Protein Kinases of the Thylakoid Membrane*

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The claim of Racker and co-workers (Lin, Z. F., Lucero, H. A., and Racker, E. (1982) J. Biol. Chem. 257, 12153–12156 and Lucero, H. A., Lin, Z. F., and Racker, E. (1982) J. Biol. Chem. 257, 12157–12160) that two protein kinases, designated CPK₁ (25 kDa) and CPK₂ (38 kDa), are present in spinach thylakoid membranes was investigated in light of results from this laboratory (Coughlan, S. J., and Hind, G. (1986) J. Biol. Chem. 261, 11378–11385) showing that 75–80% of the measurable protein kinase activity of isolated thylakoids is attributable to a protein kinase of 64 kDa apparent molecular mass.

Extraction of thylakoid membranes with octyl glucoside/cholate according to the procedure of Lin et al. (Lin, Z. F., Lucero, H. A., and Racker, E. (1982) J. Biol. Chem. 257, 12153–12156) released proteins assignable to CPK₁ and CPK₂ on the basis of photoaffinity labeling with 8-azido-[32P]ATP. The 64-kDa protein kinase was present in this extract and accounted for >80% of the total phosphotransferase activity towards lysine-rich histone as substrate; it was not labeled by the photoaffinity reagent. The three presumptive kinases were purified by ammonium sulfate precipitation, sucrose density gradient centrifugation, hydroxyapatite chromatography, and affinity chromatography. CPK₁ was specifically eluted from Cibacron blue-Sepharose by 10 mM ATP; it electrophoresed on denaturing polyacrylamide gels as a single band with apparent molecular mass of 25 kDa. Its specific activity toward lysine-rich histone as substrate was -250 pmol of phosphate transferred (mg protein)⁻¹ min⁻¹. The 64-kDa protein kinase was eluted from the affinity column by 1% (w/v) lithium dodecyl sulfate or from a histone III-δ-Sepharose affinity column by 0.25 M NaCl. Its specific activity towards lysine-rich histone was 100–200 times greater than that of CPK₁. CPK₂ eluted from the Cibacron blue affinity column in 10 mM NADP⁺; it had an apparent molecular mass of 38 kDa, possessed NADP-dependent diaphorase activity (specific activity: 225 nmol of ferricyanide reduced (mg protein)⁻¹ min⁻¹), and was cross-reacted with immunoglobulin raised against purified ferredoxin:NADP⁺ oxidoreductase, with which it was thus identified. Kinase activity was not detectable in CPK₃ or in reduced isolated by conventional procedures.

Protein kinases catalyze the transfer of the γ-phosphate of ATP or certain other trinucleotides to serine, threonine, or tyrosine residues in proteins (1, 2). These enzymes are intimately involved in the control of metabolism in animal cells (3). Relatively little is known about the properties and functions of protein kinases in plants (4, 5), with one notable exception: the well-documented effectuation of State transitions in the thylakoid by reversible phosphorylation of LHC.¹

The responsible protein kinase is associated with the thylakoid membrane (5) and is regulated by the redox status of the plastoquinone pool (6).

Lin et al. (7) and Lucero et al. (8) reported the isolation and partial purification of two protein kinases from spinach thylakoid membranes. One had an apparent molecular mass of 25 kDa (CPK₁) and the other 38 kDa (CPK₂), as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Since neither enzyme was able to utilize LHC as a substrate, the discovery of a third kinase was anticipated.

This enzyme has now been isolated and purified (9, 10). It has an apparent molecular mass of 64 kDa and phosphorylates isolated LHC at a modest rate (9, 10). Evidence for the existence of CPK₁ and CPK₂ was not obtained in these studies, even though the isolation protocols were similar (detergent solubilization of the thylakoid membrane by octyl glucoside/cholate). In order to reconcile the two bodies of data, we examined thylakoid membranes, octyl glucoside/cholate extracts, and partially or completely purified substrates with special attention to the reliability of 8-azido-[32P] ATP in detecting each of the three putative kinases. CPK₁ and CPK₂ were also purified to homogeneity for the first time, by applying affinity column chromatography to the final products from the procedure of Lin et al. (7).

EXPERIMENTAL PROCEDURES

Preparation and Extraction of Thylakoid Membranes—Spinach thylakoids were prepared as previously described (10) with omission of the wash in 2 M NaBr. A concentrated suspension of membranes (4 mg chlorophyll/ml) was treated with dithiothreitol, extracted with octyl glucoside/cholate, and centrifuged, followed by precipitation of the supernatant with ammonium sulfate as in Lin et al. (7). The precipitate from 55% (saturation) ammonium sulfate and is regulated by the redox status of the plastoquinone pool (6).

Sucrose Density Gradient Centrifugation—15 mg of protein (1 ml) were dialyzed for 4 h against 1 liter of medium at 0°C. The desalted extract (1–2 ml) was applied to the top of a 10–30% (w/v) sucrose gradient (12 ml). After centrifugation in an IEC SB-283 swinging bucket rotor for 14 h at 140,000 x g, 0°C (IEC B60 ultracentrifuge), 28 fractions (0.5 ml) were collected dropwise from the bottom of the gradient.

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¹The abbreviations used are: LHC, light-harvesting chlorophyll a/b protein complex of photosystem II; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; CPK₁, chloroplast protein kinase 1 and 2; Mes, 2-(N-morpholino)ethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; Tricine, N-tris(hydroxymethyl)methylglycine.

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the bottom of the tube. The pellet was redissolved in 0.5 ml of 0.1% Triton X-100, 10 mM sodium Tricine (pH 8.0). Samples were frozen and stored as above.

Hydroxylapatite Chromatography—The peak of kinase activity was pooled (1.8 mg of protein in 1.2 ml) and adsorbed onto a 1-ml hydroxylapatite column (0.5 x 6 cm) previously equilibrated with 10 mM octyl glucoside, 0.1% cholate, 0.5 mM dithiothreitol, 0.1 mM PMFS, 10 mM sodium Tricine (pH 8.0). The column was washed with 2 ml of equilibration medium and then eluted with a linear 0–0.5 M KH2PO4 gradient (14 ml) in medium (flow rate, 5 ml/h). 28 fractions of 0.5 ml were collected and dialyzed overnight against 2 liters of 0.1% cholate, 0.1 mM ATP, 0.5 mM dithiothreitol, 0.5 mM EDTA, 0.1 mM PMFS, 10 mM sodium Tricine (pH 8.0).

Photoaffinity Labeling of Membranes with 8-Azido-[γ-32P]ATP—Samples were incubated with 4 μCi of 8-azido-[γ-32P]ATP, radiolabeled in the α or γ position, for 5 min on ice in the dark, and then were irradiated for 10 min in the light from a General Electric germicidal lamp G15 T8 filtered through plate glass (4 J m⁻² s⁻¹ at the sample surface).

Analytical Techniques—Polyacrylamide gel electrophoresis was routinely performed in sodium dodecyl sulfate as in Ref. 11. Gels were fixed and stained with Coomassie Brilliant Blue (12) or Silver (13). Protein was determined according to Bradford (14) or when detergent would interfere, Bensadoun and Weinstein (15). Protein kinase activities (7) and ferredoxin:NADP⁺ oxidoreductase activity (16) were measured by standard procedures. Chlororhyt concentration was determined by the method of Arnon (17).

Materials—8-Azido-[γ-32P]ATP, specific activity 22 mCi/μmol, 300 μCi in 167 μl of MeOH, and 8-azido-[α-32P]ATP, specific activity 5.3 mCi/μl, 120 μCi in 370 μl of MeOH were obtained from ICN Tracerlab. Cytochrome c oligomers used as molecular mass markers were from U.S. Biochemical, other markers were from Bio-Rad. Octyl glucoside was obtained from Behring Diagnostics and other reagents from Sigma.

RESULTS AND DISCUSSION

When thylakoid membranes were photoreacted with 8-azido-[γ-32P]ATP and analyzed by denaturing gel electrophoresis and autoradiography, two strongly labeled peptides were revealed (Fig. 1). With reference to a mixture of cytochrome c oligomers as molecular mass markers, their relative masses were ~38 and 23 kDa. The control lane showing thylakoid peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide. Labeling of the 23-kDa peptides, however, suggests a higher mass for the latter, close to that of the 25-kDa LHC peptide.

In the converse experiment, shown in Fig. 4, P300 eluate was fractionated at the outset by density gradient centrifugation, then individual fractions were photoreacted with 8-azido-[α-32P]ATP and analyzed as before. The principal labeling in this case was of the 23-kDa peptide (lanes 10–15) and the 38-kDa peptide (lanes 10–13). Again, this labeling required light and was prevented by prior addition of 1 mM ATP or of 1 mM 8-azido-ATP (not shown). The 38-kDa peptide was identified as ferredoxin:NADP⁺ oxidoreductase on the basis of its copurification with diaphorase activity and immune blotting with rabbit immunoglobulin raised to the purified enzyme from spinach (data not shown). Peptides of 42, 34, and 32 kDa showed minor labeling; however, there was no evident incorporation of label into 19- or 16-kDa components. No labeling of the cytochrome b/f complex was found. Comparable data were obtained in experiments corresponding to Figs. 2 and 4, in which the position of the radiolabel was interchanged or the temperature during incubation varied between 0 and –196 °C. None of the labeled peptides corresponded to the peak of histone kinase activity (not shown) centering upon fraction 10; furthermore, there was no measurable incorporation of radiolabel from either 8-azido-[α-32P]ATP or 8-azido-[γ-32P]ATP into a 64-kDa peptide previously identified as an active protein kinase (10).

A direct attempt was made to isolate and purify the two
FIG. 2. Photoaffinity labeling of P300 eluate with 8-azido-[γ-32P]ATP. Analysis of labeled peptides by autoradiography of gradient fractions resolved on a denaturing polyacrylamide gel. 5 μl of 8-azido-[γ-32P]ATP in methanol was blown to dryness under argon (1 min, in dark, on ice). P300 eluate (1.6 nmol of cytochrome f (230 pg of protein) in 50 μl) was added to the sample and the sample was immediately added and the sample was irradiated as described under “Experimental Procedures.” ATP (1 nmol) was added then the sample was desalted through a 1-ml Sephadex G-75 column (6 × 0.3 cm) equilibrated with 10 mM CHAPS, 10 mM sodium Mes (pH 6.5). A further 150 μl of untreated P300 eluate was added, and the mixture was analyzed on a sucrose density gradient as described under “Experimental Procedures,” except that the gradient was prepared and centrifuged as in Ref. 10. Aliquots (30 μl) of each 500 μl of gradient fraction were mixed with 30 μl of buffer containing 4% sodium dodecyl sulfate and electrophoresed as described under “Experimental Procedures” and Fig. 1. Lane 0, pellet; lanes 1-16, gradient fractions (bottom to top). The molecular mass markers were lysozyme (14.4 kDa), soybean trypsin inhibitor (21.5 kDa), carbonic anhydrase (31.0 kDa), ovalbumin (45.0 kDa), serum albumin (66.2 kDa), and phosphorylase B (92.5 kDa).

FIG. 3. Photoaffinity labeling of P300 eluate with 8-azido-[γ-32P]ATP, followed by sucrose density gradient fractionation. Silver stained electrophoresis of gradient fractions. Experimental conditions were as given for Fig. 2.

As previously reported (7, 9, 10), considerable loss of total kinase activity occurs on solubilization of the thylakoid membrane in detergent, owing to the inhibitory effect of octyl glucoside. Although this makes precise quantification of the yield difficult, it is evident (Table I) that no measurable protein kinase activity remained in the detergent-insoluble membrane fragments pelleted by ultracentrifugation. Solubilized kinase activity was precipitated by 55% (saturation) ammonium sulfate (Table I). Separation of kinase activity from the coupling factor complex was effected by sucrose density gradient centrifugation (not shown), as originally reported (7). Fractions containing the peak of kinase activity and coincident diaphorase activity were pooled and examined by denaturing gel electrophoresis (Fig. 6, lane A), which revealed numerous peptide components.

This material was adsorbed on hydroxylapatite and eluted with a linear 0-0.5 M phosphate gradient (7). All the protein was initially bound to the column. Almost half of the protein eluted at 50 mM phosphate (Figs. 5 and 6, lane 5), together with most of the kinase activity, the 32-kDa peptide as the major band staining with Coomassie Blue, and numerous other peptides present in minor amounts (Fig. 6). The 38- and 16-kDa peptides eluted at 150 and 300 mM phosphate, respectively, the former being associated with a peak in diaphorase activity.
Fig. 4. Photoaffinity labeling of individual sucrose gradient fractions with 8-azido-[α-32P]ATP. Autoradiograph of fractions after denaturing electrophoresis. Aliquots (25 μl) of gradient fractions prepared as in Fig. 2 were reacted with 5 μCi (640 pmol) of 8-azido-[α-32P]ATP in 5 μl of 0.1 M EDTA. Samples were irradiated as described under “Experimental Procedures”; 1 nmol of ATP was added, followed by trichloroacetic acid (25% (w/v) final concentration). After 30 min on ice, the samples were centrifuged (12,000 × g, 10 s); the pellets were rinsed with 250 μl of 5% (w/v) trichloroacetic acid and prepared for electrophoresis by dissolving in 40 μl of the medium given in Fig. 1, supplemented with 10 μl of 1 M Tris chloride (pH 8.0).

**Table I**

| Fraction                     | Volume (ml) | Protein (mg) | Diaphorase* | Histone III protein kinase |
|------------------------------|-------------|--------------|-------------|---------------------------|
|                              |             |              | Total activity | Total activity[^h] | Specific activity[^h] | Yield[^g] | Purification[^h] |
| Thylakoids                   | 270         | 600          | 2,500        | 19                        | 32                   | 100       | 1               |
| Octylglucoside/cholate extract | 80         | 500          | 3,200        | 0.5                      | 1                    | 2.6       |
| Supernatant                  | 75          | 105          | 2,925        | 0.5                      | 1                    | 2.6       |
| Pellet                       | 400         | 100          | 0            |                           |                      |           |
| (NH₄)₂SO₄ fractions:         |             |              |              |                           |                      |           |
| 0-60%                        | 17          | 100          | 2,400        | 6-8                       | 60-80                | 32-42     | 2               |
| 60-90%                       | 2           | 1            | 90           | 0.2                       |                      |           |
| Sucrose gradient fractions 18–21 | 2.4        | 3.6 (15 mg loaded) | 100 (100 units loaded) | 0.9 (0.9 units loaded) | 250       | (100) | 8 (1) |
| Hydroxylapatite fractions 5–7 | 1.2        | 1.8 (50%)[^h] | 40 (40%)[^h] | 0.6                       | 333                   | (66)      | 10.4 (1.3) |
| Cibacron Blue fractions:     |             |              |              |                           |                      |           |
| (1) Throughput and washings  | 5.0         | 1.5          | 0            | 0                         | 0                     |           |
| (2) NADP                     | 1.0         | 0.1          | 35           | 0                         | 0                     |           |
| (3) ATP                      | 1.0         | 0.12         | 0            | 0.10                      | 830                   | (17)      | 26 (3.3) |
| (4) LiDS[^f]                 | 1.0         | 0.08         | 0            | 0                         | 0                     |           |
| Histone III fractions:       |             |              |              |                           |                      |           |
| (1) Throughput and washings  | 5.0         | 1.7          | 40           | 0.10                      |                      |           |
| (2) 0.25 M NaCl[^g]          | 1.0         | 0.02         | 0            | 0.5                       | 25,000                | (56)      | 781 (100) |

* nmol DCPI reduced min⁻¹.
[^h] nmol PO₄ transferred min⁻¹.
[^i] nmol PO₄ transferred (mg protein)⁻¹ min⁻¹.
[^g] Normalized against thylakoid activity, the values in parentheses are normalized against sucrose gradient fractions 18–21.
[^f] Yield normalized against the amount of protein and enzymatic activity loaded on the sucrose gradient.
[^e] Lithium dodecyl sulfate.
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Fig. 5. Purification of protein kinases by hydroxylapatite chromatography. Extraction of thylakoids with detergent, ammonium sulfate fractionation, sucrose density centrifugation were carried out as described under "Experimental Procedures." Fractions 18-21 from the density gradient were chromatographed on hydroxylapatite as described under "Experimental Procedures." $\bullet\bullet\bullet\bullet$, protein content (pg/fraction); $\bigodot\bigodot\bigodot\bigodot$, histone Vs kinase activity (pmol of phosphate transferred min$^{-1}$ fraction$^{-1}$). $\bigtriangleup\bigtriangleup\bigtriangleup\bigtriangleup$, ferredoxin:NADP$^+$ oxidoreductase activity (amol 2,6-dichlorophenolindophenol reduced min$^{-1}$ fraction$^{-1}$). $\Delta\Delta\Delta\Delta$, phosphate concentration (mM). Loadings (recoveries) were: protein, 1.8 (1.5) mg; histone kinase activity 0.4 (0.25) unit; reductase activity, 60 (58) units.

Fig. 6. Electrophoretic analysis of hydroxylapatite column fractions. Experimental procedures are given under "Experimental Procedures." Lane A, pooled sucrose gradient fractions 18-21; lanes 4-20, respective fractions eluted from hydroxylapatite by an increasing, linear phosphate concentration gradient.

Thus far, no obvious candidate(s) for the kinase(s) had been established. However, introduction of an affinity chromatography step resolved this issue, as in the previous report (10). Fractions 5-7 from the hydroxylapatite column, containing the majority of histone kinase activity, were pooled, made to 0.5% Triton X-100, and loaded on a Cibacron blue-Sepharose column. Of the total applied protein, 75% was recovered in the throughput and initial washings (Fig. 7), including all of the 32-kDa peptide. All protein kinase and diaphorase activity was bound to the column. Elution with 10 mM NADP$^+$

Fig. 7. Purification of protein kinases by affinity chromatography on a Cibacron blue-Sepharose column. Profile of enzymatic activities. Fractions from the hydroxylapatite column, containing the peak in kinase activity, were combined, desalted, and brought to 0.5% Triton X-100, then were loaded (~1 mg protein in 1 ml) on a Cibacron blue-Sepharose column (0.5 x 3 cm) previously equilibrated with 0.1% Triton X-100, 0.1 mM PMSF, 10 mM sodium Tricine (pH 8.0). After 10 min (4 °C) the throughput (fraction 1) was collected. The column was rinsed sequentially with this buffer and additives as follows: fractions 2 and 3, 1 ml each of buffer; fraction 4, 1 ml of buffer containing 50 mM NaCl; fraction 5, same as fraction 4, supplemented with 5 mM NADP$^+$; fraction 6, repeat of fraction 4; fraction 7, 1 ml of buffer containing 5 mM ATP; fraction 8, repeat of fraction 4; fractions 9-13, 1 ml each of buffer containing 0.1, 0.25, 0.5, 1, and 2 mM NaCl, respectively; fraction 14, 2 ml of buffer; fraction 15, 1 ml of 30 mM octyl glucoside/0.5% cholate, 10 mM sodium Tricine (pH 8.0); fraction 16, 1 ml of 1% (w/v) lithium dodecyl sulfate. Fractions 4-13 were dialyzed overnight against 0.1% Triton buffer. The open histogram shows protein content (µg/fraction), the solid area shows histone Vs kinase activity, and the hatched area shows reductase activity (units as for Fig. 5). Loadings (recoveries) were: protein, 1.8 (1.6) mg; histone kinase activity, 500 (100) units; reductase activity, 35 (32) units.
yielded the diaphorase in association with 130 μg of protein (Fig. 7, fractions 5 and 6). The calculated specific diaphorase activity was 246 μmol of 2,6-dichlorophenolindophenol reduced (mg protein)^−1 min^−1 at pH 8.0. Electrophoretic analysis showed a major band lying at an apparent molecular mass of 38 kDa (Fig. 8), identifiable by Western blotting as ferredoxin:NADP^+ oxidoreductase. No measurable protein kinase activity was associated with these column fractions.

Elution of the Cibacron blue-Sepharose column with 10 mM ATP (Fig. 8, fractions 7 and 8) yielded 10–20% of the total protein kinase activity at a specific activity of ~0.8 nmol of phosphate transferred (mg protein)^−1 min^−1 (~100 pmol of phosphate incorporated min^−1 total activity associated with 120 μg of protein). Electrophoresis revealed a major peptide of apparent molecular mass 23 kDa in these fractions. Further washing of the column with high salt or ionic detergents removed no more protein kinase activity. Lithium dodecyl sulfate (1% w/v) removed several peptides from the column including a 64-kDa component previously identified as the major thylakoid protein kinase (10).

If the hydroxylapatite column fractions containing the peak of protein kinase activity were pooled and fractionated on a histone III-S-Sepharose affinity column, as previously described (10), 95% of the total protein and 10–20% of the total protein kinase activity were recovered in the throughput and initial washings (Table I). Eluting the column with 0.25–0.5 M NaCl yielded 80–90% of the total protein kinase activity and about 1% of the total protein (Table I). Analysis of these fractions by denaturing gel electrophoresis revealed the presence of a 23-kDa peptide in the throughput and initial washings (not shown) and a 64-kDa peptide (Fig. 8) in the 0.25 M NaCl eluate. The specific kinase activity of the latter, with histone III-S as substrate, was 20–30 nmol of phosphate transferred (mg protein)^−1 min^−1, in agreement with a previously reported value for this enzyme (10). Thus, all protein kinase activity in the hydroxylapatite column fractions can be accounted for by the 23-kDa component (providing 10–20% of the total activity at a specific activity of 0.5–1.0 nmol of phosphate transferred (mg protein)^−1 min^−1) and the 64-kDa component (providing the remainder, at a specific activity of 20–30 nmol (mg protein)^−1 min^−1).

CONCLUSIONS

Most of the protein kinase activity in these extracts, as in the thylakoid itself, is attributable to the 64-kDa enzyme (10).

We show here that use of 8-azido-ATP photofluorinity labeling for identifying kinase(s) is inappropriate in that ferredoxin:NADP^+ oxidoreductase, and other polypeptides devoid of phosphotransferase activity are heavily labeled whereas the 64-kDa protein kinase is not, either in extracts or in the membrane. An explanation for this is presented in Fig. 9, which is a Dixon plot showing the effect of 8-azido-ATP on the rate of histone phosphorylation by the purified 64-kDa kinase, in the presence of different ATP concentrations. The photofluorinity reagent proved to be only a weak competitive inhibitor of the enzyme with a K_i of ~200 μM, compared to a 10-fold lower K_M for ATP. The same values were found for the membrane-bound kinase, activated either by light or chemical reductant (data not shown). This low affinity, together with the low abundance of 64-kDa kinase in thylakoid membranes (~0.02% of the total membrane protein) and the low efficiency of cross-linking (between 0.01–0.1%), presumably weighs against the formation and detection of a cross-linked product between the kinase and 8-azido-ATP.

![Silver stain of histone III-S-Sepharose affinity column fractions](image-url)
Our conclusions agree with those of Lin et al. (7) who proposed that a kinase other than CPK₁ or CPK₂ was responsible for phosphorylating LHC: we have shown that the 64-kDa enzyme could have this role (10). We also confirm here, the existence of CPK₁ as a peptide of apparent molecular mass 23 or 25 kDa (depending on the reference adopted) possessing weak kinase activity toward lysine-rich histone. This enzyme has been purified to homogeneity, using their protocol (7) followed by affinity chromatography on Cibacron blue-Sepharose.

We cannot confirm the existence of CPK₂, however. The 38-kDa peptide designated CPK₂ on the basis of photoaffinity labeling is devoid of kinase activity and can be identified as ferredoxin:NADP⁺ oxidoreductase. We have isolated this enzyme by several conventional procedures and confirmed its lack of phosphotransferase activity. It seems reasonable to conclude that the activity denoted CPK₂ in the preparation of Lin et al. (7) was due to contamination with 64-kDa protein kinase. Some, though not all, of the phosphotransferase activity assigned to CPK₁ could have this same origin, for Lucero et al. (8) showed that autophosphorylation of their crude CPK₁ fraction led to the labeling of a ~60-kDa component.

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