Purification and Characterization of a Casein Kinase 2-Type Protein Kinase from Pea Nuclei

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ABSTRACT

Almost all the polyamine-stimulated protein kinase activity associated with the chromatin fraction of nuclei purified from etiolated pea (Pisum sativum L.) plumules is present in a single enzyme that can be extracted from chromatin by 0.35 molar NaCl. This protein kinase can be further purified over 2000-fold by salt fractionation and anion-exchange and casein-agarose column chromatography, after which it is more than 90% pure. The purified kinase has a specific activity of about 650 nanomoles per minute per milligram protein in the absence of polyamines, with either ATP or GTP as phosphoryl donor. Spermidine can stimulate its activity fourfold, with half-maximal activation at about 2 millimolar. Spermine and putrescine also stimulate activity, although somewhat less effectively. This kinase has a tetrameric \(a_2b_2\) structure with a native molecular weight of 130,000, and subunit molecular weights of 36,000 for the catalytic subunit (\(a\)) and 29,000 for the regulatory subunit (\(b\)). In western blot analyses, only the \(a\) subunit reacts strongly with polyclonal antibodies to a Drosophila casein kinase II. The pea kinase can use casein and phosphatidylserine as artificial substrates, phosphorylating both the serine and threonine residues of casein. It has a pH optimum near 8.0, a \(V_{\text{max}}\) of 1.5 micromoles per minute per milligram protein, and a \(K_m\) for ATP of approximately 75 micromolar. Its activity can be almost completely inhibited by heparin at 5 micrograms per milliliter, but is relatively insensitive to concentrations of staurosporine, K252a, and chlorpromazine that strongly antagonize Ca\(^{2+}\)-regulated protein kinases. These results are discussed in relation to recent findings that casein kinase 2-type kinases may phosphorylate trans-acting factors that bind to light-regulated promoters in plants.

Protein kinases play a variety of important roles in nuclear metabolism. As examples, the phosphorylation of lamins is necessary for their disassembly during prophase of mitosis (13), the phosphorylation of histone H1 is critical for the process of chromosome condensation (17), and the phosphorylation of the large subunit of RNA polymerase II helps to promote the propagation of the transcription complex for the synthesis of pre-mRNA (10). In animals, cyclic AMP-dependent protein kinases can help regulate gene expression by phosphorylating nuclear factors (9). In plants, too, the phosphorylation/dephosphorylation of trans-acting factors regulates their binding to promoter sequences (4) and to silencer regions (12). Based on in vitro assays, one or more of the protein kinases that phosphorylate such factors appears to be present in nuclei (25).

At least one of the protein kinases in plant nuclei that control nuclear protein phosphorylation is calcium-dependent (16). However, calcium-independent protein kinases in plant nuclei have also been described (23). At least one of these kinases has the classic properties of a CK\(^2\)-type protein kinase in that it is stimulated by polyamines, inhibited by heparin, and can use GTP as well as ATP as phosphoryl donors (6). Interest in CK-2-type kinases in plants has been enhanced by recent reports that this type of protein kinase participates in controlling the binding of the AT-1 nuclear factor to a consensus sequence in certain light-regulated promoters (4). The research described in this report was designed to follow up on the earlier studies (6) by purifying to near homogeneity and characterizing the biochemical properties of the major CK-2 kinase present in the chromatin fraction of pea nuclei.

MATERIALS AND METHODS

Plant Growth

Seedlings of pea (Pisum sativum L. cv Alaska) were grown in the dark for 7 d at 23 ± 3°C.

Materials

Percoll, ATP (Tris salt), GTP (sodium salt), PMSF, phenazine, chlorpromazine, dephosphorylated casein, calf thymus histone (type III-S), and cellulose phosphate were all obtained from Sigma (St. Louis, MO). Staurosporine and K252a were from Kamiya Biomedical Corporation (Thousand Oaks, CA). The [\(\gamma\]P]ATP (850 cpm/pmol) and [\(\gamma\]P]GTP (350 cpm/pmol) were obtained from New England Nuclear. Myelin basic protein peptide 4–14 (28) was synthesized on an Applied Biosystems (Foster City, CA) automated solid phase peptide synthesizer by the Protein Sequencing Center of the University of Texas at Austin and then purified by reverse-phase HPLC on a Beckman C-18 column before use. The synthetic peptide substrate considered specific for casein kinase II (14) (RRREEETEEE) was purchased from Peninsula Laboratories (Belmont, CA). All buffers were prepared with water purified by a Milli Q water purification system (Millipore Corp.).

1 Supported in part by National Science Foundation grant DCB-9106245.

2 Abbreviations: CK, casein kinase; \(W_s\), \(N\)-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide; \(W_q\), \(N\)-(6-aminohexyl)-1-naphthalene sulfonamide.
Preparation of Nuclei and Chromatin Fraction

Isolation of nuclei from the plumes of 7-d-old etiolated pea seedlings was done as described by Datta et al. (5). Typically, 200 g of plumes was used as starting material. The chromatin fraction was prepared from purified nuclei as described by Chen et al. (1). Briefly, gradient-purified nuclei were lysed by incubating them for 10 min at 4°C in 5 mL of buffer I (60 mM Heps, pH 7.5, 1 mM EDTA, 0.5% Triton X-100, 0.5 mM PMSF in DMSO, 1.14 mM ascorbic acid, 0.2 mM ammonium molybdate, 10 mM DTT), and then the extract was centrifuged at 9000g for 1 min. The pellet of this centrifugation was referred to as the chromatin fraction of the nuclei.

Extraction and Column Chromatography of the CK-2 Kinase

All procedures were carried out at 4°C, except the HPLC chromatography steps, which were at room temperature (22 ± 3°C). Extraction of the chromatin fraction with 0.35 M NaCl and the two subsequent ammonium sulfate fractionations of this extract were exactly as described in Li et al. (16). The pellet from the second ammonium sulfate precipitation step was dissolved in 0.5 mL of buffer A (30 mM Heps, pH 7.5, 5 mM MgCl₂, 50 mM NaCl), dialyzed against buffer A for 1 h, clarified by centrifugation (9000g for 5 min), and then applied to a DEAE-5PW column (0.75 × 7.5 cm, Beckman Instruments, San Ramon, CA) that had been previously equilibrated with buffer A. Unbound proteins were washed away with 15 column volumes of buffer A, the column was eluted with buffer A plus 0.36 M NaCl until the eluent was protein-free, and then some of the proteins that remained bound were step-eluted with buffer A plus 0.9 M NaCl. Fractions eluted with 0.36 M NaCl that had spermine-stimulated protein kinase activity were combined, dialyzed against buffer B (30 mM Heps, pH 7.5, 0.4 M NaCl, 0.5% aprotinin, 0.5 mM PMSF), layered onto a 10-mL linear gradient of 5 to 20% sucrose in buffer B, and centrifuged for 15 h at 35,000 rpm (150,000g) in an SW 41 rotor. After centrifugation, 0.5-mL fractions were collected from top to bottom of the tubes.

The peak protein kinase fraction from the sucrose gradient was dialyzed against buffer C (10 mM sodium phosphate buffer, pH 7.5, 0.5% aprotinin, 0.5 mM PMSF) for 1 h and then applied to a phosphocellulose column (1.5 × 5 cm). The column was washed with two bed volumes of buffer C, then with buffer C plus 1 M NaCl. Fractions with the highest protein kinase activity were pooled and dialyzed against buffer D (buffer C with 0.25 M NaCl) and applied to a second phosphocellulose column equilibrated with buffer D. The column was washed with buffer D until the eluted material had an A280 of less than 0.01, then bound protein was eluted with buffer C plus 1.25 M NaCl. Fractions with the highest kinase activity were pooled, dialyzed against buffer E (30 mM Heps, pH 7.5, 0.1 M NaCl, 10% glycerol, 0.5% aprotinin, 0.5 mM PMSF), and applied to a casein-agarose column (2 × 10 cm) equilibrated in buffer E. After the column was washed with several bed volumes with buffer E, the bound CK-2 kinase was eluted from the column with buffer E containing 0.6 M NaCl.

To assess the native mol wt of the protein kinase, peak activity fractions from the casein-agarose column were dialyzed against buffer F (10 mM sodium phosphate buffer, pH 7.0, 0.1 M NaCl) and then applied to an HPLC molecular sieve column (0.75 × 30 cm, Ultraspherogel SEC 3000, Beckman Instruments) previously equilibrated in buffer F.

Protein Kinase Assays

Quantitative assays of protein kinase activity were made by measuring the incorporation of [32P]P from [γ-32P]ATP or GTP into a protein or peptide substrate. This assay was carried out at room temperature, following procedures described by Li et al. (16). Except where specified, all assays were carried out with preparations that had been purified through the casein-agarose purification step and adjusted to a protein concentration of 10 μg/mL. The assay was started by adding 10 μL of enzyme solution to 40 μL of a reaction buffer, giving a final concentration of 25 mM Tris-HCl, pH 8.0, 10 mM MgCl₂, 1 mM casein (or other protein or peptide substrate), 100 μM [γ-32P]ATP (850 cpm/pmol) or GTP (350 cpm/pmol). Following incubation of the assay mixture for 15 min, 30 μL was withdrawn and spotted onto 2.1-cm diameter phosphocellulose filter strips (Whatman P81). The filter strips were washed sequentially with 75 mM H₃PO₄ (three changes, 5 min each wash), 95% ethanol (1 min), and ethyl ether (1 min). Each filter was air dried and then added to a scintillation vial containing 5 mL aqueous counting scintillant (Amersham) and counted in a Packard scintillation counter.

SDS-PAGE

Samples with the highest specific activity from the casein-agarose column were desalted and concentrated by a Centricon-10 concentrator, mixed with SDS-PAGE sample buffer, boiled for 2 min, applied to a 10% SDS polyacrylamide gel, and electrophoresed (15). After electrophoresis, protein bands were visualized by a silver-stain method (20). When labeled samples were assayed, the phosphorylation reaction was carried out as described for the protein kinase assay. The reactions were stopped by boiling the samples in SDS-PAGE sample buffer. The gels were fixed with 15% TCA/50% methanol, stained with Coomassie blue, destained, dried, and autoradiographed using Kodak AR film at −70°C.

Detection of Protein Kinase Activity in Gels after SDS-PAGE

These procedures were carried out as described by Guo and Roux (11). Briefly, the samples to be assayed after SDS-PAGE were heated to 80 to 90°C for 2 min in electrophoresis sample buffer and electrophoresed as described above, except that 1 mg/mL casein was included in the separating gel before polymerization. After electrophoresis, SDS was removed from the gel by washing it with 25 mM Tris-Cl (pH 7.5). Then, phosphorylation was initiated by incubating the gel in a reaction buffer containing [γ-32P]ATP (850 cpm/pmol) for 5 h as described (11).
Assay of Phosphorylated Amino Acids in Casein

The procedure used for this assay was similar to that described by Putnam-Evans et al. (22). [32P]Casein that had been phosphorylated by reaction with the pea CK-2 protein kinase was partially hydrolyzed in 6 M HCl for 1 h at 110°C, dried under vacuum, resuspended in 10 μL of Milli-Q purified water, and co-chromatographed with phosphoamino acid standards (1 mg/mL each of L-phosphoserine, L-phosphothreonine, and L-phosphotyrosine) by paper electrophoresis (Whatman paper No. 1) in pyridine/acetic acid/water (5:50:945, v/v/v) at 2 kV for 90 min. The positions of the standards were visualized by spraying the paper with 0.05% ninhydrin in ethanol:acetic acid (3:1, v/v). The positions of the labeled amino acids were detected by autoradiography.

Western Blot Analysis

The cross-reactivity of the pea CK-2-like kinase with a polyclonal antibody raised to a Drosophila CK-2 (3) (kindly provided by Dr. Claiborne Glover III, University of Georgia, Athens) was tested by western blot analysis, using the transfer and immunostaining procedure described by Li et al. (16).

Protein Determination

Protein concentrations were determined with the Bio-Rad protein assay kit using BSA as a standard.

RESULTS

Purification of Nuclei and Chromatin Fraction

By light and electron microscopic examination, the preparation of purified nuclei was judged to be free of contamination by other intact organelles (5). After extraction of the nuclei with Triton X-100 and EDTA, the remaining chromatin fraction contained nuclear scaffolding proteins in addition to the chromatin, but it was essentially free of the nuclear envelope and other membranes (data not shown).

Purification of Protein Kinase

A summary of the purification protocol is presented in Table I. There are two major protein kinases in the crude chromatin fraction of pea nuclei. One is the Ca2+-dependent protein kinase described by Li et al. (16), and the other is the CK-2-like, polyamine-stimulated protein kinase described in this paper. When the proteins extracted from the chromatin fraction were chromatographed on the DEAE-5PW column in the presence of 0.25 M NaCl, the Ca2+-dependent protein kinase did not bind to the column, whereas the CK-2-like kinase did bind and could be eluted by 0.36 M NaCl (Fig. 1A). On phosphocellulose columns I and II, the CK-2-like kinase activity appeared in the flow-through fraction in the absence of salt (I) and bound to the column in the presence of 0.25 M NaCl (II). It could then be washed from the column by 1.25 M NaCl (Fig. 1B). Taking advantage of the fact that the casein-phosphorylating activity of the kinase could be stimulated by 100 mM NaCl, the kinase was bound to the

Table I. Purification of the CK 2-Like Protein Kinase from Nuclei of 200 g Pea Plumules

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Total Protein (mg)</th>
<th>Total Kinase Activity (nmol/min)</th>
<th>Specific Activity (nmol/min mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatin extract</td>
<td>48.9</td>
<td>13.0</td>
<td>0.3</td>
</tr>
<tr>
<td>DEAE-SPW column</td>
<td>9.6</td>
<td>10.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Sucrose gradient</td>
<td>4.0</td>
<td>8.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Phosphocellulose I</td>
<td>3.3</td>
<td>6.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Phosphocellulose II</td>
<td>0.5</td>
<td>4.3</td>
<td>8.6</td>
</tr>
<tr>
<td>Casein-agarose column</td>
<td>0.006</td>
<td>3.9</td>
<td>650</td>
</tr>
</tbody>
</table>

Figure 1. Elution profiles of A280 and protein kinase activity from the (A) DEAE-5PW, (B) phosphocellulose II, (C) casein-agarose, and (D) Ultraspherogel SEC 3000 molecular sieve columns. The numbers above the arrows in D are the mol wts × 10^-12 of the proteins used as standards, and the arrows mark the positions of the elution peak of these standards. For all four columns, the volume of sample loaded was 0.25 mL, and the volume of each fraction collected was 0.2 mL. The flow rates were 0.5 mL/min for A and D and 0.1 mL/min for B and C.
casein-agarose column in the presence of 0.1 M NaCl and then eluted with 0.6 M NaCl (Fig. 1C).

Native and Subunit Mol Wts

As judged by chromatography on a calibrated molecular sieve column, the kinase had a native mol wt of about 130,000 (Fig. 1D). The SDS-PAGE silver-staining pattern of the 130-kD protein collected from the activity peak off the molecular sieve column was identical to that shown in Figure 2, lane A. As judged by SDS-PAGE and silver-staining, the CK-2-like kinase purified through the casein-agarose step was more than 90% pure and had a heteromeric structure with subunits of 29,000 and 36,000 mol wt, present in approximately equimolar concentrations (Fig. 2, lane A). When the subunits were renatured in the gel after SDS-PAGE, the 36-kD protein band showed kinase activity, indicating that it was the catalytic subunit (Fig. 2, lane B). Considered together, the native and subunit mol wts of the kinase indicated that it probably had a tetrameric \(\alpha_2\beta_2\) structure characteristic of many CK-2-type kinases.

Substrate Specificity

Under the assay conditions chosen, the CK-2-like kinase favored casein and phosphat as artificial substrates, but it could also phosphorylate, with a much lower efficiency, histone, BSA, the myelin basic peptide favored as a substrate by protein kinase C (28), and the decapptide considered specific for CK-2-type kinases (14) (Table II). For the phosphorylatable substrates, both ATP and GTP served as phosphoryl donors (Table II).

When the partial acid-hydrolysate of \([\text{32P}]\text{casein}\) was examined, both serine and threonine residues, but not tyrosine residues, were labeled. Assuming similar rates of release and destruction of the two modified residues during hydrolysis, serine was the predominant site of phosphorylation (Fig. 3).

![Figure 2](image-url)

Figure 2. A, Peak fraction of protein kinase activity from casein-agarose column (Fig. 1C) after SDS-PAGE and silver-staining. Total sample load was about 500 ng. B, Same sample as in A. After SDS-PAGE, sample was renatured in the gel and reacted with \([\text{32P}]\text{ATP}\). After washing out unbound label, the gel was dried and analyzed by autoradiography. The numbers along the left margin are the mol wts \(\times 10^9\) of the standards used.

Table II. Comparison of CK 2-Like Protein Kinase Activity with Different Artificial Substrates and with ATP and GTP as Phosphoryl Donors

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP (nmol/min-mg)</td>
</tr>
<tr>
<td>Casein</td>
<td>667</td>
</tr>
<tr>
<td>Phosphat</td>
<td>661</td>
</tr>
<tr>
<td>Histone</td>
<td>261</td>
</tr>
<tr>
<td>BSA</td>
<td>260</td>
</tr>
<tr>
<td>Myelin basic peptide 4-14</td>
<td>241</td>
</tr>
<tr>
<td>CK-2 substrate peptide</td>
<td>220</td>
</tr>
</tbody>
</table>

Polyamine and Poly-L-Lys Stimulation of Kinase Activity

All three polyamines tested stimulated the activity of the pea CK-2-like kinase, with spermidine inducing the highest activity (Fig. 4). The stimulations by spermidine and spermine peaked at 5 to 10 mM, but putrescine continued to show increased stimulatory effects through 20 mM, which is still within the range reported to occur in plant cells (24). Among the basic polypeptides tested, poly-L-Lys, but not poly-L-Arg or protamine, was stimulatory at 10 \(\mu\)g/mL (Table III). Poly-L-Arg and protamine were also not stimulatory at 1 and 5 \(\mu\)g/mL (data not shown).

Inhibitor Profile

Using casein as an artificial substrate, the sensitivity of the CK-2-like kinase to various potential inhibitors was tested (Table III). Kinase activity was very sensitive to inhibition by heparin, but was relatively insensitive to such inhibitors of Ca\(^{2+}\)-regulated protein kinases as staurosporine (16), K252a

![Figure 3](image-url)

Figure 3. Phosphoamino acid analysis of \(\text{32P}\)-labeled casein. Casein was labeled in vivo by purified pea CK II using \([\gamma-\text{32P}]\text{ATP}\) as the phosphoryl donor, partially acid-hydrolyzed, and analyzed as described in "Materials and Methods."
(26), chlorpromazine (11, 16), and W5 (22), and was actually stimulated by 0.1 mM NaCl, which has been reported to inhibit a wheat germ CK-2-like kinase (27). Calcium chelation by EGTA did not inhibit activity.

**Ionic Requirements for Activity**

The CK-2-like kinase had a broad pH optimum near 8.0. Relative to its activity at 8.0, its activity at pH 7.0 was about 50% and its activity at pH 6.5 was about 33%. The enzyme required Mg$^{2+}$ (Mg$^{2+}$-ATP), and Mn$^{2+}$ could not substitute for Mg$^{2+}$ (data not shown), but Ca$^{2+}$ was not required (Table III). In a plot of [Mg$^{2+}$] versus kinase activity under standard assay conditions, except varying the [Mg$^{2+}$] from 1 to 20 mM, the optimum [Mg$^{2+}$] was 10 mM (data not shown).

**Kinetic Properties**

The $K_m$(ATP) was about 74 $\mu$M (Fig. 5A), and for casein was approximately 1 mg/mL (Fig. 5B). The $V_{max}$ for both was about 1.5 $\mu$mol/min-mg protein.

**Western Blot Analysis**

Under the experimental conditions used, the $\alpha$ subunit (36 kD) of the pea CK-2-like kinase cross-reacted well with the anti-*Drosophila* CK-2 polyclonal antibodies under staining conditions in which the $\beta$ subunit (29 kD) showed no detectable binding (Fig. 6). The staining of the $\alpha$ subunit appeared to be a doublet, suggesting that it had two reactive forms.

Table III. **Effects of Various Agents on CK 2-Like Protein Kinase Activity**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration</th>
<th>Relative Activity % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly-L-lysine</td>
<td>10 $\mu$g/mL</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>1 $\mu$g/mL</td>
<td>158</td>
</tr>
<tr>
<td>Poly-L-arginine</td>
<td>10 $\mu$g/mL</td>
<td>95</td>
</tr>
<tr>
<td>Protamine</td>
<td>10 $\mu$g/mL</td>
<td>98</td>
</tr>
<tr>
<td>Heparin</td>
<td>0.1 $\mu$g/mL</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>5 $\mu$g/mL</td>
<td>5</td>
</tr>
<tr>
<td>Staurosporine</td>
<td>1 $\mu$M</td>
<td>77</td>
</tr>
<tr>
<td>K252a</td>
<td>1 $\mu$M</td>
<td>100</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>10 $\mu$M</td>
<td>102</td>
</tr>
<tr>
<td>W5</td>
<td>150 $\mu$M</td>
<td>130</td>
</tr>
<tr>
<td>W6</td>
<td>500 $\mu$M</td>
<td>110</td>
</tr>
<tr>
<td>EGTA</td>
<td>2 nM</td>
<td>95</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>100 $\mu$M</td>
<td>103</td>
</tr>
<tr>
<td>NaCl</td>
<td>100 mM</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>200 mM</td>
<td>75</td>
</tr>
</tbody>
</table>

Figure 4. **Effect of putrescine (■), spermidine (▲), and spermine (●)** on the protein kinase activity of purified CK-2-like kinase from pea nuclei. Activity was assayed as described in "Materials and Methods." Each point represents the mean of triplicate determinations that all agreed with each other within 15%.

Figure 5. Assays of initial velocities of protein kinase activity of purified pea nuclear CK-2-like kinase. All assays were performed at 25°C in 50 mM Tris, pH 8.0, 10 mM MgCl$_2$, using 10 ng of purified pea kinase. A, $1/V$ versus $1/[$ATP$]$, at different fixed concentrations of casein: 0.2 (○), 0.5 (▲), 1 (●), and 2 (△) mg/mL. B, $1/V$ versus $1/[$casein$]$ at different fixed concentrations of ATP: 10 (○), 15 (▲), 25 (●), and 100 (△) $\mu$M.
DISCUSSION

The first reports of highly purified protein kinases from higher plant nuclei were by Murray et al. (18, 19). Although these appeared to be CKs, one of them could not use GTP as a phosphoryl donor (18) and the other did not bind to DEAE under relatively low salt conditions (19); thus, both were likely to be CK-1-type kinases. Later, Erdmann et al. (7) reported the isolation of both an NI (=CK-1) and an NII (=CK-2) protein kinase from the nuclei of cultured tobacco cells. However, these kinase preparations were not pure enough to characterize very thoroughly. The same is true for the CK-2-type kinase preparation partially purified by Datta et al. from pea nuclei (6). Yan and Tao (27) have also purified and characterized a CK from a higher plant source, wheat germ, but the subcellular locale of this kinase was not tested, and spermine inhibited rather than stimulated this kinase except at [Mg] below 2 mM.

The pea CK-2-type kinase preparation described here is highly purified, and thus is suitable for detailed biochemical characterization and comparison with purified animal CK-2 kinases. According to the criteria reviewed recently by Pinna (21), the pea nuclear protein kinase is CK-2-like in several respects: (a) native and subunit mol wts; (b) ability to be stimulated by polyclations, such as polyamines, and high sensitivity to inhibition by polyamines, such as heparin; (c) ability to use ATP or GTP as phosphoryl donor; (d) preference for casein or phosphotin and ability to use RRREEETEEE (14) as artificial substrates; (e) selectivity for serine and threonine as the side chains phosphorylated; (f) binding properties to phosphocellulose; and (g) kinetic (K_m, V_max) properties.

Further evidence that the pea nuclear kinase has a structure highly similar to that of animal CK-2-type kinases is that it specifically binds anti-Drosophila CK-2 kinase antibodies in a western blot analysis. This result is not due merely to there being a generic kinase catalytic site in both the pea and Drosophila kinases, because the 90-kD Ca^{2+}-dependent protein kinase in pea nuclei described by Li et al. (16) was present in the sample loaded in lane A of Figure 6 but did not react with the anti-Drosophila CK-2 antibodies. The anti-Drosophila CK-2 kinase antibodies will also recognize calf thymus and yeast CK-2 kinases but not a CK-1 kinase tested (3 and C. Glover, personal communication). Sequences that are highly conserved among widely divergent species in both the α and β subunits of CK-2 kinases have been identified (21) and may be among those recognized in the pea CK-2-like kinase by the anti-Drosophila antibodies. CK-2 enzymes are themselves phosphoprotein kinases (21), so the minor immunostained band above the 36-kD band in Figure 6 may represent an alternative phosphorylated or dephosphorylated form of the pea kinase, or possibly another, less-abundant CK-2-like kinase from pea nuclei with nearly identical chromatographic properties to the major one. This minor form is not apparent after silver-staining or on the activity gel (Fig. 2), but the latter stains are broad and may have obscured minor bands with a similar M_r.

The protocol used to purify the pea CK-2-like protein kinase is similar to that used to purify animal CK-2-like kinases. The final yield (approximately 30 ng/g fresh weight of plumes) represents approximately 30% of the initial level of kinase activity released from the chromatin fraction by 0.35 M NaCl. We have preliminary evidence that additional CK-2 kinase activity is associated with the envelope fraction of pea nuclei (our unpublished observations) and that there is a CK-2 kinase associated with the cytoplasmic fraction of homogenized pea plumes (S. Zhang, S.J. Roux, unpublished). In bovine adrenocortical cells, there is a significant shift of the intracellular distribution of CK-2 kinase from the cytoplasm into nuclei during active proliferation of these cells in culture (8). Thus, the possibility of regulated exchange between cytoplasmic and nuclear isoforms of CK-2 should be considered in plant cells as well.

Interest in CK-2-type kinases in pea seedlings has been stimulated by the report of Datta and Cashmore (4). They found that a GTP-utilizing protein kinase could phosphorylate the DNA-binding factor AT-1 and thus regulate its affinity for an AT-rich box within promoters of genes for the small subunit of Rubisco. In animal cells, trans-acting factors represent only one class of nuclear proteins that are phosphorylated by CK-2 kinases. As reviewed by Pinna (21), others include various high mobility group proteins, RNA polymerases I and II, DNA topoisomerases I and II, nucleolin, and calmodulin. Datta et al. (6) initially characterized the most prominent mol wt species of nuclear proteins whose phosphorylation was stimulated by polyamines. We have raised antibodies to some of these proteins and have begun to study them. It will be of interest to determine whether any of them fall into the categories of proteins known to be phosphorylated by CK-2-type kinases in animal cells.

The question of whether or not the regulation of CK-2 kinases by polyamines is physiologically significant remains controversial. Certainly the level of polyamines needed to stimulate CK-2 kinases (about 5 mM) is within the range found in plant cells (24). There is evidence that polyamines may serve as physiological effectors in some instances (21), but not in others (2). It seems probable that CK-2 activity

Figure 6. Western blot analysis of pea nuclear CK-2-like protein kinase, using for the immunostain polyclonal antibody prepared to Drosophila CK-2 (lanes A and B) and nonimmune rabbit serum (lanes C and D). Two different protein kinase preparations were analyzed: lanes A and C, crude nuclear proteins (10 µg total protein loaded per lane); lanes B and D, purified protein kinase (0.5 µg protein loaded per lane).
may not be under the strict control of one kind of effector, but may be regulated differently under different physiological circumstances.

LITERATURE CITED