Cyclic Nucleotide-independent Protein Kinases from Rabbit Reticulocytes

PURIFICATION OF CASEIN KINASES*

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Two cyclic AMP-independent protein kinases have been isolated from rabbit reticulocytes. These enzymes have been resolved from the cyclic AMP-regulated activities by ion exchange chromatography on DEAE-cellulose and phosphocellulose and assayed using casein as substrate. For simplicity, the casein kinases were numbered in order of elution from DEAE-cellulose. Casein kinase I (CK I) bound to phosphocellulose and to sulfopropyl-Sephadex at low ionic strength at pH 6.8. Casein kinase II (CK II) did not adhere to phosphocellulose in the absence of monovalent cations, but bound when the concentration of these ions was raised to 0.25 M. This differential chromatography of CK II on phosphocellulose was used in the purification of the enzyme. Both CK I and CK II activities were purified further by hydroxylapatite chromatography. In the phosphorylation of casein, CK I preferentially utilized ATP over GTP. The $K_m$ for ATP and GTP was determined to be $13 \mu M$ and $900 \mu M$, respectively. CK II utilized both ATP and GTP in the phosphotransferase reaction with a $K_m$ for ATP of $10 \mu M$ and $40 \mu M$ for GTP.

Analysis of the highly purified CK II by polyacrylamide gel electrophoresis in sodium dodecyl sulfate showed three major bands of molecular weight 42,000, 38,000, and 24,000. The 24,000 molecular weight band was self-phosphorylated when the enzyme was incubated with magnesium and either ATP or GTP. In a similar experiment, a single protein band of 37,000 daltons was observed with CK I which was self-phosphorylated by incubation with magnesium and ATP. Velocity sedimentation experiments yielded a sedimentation coefficient of 3.2 S for CK I and 7.5 S for CK II. Preincubation of CK II with $[\gamma-^{32}P]ATP$ followed by sucrose gradient centrifugation yielded a single, enzymatically active peak of 7.5 S which coincided with the radioactivity. A molecular weight of 144,000 ± 10% was estimated for CK II by sedimentation-equilibrium which in combination with gel electrophoresis data suggests a heterogeneous subunit structure.

A number of enzyme activities which catalyze the post-translational phosphorylation and dephosphorylation of proteins have been detected in diverse eukaryotic cells (1). These include cyclic nucleotide-regulated and cyclic nucleotide-independent protein kinases. The cyclic nucleotide-independent protein kinases are a class of enzymes whose function is not under direct control of either cAMP or cGMP and are not controlled by the same regulatory proteins as the cAMP-regulated enzymes.

Cyclic nucleotide-independent protein kinases which phosphorylate casein have been partially purified from a variety of tissues, including rat liver (2-9), human lymphocytes (10), calf brain (11), dogfish skeletal muscle (12), mouse placental cells (13), and rabbit reticulocytes (14) and erythrocytes (14, 15). Highly purified casein kinase activities have been reported recently from yeast (16), Novikoff ascites tumor cells (17), and rat liver (18). The physiological function of these enzymes remains a subject for speculation.

This paper deals with the purification and properties of two cytoplasmic cyclic nucleotide-independent protein kinases from rabbit reticulocytes.

EXPERIMENTAL PROCEDURES

Materials

ATP and GTP were obtained from P-L Biochemicals. Casein, histone (type II-A), phosphocellulose, sulfopropyl-Sephadex (SP-C50), and cellulose were obtained from Sigma. DEAE-cellulose was purchased as DE52 from Whatman. Hydroxylapatite was obtained from Bio-Rad.

Methods

Lysate Preparation—The preparation of the reticulocyte lysate has been described previously (19).

Protein Kinase Assay—The assay for protein kinase was carried out as previously described (20).

Determination of $K_m$ for ATP and GTP—Initial velocity data were obtained at 30°C under optimal assay conditions as described above and in Table II. The reaction volume was increased in order to allow removal of 0.05 ml aliquots at zero time and every 10 min thereafter for 30 min. A control which contained water instead of enzyme was included and the control counts were subtracted for each time point. The product-time curves were linear in every case over the time course of the experiment. Values of $K_m$ for both CK I and CK II were obtained from Lineweaver-Burk plots of the data. Specific activities of the stock nucleotide triphosphates were determined in triplicate by absorbance and counting on filter papers (20).

Self-phosphorylation—Casein kinase I and II were phosphorylated by incubating each enzyme in 50 mM Tris-HCl, pH 7.4 (30°C), which contained 10 mM MgCl₂ and 0.14 mM $[\gamma-^{32}P]ATP$ (612 dpm/pmol) for 30 min. The reactions were initiated by the addition of ATP and terminated by placing the tubes in an ice bath. Unreacted ATP was removed by dialysis against 1000 volumes of buffer with one change midway through the dialysis. To aid in the removal of ATP, a second dialysis bag which contained 0.5 g of Norit was included in the dialysis vessel.

Protein Determination—Protein was determined by the method of Lowry et al. (21) with bovine albumin as a standard.

Preparation of $[\gamma-^{32}P]ATP$ and $[\gamma-^{35}P]GTP$—Nucleotide triphosphates labeled in the $\gamma$ position were prepared routinely according to the procedure of Walsh et al. (22) as described in detail elsewhere.

The abbreviations used are: CK I and CK II, casein kinase I and casein kinase II, respectively.

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Typical preparations yielded specific activities of 1500 to 2000 dpm/pmol. These showed no detectable contamination with NDP or PEI-cellulose. Less than 1% of the radioactivity was present as NMP when subjected to ascending thin layer chromatography. Typical preparations yielded specific activities of 1500 to 2000 dpm/pmol prior to use.

Protein and radioactivity were quantified by scanning the gel and autoradiogram with a densitometer (EC Apparatus Corp.).

Analytical Centrifugation—Sedimentation–equilibrium experiments were performed in the optical centrifuge by layering 0.01 ml (5 µg) of CK II onto a column of 25 mM potassium phosphate buffer, pH 7.0, which contained 500 mM NaCl and 1 mM 2-mercaptoethanol. An Epon-filled, double sector centerpiece was used which contained a well similar to that described by Vinograd et al. (97). Data were collected at 236 nm by means of a UV scanner and photelectric detector. Use of the short wavelength with double beam operation was made possible by employing a cylindrical lens between the monochromator and the entrance window to the centrifuge chamber. A sedimentation coefficient was calculated from the movement of the maximum and corrected to conditions for 20°C and water. Sedimentation–equilibrium experiments were performed at 9949 rpm in a low speed rotor according to the method of Chervenka (28). Sedimentation–velocity experiments were also performed on 5 to 20% sucrose gradients with an SW-60 rotor at 60,000 rpm for 4 h at 5°C. Ovalbumin (3.6 S) and catalase (11.3 S) were included as internal standards.

![FIG. 1. Resolution of cAMP-dependent and cAMP-independent protein kinases by DEAE-cellulose chromatography. Fractions were diluted 1:10 with 0.1% bovine serum albumin and then assayed with casein (●) or histone and cAMP (○) with ATP as described under "Experimental Procedures."](http://www.jbc.org/)

**DEAE-cellulose Chromatography**—Two liters of the postribosomal supernatant from rabbit reticulocytes were mixed with 320 ml of 10-fold concentrated Buffer A (20 mM Tris-HCl, pH 7.4 (4°C), 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.02% sodium azide) and then mixed with 1 liter (settled volume) of Whatman DE52 cellulose. The slurry was filtered with a suction through Whatman No. 1 filter paper on a large Buchner funnel and washed eight times with 250-ml portions of cold Buffer A. This procedure removed the majority of the hemoglobin. The washed gel was then poured into a column (60 × 5 cm) and washed with 200 ml of Buffer A. A linear gradient of 3.5 liters which ranged from 0 to 0.5 M NaCl in Buffer A followed. This and all subsequent gradients were determined by conductivity at 0°C and compared with the appropriate standards. Samples (22 ml) were collected and aliquots were diluted (1:10) and assayed for cAMP–dependent protein kinase activity with casein and [γ-32P]ATP. The fractions were also assayed for cAMP–dependent protein kinase activity by measuring the incorporation of inorganic phosphate into histone in the presence of cAMP and [γ-32P]ATP. Fractions 87 to 107 containing CK I were pooled and dialyzed against Buffer B (25 mM KH2PO4/K2HPO4, pH 6.8, 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.02% sodium azide) and then mixed with 1 liter (settled volume) of 0.1% bovine albumin, pH 7.

**Sucrose–Sephadex Chromatography**—The CK I from the phosphocellulose step was pooled and dialyzed against Buffer B. Then either it was applied directly to a sucrose–Sephadex column (5 × 9.6 cm) or, alternatively, the enzyme was concentrated by batch elution with Buffer B containing 1.25 M NaCl from 3- to 5-ml columns of phosphocellulose. After dialysis against Buffer B, it was applied to the sucrose–Sephadex column. The column was washed with one column volume of Buffer B and eluted with a 400-ml linear gradient of 0 to 1.25 M NaCl in Buffer B.

**Chromatography on Hydroxylapatite**—For this step, 25 ml of hydroxylapatite slurry (Bio-Rad HT) were gently mixed with a cel- lulose (Sigma Cell 50) slurry prepared by adding 2 g of the dry powder to 200 ml of 5 mM potassium phosphate, pH 7. After 1 h the mixture was filtered and stored at 4°C until ready for use. The material was then packed into a 2.6 × 2 cm column and eluted with Buffer B containing 1.0 M NaCl in Buffer B. Fractions 87 to 107 containing CK II were pooled and dialyzed against Buffer B (25 mM KH2PO4/K2HPO4, pH 7.0, 1 mM EDTA) and then mixed with 1 liter (settled volume) of 0.1% bovine albumin, pH 7. The fractions were then assayed for cAMP–dependent protein kinase activity with casein and [γ-32P]ATP. The fractions were also assayed for cAMP–dependent protein kinase activity by measuring the incorporation of inorganic phosphate into histone in the presence of cAMP and [γ-32P]ATP. Fractions 87 to 107 containing CK I were pooled and dialyzed against Buffer B (25 mM KH2PO4/K2HPO4, pH 6.8, 1 mM EDTA, 10 mM 2-mercaptoethanol, 0.02% sodium azide) and then mixed with 1 liter (settled volume) of 0.1% bovine albumin, pH 7.

**Identification of Protein Kinase Activities after Chromatography on DEAE-cellulose**—The postribosomal supernatant fraction from rabbit reticulocytes was chromatographed on DEAE-cellulose and protein kinase activities were identified by monitoring the incorporation of radioactive phosphate into casein and histone (Fig. 1). Two peaks of cyclic AMP–dependent protein kinase activity were detected which phosphorylated casein and used ATP as the phosphate donor. These enzymes were eluted from DEAE-cellulose in the range 0.075 to 0.130 M and 0.170 to 0.220 M KCl and have been designated casein kinase I (CK I) and II (CK II) according to their order of elution. CK I was not observed when the initial step in purification involved precipitation with 50% ammonium sulfate. However, when the postribosomal supernatant fraction was batch adsorbed to DEAE-cellulose, this enzyme...
Purification of Casein Kinases

Fraction No. Fraction No

**Fig. 2.** A, phosphocellulose chromatography of CK I; the enzyme was applied in the absence of monovalent cations. B, phosphocellulose chromatography of CK II; the enzyme was applied in 0.25 M NaCl. Fractions were assayed with casein and either ATP (●) or GTP (○) or histone with ATP in the presence of cyclic AMP (□). Absorbance was monitored at 280 nm (▲).

Matic activity was routinely detected. CK II has been previously reported in rabbit reticulocytes (formerly identified as IIIa) (14, 29), and an activity with a similar elution pattern has also been described in rabbit erythrocytes (14, 15). The first peak of cyclic AMP-dependent protein kinase activity emerged between 0.03 M and 0.06 M KCl and was similar to the type I enzyme as identified by Corbin et al. (30). The second peak of cyclic AMP-dependent protein kinase corresponds to the type II enzyme and was eluted between 0.11 M and 0.16 M KCl.

**Phosphocellulose Chromatography of CK I and CK II—**

The DEAE-cellulose fractions 87 to 107 and 115 to 145 were chromatographed separately on phosphocellulose. CK I was eluted as a single symmetrical peak from 0.50 M to 0.75 M NaCl. (Fig. 2A). A minor amount of casein kinase activity did not adhere to the phosphocellulose resin. This enzyme comprised only 10% of the total casein activity applied to the phosphocellulose column. It had chromatographic properties which were similar to CK II and undoubtedly corresponded to a small contaminating amount of that enzyme.

CK II behaved somewhat anomalously on phosphocellulose. In the absence of monovalent cations, about 90% of the protein kinase activity did not adhere to the resin. The fraction which did not bind to the initial phosphocellulose column was dialyzed against Buffer B containing 0.25 M NaCl and applied to a second phosphocellulose column. At this salt concentration, the enzyme adhered to the resin and was eluted as a single peak of activity from 0.70 M to 0.85 M NaCl (Fig. 2B). This anomalous behavior on phosphocellulose was used to enhance the purification of the enzyme. At this point the cyclic AMP-dependent activities were well resolved from the cyclic AMP-independent casein kinase since both type I and type II cyclic AMP-dependent kinases were not bound to phosphocellulose under these conditions.

**Chromatography of CK I on Sulfopropyl-Sephadex—**CK I adhered to the resin and appeared in fractions eluting between 0.3 M and 0.5 M NaCl (Fig. 3). Binding of the protein kinase to the column was very dependent on pH as the enzyme did not bind to the resin when the pH was raised to 7.1. CK I was extremely unstable after this step in the purification, and it was pooled and concentrated immediately to help stabilize the activity.

**Chromatography on Hydroxylapatite—**The final step in the purification of both CK I and CK II was chromatography of the enzymes on hydroxylapatite. Both enzymatic activities adhered to the resin. CK I was removed by 0.26 to 0.32 M

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**TABLE I**

| Purification of casein kinases I and II from rabbit reticulocytes |
|---------------------------------------------------------------|
| **Enzyme** | **Step** | **Protein Units** | **Specific activity** | **Purification** |
| CK I | 1. DEAE-cellulose | 1,632 | 240 | 1,470 | 1 |
| | 2. Phosphocellulose | 39 | 71 | 16,200 | 12 |
| | 3. Sulfopropyl-Sephadex | 0.7 | 9.8 | 140,000 | 95 |
| | 4. Hydroxylapatite | 0.05 | 2.5 | 500,000 | 340 |
| CK II | 1. DEAE-cellulose | 3,038 | 1,060 | 3,490 | 1 |
| | 2. Phosphocellulose I | 1,940 | 1,040 | 5,360 | 1.5 |
| | 3. Phosphocellulose II | 11 | 660 | 340,000 | 5/7 |
| | 4. Hydroxylapatite | 2.6 | 380 | 1,460,000 | 418 |

*One unit was defined as that amount of enzyme which catalyzed the incorporation of 1 pmol of inorganic phosphate from [γ-32P]ATP into dephosphorylated casein per min at 30°C.

*Units per mg of protein.

*Purification was calculated starting with the DEAE-cellulose step rather than the postribosomal supernatant since it is difficult to determine the amount of activity contributed by CK I and CK II in the supernatant.
while CK II was eluted between 0.18 and 0.31 M potassium phosphate (Fig. 4). After a brief dialysis against Buffer B, the enzymes were concentrated by batch chromatography from 1-ml hydroxylapatite columns. They were stored at 4°C in Buffer B which contained 0.4 M potassium phosphate, pH 6.8. A typical purification of CK I and CK II is summarized in Table I.

Analysis of CK I and CK II by Polyacrylamide Gel Electrophoresis—CK I yielded a major band of molecular weight 37,000 when electrophoresed on polyacrylamide gels containing sodium dodecyl sulfate. Preincubation with [γ-32P]ATP followed by electrophoresis and autoradiography resulted in one phosphorylated band corresponding to the 37,000 molecular weight protein (Fig. 5A). CK II was analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, and three major bands with molecular weights of 42,000, 38,000, and 24,000 were observed as shown in Fig. 5B. When the enzyme was incubated with [γ-32P]ATP and analyzed by gel electrophoresis followed by autoradiography, radioactive phosphate was detected and associated exclusively with the 24,000-dalton protein. Only one-third as much phosphate was incorporated during the 30-min incubation period when equal concentrations of GTP were substituted for ATP.

Variable amounts of CK II ranging from 6 μg to 30 μg were electrophoresed, stained with Coomassie blue R-250, and scanned with a densitometer (547 nm) to determine the relative amount of protein in each band. Integration of the traces and correction for dye binding on a weight basis (31) yielded an average molar ratio of 1.3:1.0:1.6 for the 42,000, 38,000, and 24,000 molecular weight subunits, respectively.

Analytical Ultracentrifugation—CK II was centrifuged to equilibrium (based on the identical distributions obtained after 16 and 21 h of centrifugation). The long column technique of Chervenka (28) allowed data collection over a
FIG. 6. Band sedimentation in the optical ultracentrifuge. A solution containing 5 μg of CK II was layered at low speed on a buffered 0.5 M NaCl column as described under "Methods" and the rotor accelerated to 59,780 rpm. The direction of sedimentation was from left to right. Scans were made at 4-min intervals with the monochromator set at 236 nm. A, initial absorbance trace after layering; B, after 66 min at speed.

full 3 mm of the column and the logarithmic plot was linear over 70% of the distribution. A value of 144,000 ± 14,000 was calculated for the apparent weight average molecular weight of CK II. The stated error arises largely from the uncertainty in the value chosen for the apparent isopotential specific volume. In the absence of density data, we have assumed a value of 0.74 ± 0.02 ml/g. Near the bottom of the column, a limiting value of about 220,000 was calculated from the slope of the logarithmic plot (32). Sedimentation velocity experiments with CK II in the optical centrifuge showed a single peak sedimenting with an $s_{0.5}$ value of 7.5 (Fig. 6). Insufficient quantities of CK I were available to perform experiments with the optical centrifuge and, therefore, velocity experiments had to be done with sucrose density gradients. Five velocity experiments were performed which yielded a value of 3.2 ± 0.15 for the sedimentation coefficient of CK I (Fig. 7A). In these experiments, the $s_{0.5}$ value of CK II which had been self-phosphorylated with ATP was also determined. A value of 7.5 ± 0.3 S was calculated relative to ovalbumin and catalase standards, and the enzymatic activity coincided with the radiolabel incorporated during the self-phosphorylation process (Fig. 7B). Therefore, we concluded that phosphorylation of CK II did not effect a change in the molecular weight of this enzyme. Taken together, the gel electrophoresis data and the centrifuge data suggest a structure for the CK II enzyme which would be composed of two 24,000 molecular weight subunits and one each of the 42,000 and 38,000 molecular weight subunits.

Determination of $K_m$ for ATP and GTP—Lineweaver-Burk plots were constructed for CK I and for CK II when ATP and GTP were used as phosphate donors (Fig. 8). A summary of the $K_m$ values for the enzymes is given in Table II. The $K_m$ values for ATP and GTP were determined as 10 μM and 40 μM, respectively for CK II. Corresponding values for CK I were 13 μM for ATP and 900 μM for GTP. Clearly the two enzymes were distinguishable on the basis of their reactivity with GTP.

TABLE II

| Enzyme | $K_m$ ATP (μM) | $K_m$ GTP (μM) |
|--------|---------------|---------------|
| CK I   | 13            | 900           |
| CK II  | 10            | 40            |

Conditions were 50 mM Tris-HCl, pH 7.0, 10 mM MgCl₂, 140 mM KCl and 30°C as described under "Methods." Assays were initiated by the addition of substrates to varying amounts of enzyme calculated to yield optimal incorporation over the range of experimental nucleotide triphosphate concentrations used.

FIG. 7. Sucrose gradient centrifugation of CK I and CK II. Sucrose gradients (5 to 30%) were prepared in Buffer B containing 0.5 M NaCl. A, CK I (700 enzyme units); B, CK II (8000 enzyme units). Arrows indicate the positions of ovalbumin (left) and catalase (right). Enzyme activity assayed with casein and ATP ( ). Self-phosphorylation of CK II (O); 0.05-ml aliquots of the individual fractions were spotted on filter paper (1 × 2 cm) and counted directly in toluene scintillation fluid.

FIG. 8. Lineweaver-Burk plots for CK I and CK II. A, CK II (0.035 μg) and ATP; B, CK II (0.16 μg) and GTP; C, CK I (0.07 μg) and ATP; D, CK I (0.32 μg) and GTP. The reaction was initiated as described under "Methods" by addition of substrates to varying amounts of enzyme calculated to yield optimal incorporation over the range of experimental nucleotide triphosphate concentrations used.
DISCUSSION

Previous studies on the cAMP-independent protein kinases from the prothorosomal supernatant fraction from rabbit reticulocytes had shown the presence of a single peak of activity eluting from DEAE-cellulose (14). This peak was termed III_c and was identical with CK II described here. CK I was not observed when an ammonium sulfate precipitation preceded the DEAE-cellulose chromatography step. Thus, this is the initial report on the second cytoplasmic casein kinase activity, although previous studies had shown at least two casein kinase activities were associated with the protein-synthesizing complex. Centrifugation through 0.5 M NaCl dissociated these latter activities from the complex (90). Kumar and Tao (15) have reported two cAMP-independent protein kinase activities from rabbit erythrocytes. These enzymes were similar chromatographically to CK II and utilized both ATP and GTP in the phosphotransferase reaction. The K_μ values for ATP and GTP differed significantly from those reported here; however, this may be due to the fact that their studies were carried out at pH 9.0. We have observed that CK II aggregated when the monovalent salt concentration was less than 0.5 M. This may account for the very high molecular weight values observed by Kumar and Tao (15) and suggests that the two peaks may be different aggregation states of CK II.

The anomalous behavior of CK II on phosphocellulose has been noted. When the small amount of activity (usually less than 10%) which binds under conditions of high salt was carried through the sulfopropyl-Sepharose and hydroxylapatite steps, a high molecular weight contaminant was found to co-chromatograph. This contaminant was not present in the phosphocellulose flow-through fraction which contained the majority of the CK II activity. Therefore, we have routinely included phosphocellulose chromatography at low salt in our procedure, even though only a small overall purification was realized.

Both CK I and CK II lose activity rapidly in the latter stages of purification which we attribute to the general decline in protein concentration. It is important, therefore, to maintain stock solutions of these enzymes at the highest practical concentrations of protein. We have found that this was accomplished most satisfactorily by batch elution from small (1 to 2 ml) hydroxylapatite columns (95 to 100% yield).

A subunit molecular weight of about 37,000 was determined for CK I by gel electrophoresis. Assuming a globular shape for CK I, the s value of 3.2 obtained via centrifugation in sucrose translates to about 37,000 (33). This suggests that CK I is a single subunit enzyme. The molecular weight data from gel electrophoresis and the ultracentrifuge are consistent with a heterogeneous subunit structure for CK II. An enzyme with subunit molecular weights of 42,000, 38,000, and 24,000 in a ratio of 1:1:2 as suggested by gel electrophoresis would yield a native molecular weight of 128,000 ± 6,500. This would be consistent with both the molecular weight of 144,000 ± 14,000 obtained by equilibrium centrifugation and the 7.5 S velocity coefficient. A similar structure has been proposed for a casein kinase activity purified from Novikoff ascites tumor cells (17) and rat liver (18). Our finding that only the smaller subunit (24,000) is self-phosphorylated is also similar to that found by others (17). The radiolabeling of the native 7.5 S complex confirmed the gel electrophoresis data which suggested that it is indeed a subunit of CK II. Attempts to further purify CK II by binding the enzyme to adenosine-agarose and ATP-Sepharose or by chromatography on Sephadex G-100 showed no alteration in the subunit pattern. When the time course for the self-phosphorylation of CK II was examined, 1.7 mol of phosphate were incorporated per 130,000 g of CK II, with 97% of the total radioactivity in the 24,000 dalton subunit. This result would be consistent with the proposed αβ2 structure with one phosphate incorporated per 24,000 molecular weight β subunit. No reduction in enzymatic activity was detected over the time course of the self-phosphorylating experiment measured in the standard casein assay. It is interesting that CK I is also self-phosphorylated, indicating that this may be a common type of control mechanism. It has been shown that the cyclic AMP-regulated protein kinase from bovine cardiac muscle can phosphorylate its own regulatory component (34). This modification controls the enzymatic activity by altering the rate of subunit reassociation (35). Recently the cGMP-dependent protein kinase from bovine lung has also been shown to be a substrate for its own enzymatic activity (36).

CK I and CK II differ enzymatically with respect to nucleotide specificity. CK I preferentially utilizes ATP, and CK II uses both ATP and GTP. In addition, the two protein kinases have been shown to modify different residues in the B variant of β-casein (37). These residues are different from those phosphorylated by the cAMP-regulated protein kinase from reticulocytes. CK I and CK II also differ in specificity toward translational initiation factors. The highly purified preparation of CK I phosphorylates eIF-4B and eIF-5 while CK II modifies eIF-2, eIF-3, eIF-4B, and eIF-5 (20).

The biological role of the cAMP-independent protein kinases remains to be fully elucidated. The recent identification of the hemic-controlled repressor (HCR) as a cyclic nucleotide-independent protein kinase (38-42) along with the discovery that both CK I and CK II phosphorylate certain initiation factors suggest a possible role in the regulation of eukaryotic protein synthesis for these enzymes.

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Purification of Casein Kinases

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Cyclic nucleotide-independent protein kinases from rabbit reticulocytes.
Purification of casein kinases.
G M Hathaway and J A Traugh

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