobacco (*Nicotiana tabacum* L. cv Turkish Samsun), pea (*Pisum sativum* L. cv Feltham First) and spinach (*Spinacia oleracea* L. cv Northland). The greatest stimulatory effect was observed at Zn^{2+} concentrations of 1 to 2 millimolar; higher concentrations were inhibitory. The stimulatory effect of Zn^{2+} was independent of Mg^{2+} concentration from 1 to 5 millimolar and thus does not appear to be due to the formation of a Zn^{2+}-ATP complex. Phosphorylation of histones IIA, an exogenous protein substrate, was inhibited by 2 millimolar Zn^{2+}. At low levels of ATP, Zn^{2+} not only stimulates general endogenous protein phosphorylation, but also the phosphorylation of the apoproteins of the light-harvesting chlorophyll *a*/*b*-protein complex. However, under these conditions Zn^{2+} inhibits the ATP-induced quenching of photosystem II fluorescence and the increase in the ratio of photosystem I to photosystem II fluorescence which are both characteristic of the State 1-State 2 transition. These results suggest that phosphorylation of the light-harvesting chlorophyll *a*/*b*-protein complex may not directly bring about the State 1-State 2 transition.

Higher plants can regulate the distribution of harvested light energy to either PSI or PSII (6). Approximately half of the photosynthetic pigments are contained in the LHCP,[2] a family of pigment-protein complexes which comprise the major antenna system in the photosynthetic apparatus (24). The ability to alter the proportion of light energy directed from the LHCP to either PSI or PSII has been proposed to be an important mechanism for the optimization of the rate of photosynthetic electron transport (2) or in the regulation of steady state photosynthetic energy production (18). The process whereby the photosynthetic apparatus makes such changes in the distribution of light energy is termed the State 1-State 2 transition (6). It is now well established that this transition can be brought about by reversible phosphorylation of thylakoid proteins (2, 9-11, 22). The protein kinase and phosphatase activities that are involved in the phosphorylation and dephosphorylation of thylakoid polypeptides are integral membrane proteins (1, 8).

The apoproteins of the LHCP are the major substrates of the thylakoid protein kinase activity (1, 7). Several groups, having shown a correlation between phosphorylation of the LHCP apoproteins and the State 1-State 2 transition, suggested that LHCP phosphorylation is the specific mechanism that brings about the State 1-State 2 transition (2, 9, 12, 22). We have recently found that Zn^{2+} is capable of dramatically stimulating phosphorylation of the thylakoid membrane proteins (19). It would be expected that if phosphorylation of LHCP mediates the State 1-State 2 transition and Zn^{2+} stimulates this phosphorylation, then addition of Zn^{2+} to thylakoids should also stimulate the State 1-State 2 transition. We have examined the nature of the Zn^{2+} effect and report data which demonstrated that even though Zn^{2+} stimulates phosphorylation of the LHCP apoproteins, the State 1-State 2 transition is inhibited.

MATERIALS AND METHODS

Tobacco (*Nicotiana tabacum* L. cv Turkish Samsun), spinach (*Spinacia oleracea* L. cv Northland), and pea (*Pisum sativum* L. cv Feltham First) were grown from seed in a soil-vermiculite mixture in a glass house. Thylakoid membrane preparation from leaves and assay of total thylakoid protein kinase activity were as previously described (18, 19), except that the amount of labeled ATP in the assay was increased to 10 μCi.

Assessment of specific phosphorylation of the LHCP apoproteins was accomplished by halting the kinase assay by adding a final concentration of 3% (w/v) SDS. The samples, each containing 6 μg of Chl, were then electrophoretically fractionated on a 10% (w/v) acrylamide gel (15). Following electrophoresis, the gels were dried and the LHCP apoproteins, two closely migrating phosphoproteins of M_r approximately 28,000, were located by autoradiography. The ^{32}P-labeled LHCP bands were excised and the amount of phosphate incorporated into them was measured from Cerenkov radiation in a scintillation counter. Assessment of phosphorylation of individual thylakoid polypeptides was carried out using the assay conditions described above, except that the Chl concentration in each 0.1 ml incubation volume was increased to 50 μg and each contained 200 μCi of [$\hat{\gamma}$-^{32}P] ATP. Incubations were terminated after 3 min with 3% (w/v) SDS, and duplicate samples were electrophoretically fractionated on gels containing 7.5 or 12.5% (w/v) acrylamide and processed as above. Use of these two acrylamide concentrations facilitated fractionation of high and low mol wt polypeptides, respectively. Addition of labeled ATP following treatment of the membranes with SDS resulted in no detectable labeling of the fractionated

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[2] Abbreviation: LHCP, light-harvesting chlorophyll *a*/*b*-protein complexes.

polypeptides.

The ability of ATP to induce the State 1-State 2 transition was assessed by monitoring the 685 nm fluorescence at 20°C (10) using conditions identical to those in the protein kinase assay except that the Chl concentration was reduced to 10 μg ml^{-1}, no labeled ATP was included, and 1 μM nigericin was added. Fluorescence was excited with 112 μE s^{-1} m^{-2} of broad band (<500 nm) blue light in a stirred cuvette and monitored through a 685 nm interference filter (11.5 nm bandwidth) using a Hamamatsu R446 photomultiplier tube. Thylakoids were preincubated in the light for 3 min at 20°C prior to addition of ATP. The ATP-induced decrease in 685 nm fluorescence was measured 4 min after adding ATP. Under these conditions, the maximum amount of quenching observed was approximately 52% of the initial fluorescence; the concentration of ATP which produced half the maximal amount of quenching was 0.25 mM.

Simultaneous room-temperature measurement of fluorescence emission at 685 and 740 nm (14) was made using two of the light pipes in a trifurcated fiber optic system. The third pipe was used to transmit excitation radiation produced by a helium-neon laser (Spectra-Physics) through a 632.8 nm interference filter (Ealing). The photon flux density was 100 μE s^{-1} m^{-2}. Fluorescence was measured through 685 nm and 740 nm interference filters (Ealing). The change in the ratio of 740 to 685 nm fluorescence was continuously monitored for 7 min following the addition of ATP. The change is expressed relative to the ratio prior to ATP addition. Chl concentrations were determined by the method of Arnon (3). [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from Amersham.

RESULTS

We previously reported (19) that Zn^{2+} could stimulate endogenous tobacco thylakoid protein phosphorylation. We now report the effect of Zn^{2+} on the thylakoid protein kinase activity in three species. The phosphorylation of endogenous thylakoid polypeptides was investigated as a function of Zn^{2+} concentration at low (0.05 mM) or high (0.5 mM) levels of ATP. The Mg^{2+} concentration was maintained at 5 mM. The stimulatory effect of Zn^{2+} on endogenous protein phosphorylation was concentration-dependent (Fig. 1). At the low ATP concentration, the rate of protein phosphorylation was increased with increasing Zn^{2+} to approximately 2 mM, whereas higher concentrations of Zn^{2+} were inhibitory. The Zn^{2+} concentration that produced the optimal rate of protein phosphorylation was somewhat different between tobacco, pea, and spinach (Fig. 1A). In contrast, the addition of Zn^{2+} at the high ATP concentration (Fig. 1B) produced only inhibition of protein phosphorylation at Zn^{2+} concentrations of 1 mM and higher. Calcium ions at similar concentrations produced no stimulation, but rather increasing amounts of inhibition with increasing concentrations of ATP (19). Similarly, Cd^{2+} and Mn^{2+} also failed to stimulate thylakoid protein kinase activity and were much more inhibitory than Ca^{2+} (data not shown). Thus, the effects observed with Zn^{2+} appear to be relatively specific for that ion and not due to a general effect of divalent cations.

Since the usual substrate of the thylakoid protein kinase activities is an ATP-Mg^{2+} complex, it is possible that such changes in the phosphorylation rate could be caused by the formation of ATP-Zn^{2+} complexes in the assay mixture. This possibility was examined by increasing the Zn^{2+} concentration at 0.05 mM ATP in the presence of 1, 2.5, or 5 mM Mg^{2+} (*i.e.* a Mg^{2+} concentration 20-, 50-, or 100-fold greater than the ATP concentration). Increasing Mg^{2+} increased the control rate of endogenous protein phosphorylation as previously reported (17, 18) but had no obvious effect on the Zn^{2+}-induced changes on protein phosphorylation (Fig. 2).

As shown above (Fig. 1), the effect of Zn^{2+} on the thylakoid protein kinase activity was different at high and low ATP concentrations. We examined the effect of 2 mM Zn^{2+} at varying ATP concentrations from 0.025 to 0.5 mM. Phosphorylation of endogenous thylakoid proteins (Fig. 3) is stimulated by 2 mM Zn^{2+} at ATP concentrations of less than approximately 0.2 mM and inhibited at higher concentrations. The amount of Zn^{2+}-induced increase in protein phosphorylation is approximately 5-fold at 0.025 mM ATP, the lowest concentration tested. The effect of 2 mM Zn^{2+} on phosphorylation of specific proteins, the apoproteins of the LHCP, is shown in Figure 4. The results are similar to total protein phosphorylation in that the incorporation of phosphate into the LHCP is stimulated below, and inhibited above, ATP concentrations of 0.2 mM. The specific phosphorylation of the LHCP apoproteins was stimulated approximately 4-fold by 2 mM Zn^{2+} at 0.025 mM ATP.

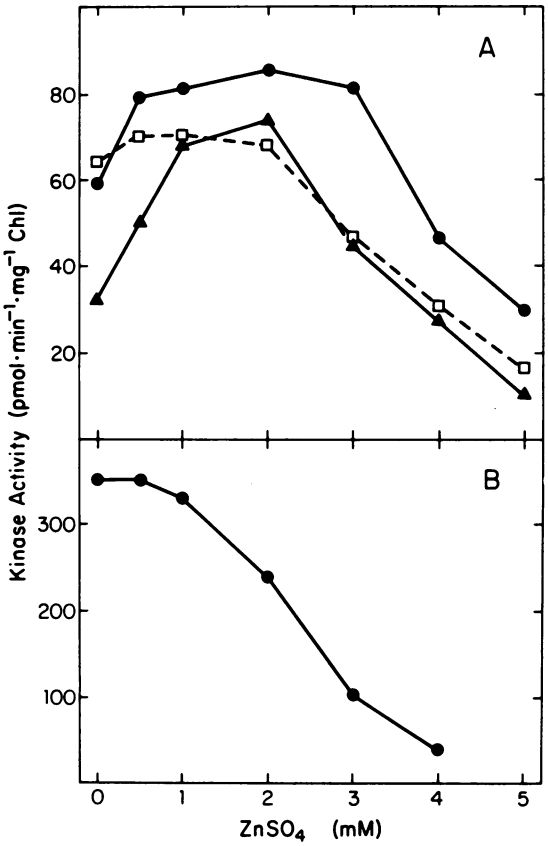

FIG. 1. Effect of $ZnSO_4$ concentration on phosphorylation of total thylakoid polypeptides. Assay components included 5 mM $MgSO_4$ and either 0.05 mM (A) or 0.5 mM (B) ATP. Thylakoid membranes were prepared from tobacco (▲), pea (□), and spinach (●).

The thylakoid membrane contains at least three distinct protein kinase activities (1, 16, 17, 19), and each activity may have a different specificity with respect to its protein substrates (16). We therefore investigated what effect Zn^{2+} has on phosphorylation of specific proteins other than the LHCP apoproteins. Histones have been used as an exogenous substrate although they do not appear to be substrates for all of the kinases (1, 16, 19). Histones (3 mg ml^{-1}) were added to the standard kinase assay containing a reduced amount of thylakoid membranes to enhance the observation of exogenous protein phosphorylation. The addition of 2 mM Zn^{2+} to histone-containing assays inhibited protein phosphorylation at all ATP concentrations tested (Fig. 5).

In order to allow greater sensitivity than in the above assays, the specific radioactivity of the ATP used in the labeling was increased. The labeled thylakoid polypeptides thus produced

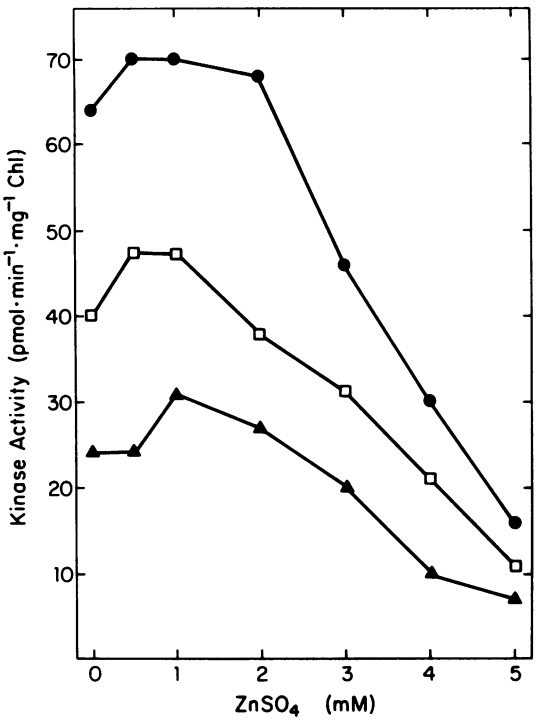

FIG. 2. Effect of $ZnSO_4$ concentration on phosphorylation of pea thylakoid membrane polypeptides. The assays contained 1 mM (▲), 2.5 mM (□), or 5 mM (●) $MgSO_4$.

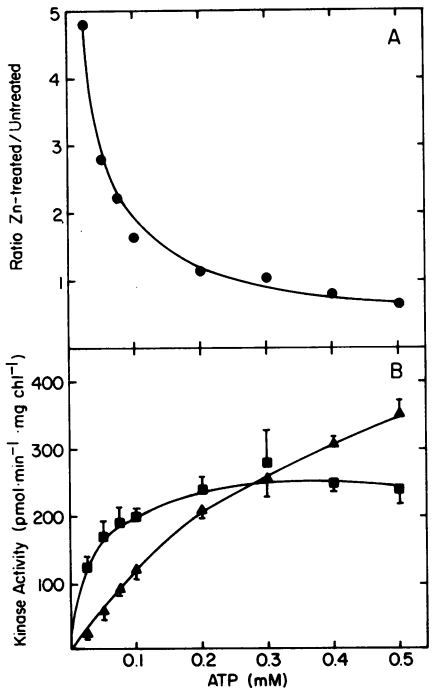

FIG. 3. Effect of 2 mM $ZnSO_4$ on phosphorylation of total endogenous thylakoid proteins as a function of ATP concentration. (▲), no $ZnSO_4$ added; (■), 2 mM $ZnSO_4$ included; (○), ratio of the rates of phosphorylation in the presence and absence of included $ZnSO_4$. The SE of the triplicate determination is indicated by the bars.

were fractionated by polyacrylamide gel electrophoresis and the amount of phosphate incorporated into the various polypeptides quantitated. Such techniques revealed at least 20 labeled polypeptides as shown for tobacco thylakoids in Figure 6. The amount of phosphate incorporated into the major labeled polypeptides is summarized for tobacco (Table I) and pea (Table II) thylakoid membranes. Although there is some variability from experiment to experiment, it can be seen that some polypeptides respond similarly to the LHCP apoproteins (M_r approximately 28,000) in that Zn^{2+} stimulates phosphate incorporation at the low ATP concentration and inhibits at the high concentration. However, the phosphorylation of some polypeptides (*e.g.* M_r = 58,000) are inhibited by or at least show a lack of stimulation by

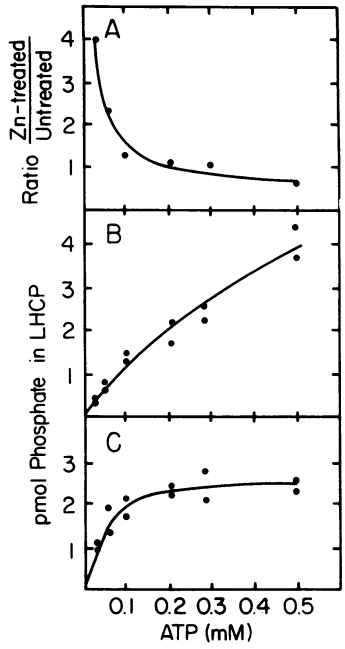

FIG. 4. Effect of 2 mM $ZnSO_4$ on phosphorylation of the LHCP apoproteins as a function of ATP concentration. A, the ratio of phosphate incorporated into the LHCP apoproteins in the presence and absence of added $ZnSO_4$. B, the amount of phosphate incorporation into the LHCP apoproteins during 2 min in the absence of included $ZnSO_4$. C, the amount of phosphate incorporation into the LHCP apoproteins during 2 min in the presence of included $ZnSO_4$. The data in B and C represent the results obtained with duplicate samples.

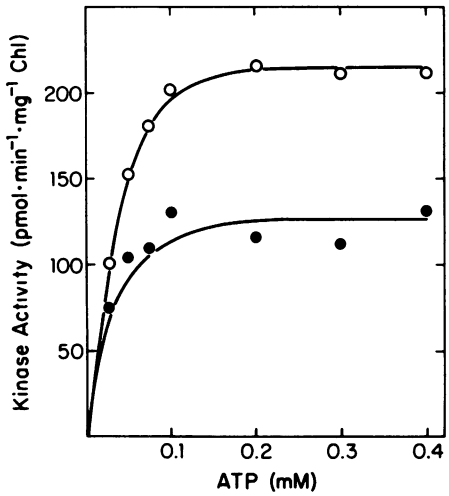

FIG. 5. Effect of 2 mM $ZnSO_4$ on tobacco thylakoid protein kinase activity measured in the presence of 3 mg ml^{-1} of histones IIA. The concentration of thylakoid membranes in the assay was reduced to 0.1 mg Chl ml^{-1} to facilitate observation of exogenous protein phosphorylation. Assay components included 5 mM $MgSO_4$. (○), no $ZnSO_4$ added; (●), 2 mM $ZnSO_4$ included.

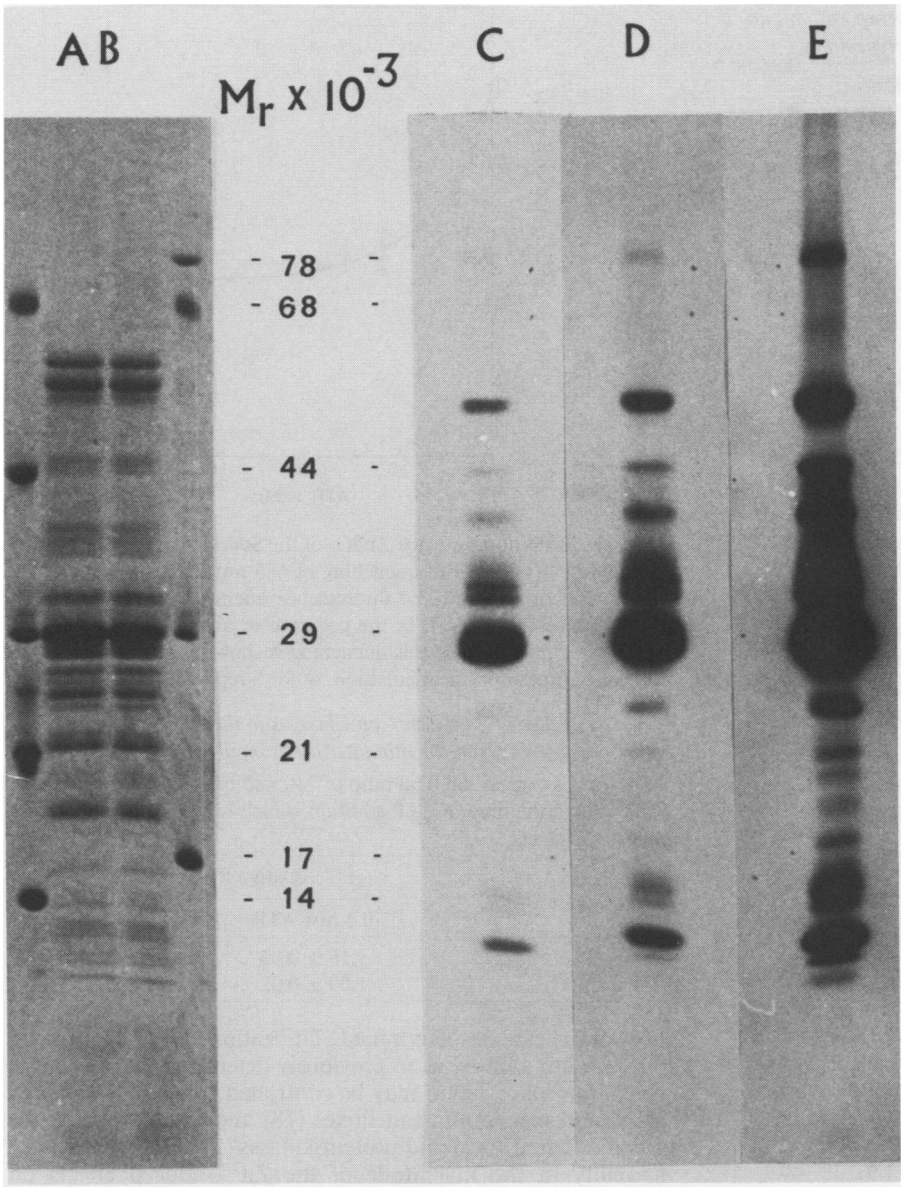

FIG. 6. Fractionation of ^{32}P-labeled tobacco thylakoid polypeptides by polyacrylamide (7.5 to 15% gradient) gel electrophoresis. A and B, gel with labeled and unlabeled membranes, respectively, stained with Coomassie brilliant blue R-250 to visualize the polypeptides. B, C, and D, visualization of the phosphoproteins by autoradiography for 2, 4, or 16 h, respectively. Numbers refer to the M_r values of marker proteins with known molecular weights.

addition of Zn^{2+}, at the low ATP concentration.

The ATP-induced quenching of PSII fluorescence from uncoupled thylakoids has been accepted to indicate a transition from State 1 to State 2 (9, 10, 12). The effect of 2 mM $ZnSO_4$ on ATP-induced 685 nm fluorescence quenching at various ATP concentrations is shown in Figure 7. In contrast to the Zn^{2+} effects on the kinase activity, PSII fluorescence quenching was inhibited by Zn^{2+} at all ATP concentrations examined. To confirm that this Zn^{2+}-induced inhibition of PSII fluorescence quenching did indeed reflect a diminished redistribution of captured quanta from PSII to PSI, the change in the ratio of 740:685 nm fluorescence emission following ATP addition (14) was monitored in the presence and absence of 2 mM Zn^{2+} (Table III). The data are reported as the change in the ratios of 740:685 nm fluorescence emission 7 min after ATP addition and are indicative of the ratio of PSI to PSII fluorescence emission (14). However, the fluorescence signals were monitored continuously during the 7-min period, and the ratios reported represent the trend of the data for all times during the measurement. Addition of either 0.1 or 1.0 mM ATP caused an increase in the ratio of PSI:PSII fluorescence and demonstrated a redistribution of absorbed quanta in favor of PSI (14). The increase in the PSI:PSII fluorescence ratio was inhibited by Zn^{2+} at both ATP concentrations demonstrating that Zn^{2+} inhibits the ATP-induced redistribution of quanta from PSII to PSI. The degree of Zn^{2+} inhibition of this phenomenon was considerably greater at the higher ATP concentration. The Zn^{2+}-induced inhibition observed at 0.1 mM ATP was reproducible, even though small. Clearly, the effect of Zn^{2+} on phosphorylation of the LHCP apoproteins does not seem to parallel the Zn^{2+}-induced modification of the State1-State 2 transition at low ATP concentrations.

DISCUSSION

The effects of Zn^{2+} on protein phosphorylation in thylakoid membranes of higher plants are complex. The type and magnitude of the effect that Zn^{2+} produces are very much dependent on its own concentration as well as that of ATP. At 0.05 mM ATP the maximum amount of stimulation of endogeneous protein phosphorylation is observed at approximately 2 mM Zn^{2+}, whereas higher concentrations are inhibitory. In contrast, Zn^{2+} concentrations above 0.5 mM inhibited endogenous protein phosphorylation when monitored using 0.5 mM ATP. The exact magnitude of change produced by Zn^{2+} addition at any given ATP concentration was somewhat variable between experiments,

Table I. *Effect of 2 mM $ZnSO_4$ on Phosphate Incorporation into Various Tobacco Thylakoid Membrane Polypeptides*

$M_r \times 10^{-3}$	Incorporation			
	50 μM ATP		500 μM ATP	
	−Zn	+Zn	−Zn	+Zn
	pmol $PO_4 \cdot min^{-1} \cdot mg^{-1}$ Chl			
Experiment 1[a]				
58	2.02	1.76	1.76	1.12
46	0.69	0.69	2.13	<0.2
42	0.53	0.64	1.92	0.3
35	1.76	2.24	9.44	2.66
33	3.62	4.05	18.8	5.01
29 + 27[c]	41.2	51.4	131	50.7
Experiment 2[b]				
58	3.09	3.15	5.60	1.86
46	0.90	0.96	1.23	<0.2
42	1.06	1.23	3.41	0.90
35 + 33[d]	4.16	4.59	17.3	8.53
29 + 27[c]	65.5	92.7	209	139
9	5.60	6.35	25.9	15.7

[a] Proteins were fractionated on 7.5% acrylamide gels.
[b] Proteins were fractionated on 12.5% acrylamide gels.
[c] The closely migrating apoproteins of the LHCP.
[d] These phosphoproteins were not sufficiently resolved to permit independent quantitation.

Table II. *Effect of 2 mM $ZnSO_4$ on Phosphate Incorporation into Various Pea Thylakoid Membrane Polypeptides*

$M_r \times 10^{-3}$	Incorporation			
	50 μM ATP		500 μM ATP	
	−Zn	+Zn	−Zn	+Zn
	pmol $PO_4 \cdot min^{-1} \cdot mg^{-1}$ Chl			
Experiment 1[a]				
58	3.68	3.34	2.14	2.79
50	1.09	1.25	10.3	4.58
44	5.75	10.6	21.5	20.3
37	5.55	7.10	41.7	26
29[c]	111	146	295	242
21	1.17	2.09	1.91	<0.7
Experiment 2[b]				
58	5.45	4.53	3.9	3.36
50 + 44[d]	8.05	8.65	42.9	27.8
37	10.85	15.2	52.5	39.6
29[c]	100	196	398	277
21	1.20	1.64	1.77	1.53
16.5	0.36	1.32	2.96	0.85
13.5	3.19	6.45	9.40	12.8
10.5	3.84	7.80	20.6	23.3
9	1.04	0.88	<0.7	<0.7

[a] Proteins were fractionated on 7.5% acrylamide gels.
[b] Proteins were fractionated on 12.5% acrylamide gels.
[c] The closely migrating apoproteins of the LHCP.
[d] These phosphoproteins were not sufficiently resolved to permit independent quantitation.

but the direction of the change (*i.e.* increase or decrease) was consistent and reproducible. Under the assay conditions used, the rate of phosphate incorporation from labeled ATP into protein was linear with time for approximately 5 min. Addition of Zn^{2+} did not affect the phosphate incorporation at zero time, but modified the rate of phosphate incorporation. By labeling for only 2 min the effect of protein phosphatase activity is minimized (19), and the initial rate of thylakoid protein phosphorylation can be determined. Utilization of such a kinetic approach has allowed us to previously determine that thylakoid protein phosphorylation may be controlled by adenylate energy charge and magnesium ion fluxes (18) and that there may be several different thylakoid protein kinases (19). We feel that the variability in the magnitude of the Zn^{2+}-induced effects on thylakoid protein kinase activity was not due to the assay procedure, but rather to changes in the plant materials used in these studies. Plant material was grown in a glasshouse, and the temperature varied considerably (between approximately 15 and 32°C) during the several months over which these experiments were conducted. The protein kinase activities appear to be labile *in vitro* at temperatures as low as 38°C (19), and the relative amount of each of the various protein kinase activities may be strongly influenced by environmental conditions such as ambient temperature during growth.

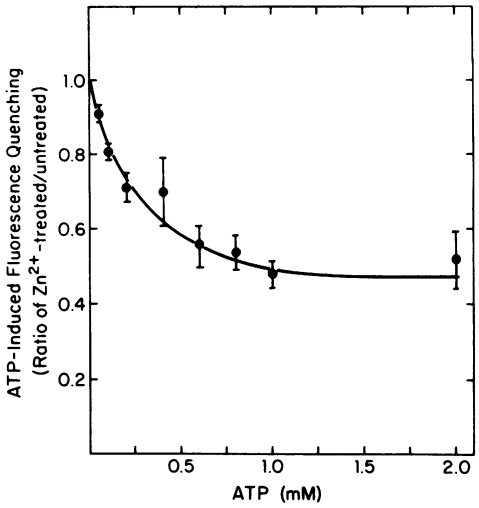

FIG. 7. Inhibition by 2 mM $ZnSO_4$ of the State 1-State 2 transition as measured by ATP-induced quenching of 685 nm fluorescence at 20°C. The amount of ATP-induced fluorescence quenching was measured 7 min after addition of ATP in the presence or absence of $ZnSO_4$. The zinc-induced inhibition of this quenching is shown as a function of the ATP concentration in the incubation. SE for 5 replicates is indicated.

Table III. *Effect of 2 mM Zn^{2+} on Changes in the Ratio of 740:685 nm Fluorescence Measured 7 min after Addition of 0.1 or 1.0 mM ATP*

The values express the final ratio of 740:685 nm fluorescence relative to the ratio at the time of ATP addition, which is made 1.0. The SE of 5 replicates is given.

	Relative F740:F685	
	0.1 mM ATP	1.0 mM ATP
$-Zn^{2+}$	1.16 ± 0.03	1.58 ± 0.11
+2 mM Zn^{2+}	1.09 ± 0.02	1.12 ± 0.03

The thylakoid protein kinase activity responds to the redox state of the plastoquinone pool. Captured quanta of light drive the photochemical reaction centers of PSII and PSI resulting in photosynthetic electron transfer and the reduction of plastoquinone molecules. Reduced plastoquinone activates the protein kinase activity (13); if the plastoquinone molecules are oxidized, the activity is inactivated with a half-time of 5 min (1). It could be argued that the effect of Zn^{2+} on the protein kinase activity results from its direct action on photosynthetic electron transfer (4). However, the thylakoid protein kinase was activated by light treatment of the thylakoid membranes in the absence of Zn^{2+} prior to addition to the assay medium. A short labeling period (2 min) was also employed to minimize any such problems. The protein kinase activity is stimulated by Mg^{2+} at concentrations

manyfold greater than the ATP concentration (17, 19). This response has been proposed to function as part of a physiological control mechanism to enable the kinase to mediate regulation of photosynthetic energy production (18). The effects of Mg^{2+} and Zn^{2+} appear to be independent of each other (Fig. 2) suggesting that the two cations may exert their respective effects at separate sites. It is also suggested that the effect of Zn^{2+} is not brought about by the formation of a stimulating or inhibiting Zn^{2+}-ATP complex since increasing Mg^{2+} did not appear to diminish the Zn^{2+}-induced changes in thylakoid protein phosphorylation.

The ability of 2 mM Zn^{2+} to stimulate endogenous protein phosphorylation at low ATP concentrations is general for thylakoid phosphoproteins collectively (Fig. 3) and also specifically for the LHCP apoproteins (Fig. 4). In contrast, the phosphorylation of exogenous histones is inhibited by 2 mM Zn^{2+} at all ATP concentrations tested. The differences observed between endogenous and exogenous protein phosphorylation may be interpreted as being due to the presence of a number of kinase activities with different protein specificities and affinities for ATP (1, 16, 17, 19). A Zn^{2+}-stimulated kinase with high affinity for ATP (K_m perhaps 0.025 mM, *cf.* 17) and a Zn^{2+}-inhibited kinase with a lower affinity for ATP (K_m greater than 0.2 mM) would explain the data. Since histones IIA are apparently phosphorylated by a Zn^{2+}-sensitive kinase with a high affinity for ATP (19 and Fig. 5), the histone kinase and the Zn^{2+}-stimulated kinase are separate activities, or the addition of Zn^{2+} is able to alter the specificity of the kinase(s) between exogenous and endogenous protein substrates. This notion of different kinases with high and low affinities for ATP and differing protein specificity is supported by the data in Tables I and II. The amount of phosphate incorporated into some polypeptides (*e.g.* the LHCP apoproteins) increases dramatically from 0.05 to 0.5 mM ATP in the absence of Zn^{2+} as expected for a low affinity enzyme. In contrast, other membrane polypeptides (*e.g.* M_r = 58,000) show little or no increase in phosphate incorporation.

There is currently much interest in the mechanism whereby harvested quantal energy is distributed between PSI and PSII. Thylakoid protein phosphorylation is correlated with the State 1-State 2 transition (2, 9, 10, 11, 22), and a number of reports conclude that phosphorylation of the LHCP apoprotein is the mediating mechanism (2, 9, 12, 22). This hypothesis was derived from the apparent lack of detectable State 1-State 2 transition in the chlorina-f2 mutant of barley (*Hordeum vulgare*) lacking the LHCP (10, 12, 23). However, the involvement of changes in membrane surface charge density (6) brought about by general protein phosphorylation, or the specific phosphorylation of some minor component, has not been conclusively excluded. Additionally, a mutant of *Scenedesmus obliquus* has recently been described (21) which is lacking the LHCP, but is still able to carry out the State 1-State 2 transition.

The ability of nonphysiological concentrations of Zn^{2+} to influence the phosphorylation of endogenous thylakoid proteins provides a probe with which to examine the role of phosphorylation of the LHCP in the State 1-State 2 transition. If, as proposed, phosphorylation of the LHCP is the mediating element in the State 1-State 2 transition, then addition of Zn^{2+} should cause parallel changes in the rate of the State 1-State 2 transition and phosphorylation of the LHCP. However, as judged from the ATP-induced quenching of PSII-derived fluorescence (Fig. 7) and changes in the ratio of PSI to PSII fluorescence emission (Table III), Zn^{2+} inhibited the State 1-State 2 transition at ATP concentrations where phosphorylation of the LHCP apoproteins was markedly stimulated. At the high ATP concentration, addition of 2 mM Zn^{2+} inhibited both LHCP phosphorylation and the State 1-State 2 transition.

The differential effect of Zn^{2+} on phosphorylation of the LHCP apoproteins and the State 1-State 2 transition at low ATP concentrations can be interpreted in several ways. It may be that the connection between LHCP phosphorylation and the State 1-State 2 transition is not direct. Phosphorylation of LHCP may be followed by one or more molecular events prior to initiation of the State 1-State 2 transition, and Zn^{2+} could act to uncouple the two phenomena from one another. Such a hypothesis is consistent with the observation that addition of cholesterol to thylakoid membranes inhibits the State 1-State 2 transition, presumably by decreasing membrane fluidity, without affecting LHCP phosphorylation (Philip Haworth, personal communication). Alternately, LHCP phosphorylation could be just one of a number of mechanisms responsible for the State 1-State 2 transition. Finally, it could be argued that it is phosphorylation of one or more proteins other than the LHCP apoproteins that bring about the quantal redistribution. A possible candidate for a phosphoprotein other than the LHCP apoproteins which might mediate the State 1-State 2 transition would be the M_r = 58,000 polypeptide. Phosphorylation of this polypeptide is not stimulated by Zn^{2+} at the low ATP concentration, and in this respect, it responds in the same manner as the State 1-State 2 transition. The M_r = 58,000 phosphoprotein is a peripheral antenna component of PSI (M. P. Skrdla, J. P. Markwell, and J. P. Thornber, in preparation) and thus could be well situated to mediate the State 1-State 2 transition. Arguments could also be made favoring the involvement of still other polypeptides, such as those of the PSII reaction center which are also phosphorylated (22).

In any event, the existence of multiple thylakoid protein kinases, some of which do not use the LHCP as substrate, indicates the thylakoid protein phosphorylation is probably involved in a number of different regulatory mechanisms. For example, phosphorylation of light-harvesting complexes may play roles in membrane structural organization (20), and thylakoid kinase activity may play an important role in chloroplast development and assembly of the thylakoid membrane (5).

In conclusion, this report shows that selected concentrations of Zn^{2+} and ATP can markedly stimulate phosphorylation of thylakoid proteins collectively and the LHCP apoproteins specifically. In contrast, identical conditions inhibit the State 1-State 2 transition. We suggest that the role of the LHCP phosphorylation in the State 1-State 2 transition should be reexamined and subjected to more intense experimental scrutiny.

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