Subunit Structure of Casein Kinase II from Bovine Testis

DEMONSTRATION THAT THE \( \alpha \) AND \( \alpha' \) SUBUNITS ARE DISTINCT POLYPEPTIDES*

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The relationship between the \( \alpha \) and \( \alpha' \) subunits of casein kinase II was studied. For this study, a rapid scheme for the purification of the enzyme from bovine testis was developed. Using a combination of chromatography on DEAE-cellulose, phosphocellulose, hydroxylapatite, gel filtration on Sephacryl S-300 and heparin-agarose, the enzyme was purified approximately 7,000-fold. The purification scheme was completed within 48 h and resulted in the purification of milligram quantities of casein kinase II from 1 kg of fresh bovine testis. The purified enzyme had high specific activity (3,000–5,000 nmol of phosphate transferred per min/mg protein) when assayed at 30 °C with ATP and the synthetic peptide RRDDDDDDDD as substrates. The isolated enzyme was a phosphoprotein with an alkali-labile phosphate content exceeding 2 mol/mg protein. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis three polypeptides were apparent: \( \alpha' \) (Mr 45,000), \( \alpha' \) (Mr 40,000), and \( \beta \) (Mr 26,000). Several lines of evidence conclusively demonstrated that the \( \alpha \) and \( \alpha' \) subunits are distinct polypeptides. Two-dimensional maps of \( \alpha' \)-tryptic peptides derived from the two proteins were related, but distinct. An antipeptide antibody was raised in rabbits against the \( \alpha' \) subunit and failed to react with either the \( \alpha' \) or \( \beta \) subunits. Direct comparison of peptide sequences obtained from the \( \alpha \) and \( \alpha' \) subunits revealed differences between the two polypeptides. The results of this study clearly demonstrate that the \( \alpha \) and \( \alpha' \) subunits of casein kinase II are not related by post-translational modification and are probably encoded by different genes.

Casein kinase II (CKII) is a widely distributed messenger-independent protein serine/threonine kinase (Hathaway and Traugh, 1982; Edelman et al., 1987). In addition to its ubiquitous distribution in different tissues and species, the enzyme has been found in different subcellular compartments. CKII or similar enzymes have been identified in the cytosol, in the nucleus (Matthews and Huebner, 1984), in mitochondria (Danuni and Reed, 1988), and associated with a microsomal fraction (Singh and Huang, 1985). Among protein kinases, the enzyme is notable for its ability to utilize GTP as well as ATP as a phosphate donor (Hathaway and Traugh, 1982). It is activated in vitro by polycationic compounds such as polyamines (Hathaway and Traugh, 1984) and inhibited by polyanionic compounds such as heparin (Hathaway et al., 1980). However, the physiological relevance of these observations remains unknown. More recently, it has been demonstrated that CKII activity is elevated in soluble extracts of cultured cells that have been treated with insulin or growth factors whose receptors are protein tyrosine kinases (Sommercorn et al., 1987; Klarlund and Czech, 1988; Carroll and Marshak, 1989; Ackerman and Osheroff, 1989).

CKII has been purified from a variety of different mammalian tissues and generally consists of a tetramer with an \( \alpha \beta \) or \( \alpha \alpha' \beta' \) structure (Hathaway and Traugh, 1979; Dahmus, 1981; DePaoli-Roach et al., 1981; Huang et al., 1982; Cochet et al., 1983; Zandomeni et al., 1988). The molecular weight of the holoenzyme is 130,000 while the molecular weights of the individual subunits as assessed by SDS-polyacrylamide gel electrophoresis are as follows: \( \alpha \) (41,000–14,000), \( \alpha' \) (37,000–42,000), and \( \beta \) (24,000–26,000). Based on the ability of the \( \alpha \) subunit to bind fluorosulfonylbenzoyl adenosine (Hathaway et al., 1981; Feige et al., 1983) and on its sequence identity with other protein kinases, the \( \alpha \) subunit has been identified as the catalytic subunit of CKII (Takio et al., 1987, Saxena et al., 1987, Chen-Wu et al., 1988; Meisner et al., 1989). The amino acid sequence of the \( \beta \) subunit was initially derived by sequencing the isolated subunit from bovine lung (Takio et al., 1987); however, the function of this subunit remains unknown. When direct sequencing of the \( \alpha \) subunits was attempted the results suggested that sequence heterogeneity existed for the \( \alpha \) subunits of bovine CKII (Takio et al., 1987). Since the sequence was derived from a mixture of the \( \alpha \) subunits, it was not possible to ascribe specific sequence to the \( \alpha \) and \( \alpha' \) bands visualized on SDS-polyacrylamide gels. Although there is evidence that the \( \alpha \) and \( \alpha' \) subunits are closely related, the relationship between the two subunits has not been resolved. Some studies have suggested that the \( \alpha \) and \( \alpha' \) subunits are the products of distinct gene products (Dahmus et al., 1984; Takio et al., 1987), as is the case in yeast (Padmanabha and Glover, 1987; Chen-Wu et al., 1988). Other investigations have implied that the two subunits are related through post-translational modification, most...
likely proteolysis (Hathaway and Traugh, 1982; Zandonemi et al., 1988).

To facilitate our studies on the regulation of this protein kinase and to extend our physical characterization of the enzyme, we have devised a rapid scheme for the purification of milligram quantities of the enzyme. Taking advantage of the high levels of CKII activity that are found in testis (Singh and Huang, 1985; Krebs et al., 1988), we have used bovine testis as our source of material. Evidence obtained by analysis of tryptic peptide maps, by differences in immunological reactivity and by direct comparison of peptide sequences derived from the two polypeptides indicated that the α and α' subunits of the bovine testis enzyme are distinct polypeptides, not related by post-translational events.

**Experimental Procedures**

**Materials**—Synthetic peptides were synthesized by Dr. P. Chou and H. Zebroski of the Chemical Synthesis Facility of the Howard Hughes Medical Institute at the University of Washington, Seattle, as described previously (Kuenzel et al., 1987). DEAE-cellulose (DE52) and cellulose (C11) were purchased from Whatman. High resolution hydroxyapatite was purchased from Calbiochem. Trypsin (treated with diphenylcarbamyl chloride), collagenase, and protease inhibitors, polyvinylpyrrolidone 40, and heparin-agarose were from Sigma. [γ-^32P]ATP and [α-^32P]ATP (specific activity 100 cpm/pmol) and 0.5 mM synthetic peptide substrate (RRRDSSDDDD) in a total volume of 50 µl. Reactions were started by the addition of 6 µl of column fraction or purified enzyme and terminated after 5–10 min by spotting 20 µl of the reaction mixture of P-81 phosphocellulose paper as described previously (Kuenzel and Krebs, 1988; Kuenzel et al., 1987). CKII containing fractions were diluted to ensure that hydrolysis of substrate did not exceed 10%. One unit of CKII activity is defined as the amount of activity required to transfer 1 nmol of phosphate into substrate/min at 30 °C.

**Tryptic Peptide Mapping**—The α and α' subunit bands of CKII were excised from Coomassie Blue-stained SDS-polyacrylamide gels and washed extensively with 10% methanol. The gel slices were dried, then iodinated using Na¹²¹I (50 µCi/gel slice) according to the method of Elder et al. (1977). After external washing with 10% methanol, the gel slices were dried and the iodinated protein bands digested extensively with trypsin by modification of the method of Elder et al. (1977). Briefer, the dried gel slices were cut into several small pieces and rehydrated in 0.5 ml of 50 mM NH₄HCO₃ containing 0.05 mg/ml trypsin. After incubation for 22 h at 37 °C, the supernatant was removed. An additional 5 µg of trypsin was added to this supernatant and digestion continued for another 2 h. The supernatants containing the peptides were then lyophilized three times with water before being resuspended in 20 µl of 1.5 mM ethylenediaminetetraacetic acid, 15 mM sodium succinate, pH 3.0, 50 µM APRT. After incubation for 22 h at 37 °C, the supernatant was removed.

**Preparation of Antipeptide Antibodies—Subunit-specific antipeptide antibodies to the α and α' subunits of CKII were prepared as follows. A synthetic peptide (SHSYDYDKYKQRI) based on sequence derived from the γ subunit of CKII (Takio et al., 1987) was coupled to bovine serum albumin using glutaraldehyde (Doolittle, 1986). The same peptide containing an N-terminal cysteine was conjugated to keyhole limpet hemocyanin using m-maleimidobenzoic acid N-hydroxysuccinimide ester (Doolittle, 1986). Mixtures of these conjugates were used to immunize New Zealand White rabbits. Similarly, a peptide (KYILDELPDEELED) based on sequence derived from the α subunit of bovine CKII was conjugated to keyhole limpet hemocyanin using m-maleimidobenzoic acid N-hydroxysuccinimide ester (Takio et al., 1987). Mixtures of these conjugates were used to immunize New Zealand White rabbits. Rabbits were given initial injections of peptide-conjugate (1.5 mg) mixed with an equal volume of Freund's complete adjuvant. The rabbits were boosted with peptide-conjugate (1.5 mg) either in phosphate-buffered saline (20 mM sodium phosphate, pH 7.2, 0.15 M NaCl) or mixed with an equal volume of Freund's incomplete adjuvant. The rabbits were bled 7–10 days after booster injections.

**Immunoblotting**—CKII (0.6 µg) was subjected to electrophoresis on 12% SDS-polyacrylamide gels and transferred to nitrocellulose (Towbin et al., 1979) using a Bio-Rad transblot apparatus. The nitrocellulose strips were blocked with 3% gelatin in Tris-buffered saline (10 mM Tris-Cl, pH 7.5, 500 mM NaCl) and were then incubated overnight with antipeptide antiserum at a dilution of 1:250 in Tris-buffered saline containing 0.05% gelatin. After washing, the nitrocellulose strips were incubated with a 1:5000 dilution of biotinylated antirabbit immunoglobulin G was utilized as secondary antibody with 4-chloro-1-naphthol as substrate. Procedures were performed according to the manufacturer's recommendation.

**Partial Sequencing of α and α' Subunits** Internal peptide se-
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sequence of the α and α′ subunits of CKII was obtained using the methodology of Aebersold et al. (1987). CKII (500 pmol) was subjected to electrophoresis on a preparative 12% SDS-polyacrylamide gel and then transferred to nitrocellulose (Towbin et al., 1979). After visualizing the α and α′ subunit bands by staining the nitrocellulose with Ponceau S, the bands were excised and placed on a Pierce Chemical Co. RP-300 C4 column (21 × 30 mm) connected to a Hewlett-Packard 1000 system equipped with an analytical workstation. The column was developed with a 60-60% acetonitrile gradient (1% acetonitrile/min) in 0.1% trifluoroacetic acid. The flow rate was 0.3 ml/min. Those peptide peaks that were judged to be suitable for sequencing were sequenced by automated Edman degradation using an Applied Biosystems model 470A gas phase sequencer. Phenylthiohydantoin-derivated amino acids were separated using an on-line microbore HPLC system (model 120A).

Other Procedures—SDS-polyacrylamide gel electrophoresis was performed using 12% gels and the buffer system of Laemmli (1970). For two-dimensional gels, the first dimension was nonequilibrium pH gradient electrophoresis (NEPHGE) performed according to the method of O'Farrell et al. (1977). NEPHGE was done for 2400 volt h using pH 3-10 ampholytes. Alkaline-labile phosphate containing samples were separated using purified CKII (1-3 nmol) as described by Guy et al. (1981), with phosphate detected according to the method of Ames (1966). Phosphorylase a and phosphorylase b were utilized as internal controls for this procedure. Protein determinations were done according to the procedure of Bradford (1976) using bovine serum albumin as standard.

RESULTS

Purification of Casein Kinase II and Subunit Characterization—Since testis contains very high CKII activity (Krebs et al., 1988), we made use of bovine testis to develop a rapid scheme for the purification of milligram quantities of the enzyme. Although the purification scheme involves five chromatographic steps, utilization of batch adsorption and elution for the first two steps facilitates rapid completion of the protocol. The procedure can be completed within 48 h and results in a yield of approximately 3 mg of pure CKII/kg of testis. The results of a typical purification are summarized in Table I. The procedure has an overall yield exceeding 30% with a purification of approximately 7000-fold. Furthermore, the specificity of the activity of the preparation exceeds that reported for most of the published purifications of CKII.

By SDS-polyacrylamide gel electrophoresis (Fig. 1a), three distinct polypeptides are apparent: α (M, 45,000), α′ (M, 40,000), and β (M, 26,000). A minor band with a molecular weight slightly less than that of α′ was also present in some preparations and appears to be a proteolytic product of one of the α subunits since it was not always present. Upon prolonged storage at 4 °C, some partial proteolysis of both the α and α′ subunits was observed when the stored enzyme was analyzed by SDS-polyacrylamide gel electrophoresis (data not shown). Under these circumstances the α and α′ subunits showed diminished apparent molecular weights and a number of smaller proteolytic products were visible. A loss of activity did not accompany the proteolytic events. The proteolytic sensitivity of the α and α′ subunits of CKII may be partially responsible for some of the confusion that exists in the literature concerning the relationship of the α and α′ subunits.

Through the use of protease inhibitors during homogenization and by completing the purification promptly, proteolysis of the α and α′ subunits during purification is almost completely prevented. This point was of particular importance for carrying out the physical comparisons of the two subunits. Two ensure that the subsequent analyses of the two subunits were conducted with intact subunits, aliquots of CKII were boiled immediately after purification in sample buffer for SDS-polyacrylamide gel electrophoresis (Laemmli, 1970). This treatment prevented further proteolysis and was compatible with the further analyses.

An an initial experiment to compare the properties of the α and α′ subunits of CKII, the enzyme was subjected to two-dimensional electrophoretic separation (Fig. 1b). In preliminary experiments (data not shown), the two subunits did not enter isoelectric focusing gels. Therefore, it was necessary to make use of NEPHGE as the first dimension for the two-dimensional separations. Examination of Fig. 1b demonstrates that the α and α′ subunits can be clearly resolved with this technique, α′ being the more basic of the two polypeptides. It is also apparent that each subunit resolves into two

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**Table I**

<table>
<thead>
<tr>
<th>Total protein</th>
<th>Total activity*</th>
<th>Specific activity</th>
<th>Yield</th>
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<tr>
<td>mg</td>
<td>units</td>
<td>units/mg</td>
<td>%</td>
</tr>
<tr>
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<tr>
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<td>Phosphocellulose</td>
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<td>24,675</td>
<td>513</td>
</tr>
<tr>
<td>Hydroxylapatite</td>
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<td>11,976</td>
<td>1,409</td>
</tr>
<tr>
<td>Sephacryl S-500</td>
<td>5.0</td>
<td>12,650</td>
<td>2,530</td>
</tr>
</tbody>
</table>

Heparin-agarose

Pool 1: 1.9 | 6,384 | 3,360 | 18

Pool 2: 1.1 | 5,643 | 5,093 | 16

Total: 3.0 | 11,927 | 4,439 | 34

*One unit of CKII is defined as the amount of enzyme required to transfer 1 nmol of phosphate from ATP into synthetic peptide substrate/min at 30 °C. The peptide RRDDDSDDDD was utilized for these determinations.

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**Fig. 1.** Electrophoretograms of purified casein kinase II. a, purified bovine testis casein kinase II (5 μg, right lane) was subjected to electrophoresis on a 0.75-mm 12% SDS-polyacrylamide minigel, which was stained with Coomassie Blue. The positions of the individual subunits are marked: α (M, 45,000), α′ (M, 40,000), and β (M, 26,000). Molecular weight markers (left lane) are as follows from top to bottom: phosphorylase b (97,400), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,000), and lysozyme (14,400). b, purified bovine testis casein kinase II (3 μg) was subjected to NEPHGE for 2,400 volt h using pH 3-10 ampholytes in a 3-mm diameter tube. NEPHGE is illustrated in the horizontal dimension with the cathode (base) to the right and the anode (acid) to the left. Following NEPHGE, the polyacrylamide gel rod was stained with silver. The subunits of casein kinase II were visualized by silver staining. The major isoelectric variants of the α and α′ subunits are marked with arrowheads.
distinct spots. This result suggests that there may be subforms of the individual subunits that may be related by post-translational modification.

As isolated, the purified enzyme contains 2.3 ± 0.2 mol of alkali-labile phosphate/mole protein as determined by direct analysis of phosphate released following treatment of CKII with 1 N NaOH. When subjected to autophosphorylation, an additional 0.3 mol of phosphate/mole holoenzyme can be incorporated into the β subunit of CKII (data not shown). This incorporation is considerably less than the 1–2 mol of phosphate/mole holoenzyme that has been previously observed by other investigators (Hathaway and Traugh, 1979; Meggio and Pinna, 1984) and suggests that the enzyme we have isolated is already heavily phosphorylated on its β subunit, which under most circumstances is the site of autophosphorylation. Consistent with this interpretation is the fact that β appears as a doublet on SDS-polyacrylamide gels (Fig. 1a) and is readily converted into a single band upon addition of MgATP (data not shown). The observation of potential subforms for the α and α′ subunits of CKII on two-dimensional gels implies that these two subunits may also contain phosphate. Indeed, under certain conditions, CKII has been reported to autophosphorylate its α subunits (Meggio et al., 1983). We do not know, however, if the isoelectric variants of α or α′ identified on two-dimensional gels (Fig. 1b) are related by phosphorylation.

**Peptide Maps and Immunological Studies of the α and α′ Subunits of Casein Kinase II**—The α and α′ subunits of CKII were separated by SDS-polyacrylamide gel electrophoresis, iodinated, and then subjected to extensive tryptic digestion. Two-dimensional maps of the resultant peptides (Fig. 2) demonstrate that there are similarities between maps of tryptic peptides derived from the two polypeptides. This observation is consistent with results previously reported (Dahmus et al., 1984). However, there are also a number of peptides that are unique to each protein. In particular, the peptide map of α contains three unique spots (marked by closed arrows in Fig. 2) and the peptide map of α′ contains five unique spots (marked by open arrows in Fig. 2). If the α′ subunit were derived from α through proteolysis, it would be expected that the α′ subunit would have at most two unique peptides. Thus, our results clearly suggest that the α and α′ subunits are related, but distinct, polypeptides.

Partial sequencing of a mixture of α and α′ subunits of bovine lung CKII revealed sequence heterogeneity (Takio et al., 1987). At that time, the relationship of the derived sequences to the α and α′ bands seen on SDS-polyacrylamide gel electrophoresis was not established. To develop subunit-specific reagents to address this issue, antipeptide antibodies were raised in rabbits against a peptide sequence (EDLY-DYIDKYNIEL) which corresponded to a region of the α and α′ subunits that showed sequence heterogeneity. This region is identical to amino acids 252–265 as encoded by cDNA clones from a Hep G2 library (Meisner et al., 1989) or from a human T-cell library. Similarly, antipeptide antibodies directed against a 14-amino acid sequence of the β subunit were produced in rabbits. Attempts to immunoprecipitate purified CKII or CKII from cell extracts with either of the antisera were unsuccessful; however, the antisera reacted with purified CKII on immunoblots. The reactivity of the antipeptide antibodies against the different subunits of purified bovine testis CKII on immunoblots is shown in Fig. 3. Antiserum from a rabbit immunized with peptide based on the α sequence clearly recognizes the α subunit (M, 45,000) but fails to react with either the α′ or β subunits. In contrast, antiserum from a rabbit immunized with peptide based on sequence derived from the β subunit reacts exclusively with the β subunit of purified CKII. From these results, we have concluded that the sequence used for immunization of rabbits was derived from the α subunit (M, 45,000). The results also reinforce the hypothesis that the α and α′ subunits are distinct since proteolytic removal of the immunoreactive sequence would produce a polypeptide considerably smaller than α′ (M, 40,000). We have not yet obtained an antipeptide antibody that is specific for the α′ subunit of CKII.

**Internal Sequence Analysis of the α and α′ Subunits of Casein Kinase II**—As indicated above, partial sequence data derived from the mixture of α and α′ subunits of bovine lung CKII had revealed sequence heterogeneity (Takio et al., 1987), however, it was not possible to ascribe individual peptide sequences to the α and α′ bands seen by SDS-polyacrylamide gel electrophoresis. Thus, to extend the analyses that we have presented in previous sections, we sought sequence information from the α and α′ subunits that had been separated on SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. The individual subunits were digested with the lysine-specific Achromobacter protease I and the cleavage products separated on a C8 reverse phase column (Fig. 4). The relationship of the peptide profiles of α and α′ is consistent with the subunits being related but distinct. Selected peaks from these profiles were subjected to sequence analysis (summarized in Fig. 5). The three peaks originating from the α subunit of CKII each proved to be mixtures of two peptides.

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Fig. 2. Two-dimensional tryptic peptide mapping of the α and α′ subunits of casein kinase II. Following separation of the α and α′ subunits of casein kinase II by SDS-polyacrylamide gels, the individual proteins were iodinated and digested with trypsin. The tryptic peptides were then applied at the origin (marked with α) and subjected to electrophoresis at pH 1.9 (horizontal dimension with anode at left) and then to chromatography (vertical dimension). Peptides derived from α (a) and α′ (b) were mapped individually. An additional peptide map (c) contained a 1:1 mixture (based on counts/min) of peptides derived from the two polypeptides. The peptides that are unique to α are marked (●) as are the peptides that are unique to the α′ subunit (△). Major peptides common to both α and α′ are marked (*).
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**FIG. 3. Immunoblotting of casein kinase II.** Purified casein kinase II (0.6 μg) was subjected to electrophoresis on 12% SDS-polyacrylamide gels and transferred to nitrocellulose. Nitrocellulose strips were probed with antipeptide antisera (1:250 dilution) that were raised against peptides based on sequences obtained from either the α subunit (lane a) or the β subunit (lane b) of casein kinase II. The blots were developed using horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G as described under “Experimental Procedures.” The positions of the α, α′, and β subunits of casein kinase II are marked.

**FIG. 4. Cleavage products of α and α′ subunits of casein kinase II.** The α and α′ subunits of bovine testis casein kinase II were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. The individual proteins were then subjected to digestion with Achromobacter protease I as described under “Experimental Procedures.” The peptides that were released from the nitrocellulose by proteolysis were then separated by reverse-phase HPLC on a 2.1 x 30 mm RP300 C18 column. The column was run at a flow rate of 0.3 ml/min and was developed with a 0–60% acetonitrile gradient in 0.1% trifluoroacetic acid over 60 min. Peptides were detected at 206 nm. The peaks that were analyzed by automated Edman degradation are marked (peaks 1–7).

**FIG. 5. Peptide sequences obtained by automated Edman degradation.** Peptides derived from the α and α′ subunits of casein kinase II were obtained as described in the legend to Fig. 4 and subjected to automated Edman degradation. When a peak contained a mixture of peptides, the percentage of the total accounted for as each peptide (a, b) is indicated (as determined at cycle 1 for peaks 1–3 and at cycle 2 for peak 4). Amino acid differences between α and α′ are underlined and indicated in bold type.

**FIG. 6. Comparison of the partial peptide sequences of bovine testis casein kinase II with the derived amino acid sequence of the α subunit of human casein kinase II.** The sequence of the α subunit of human casein kinase II (line 1) was derived from the nucleotide sequence of a cDNA isolated from a Hep G2 library (Meisner et al., 1989) and is identical to that obtained from a cDNA isolated from a human T-cell library. Amino acid sequences of peptides derived from the α (line 2) and α′ (line 3) subunits of bovine testis casein kinase II were obtained as described in previous figure legends. Amino acids that are different between the α and α′ subunits are underlined and indicated in bold type.

For peaks 1 and 2, major and minor peptides could be distinguished in each and peak 3 was an equimolar mixture of two peptides. Although the three peaks originating from the α subunit CKII each proved to be a mixture of two peptides, correct sequence assignment was possible since all six sequences derived from these peaks were previously determined unambiguously by analyses of isolated peptides from the bovine lung enzyme. The six sequences obtained from the α (M, 45,000) subunit all showed complete identity with the amino acid sequences encoded by cDNAs isolated from a Hep...
The (Y subunits of CKII can be autophosphorylated under certain conditions (Meggio et al., 1983). To date, the function of CKII is activated by treatment of 3T3-Ll adipocytes or H4-IIE hepatoma cells with insulin or by treatment of CKII isolated from rat liver nuclei (Qi et al., 1986). The demonstration that the (Y and (Y' subunits of CKII are distinct polypeptides that are not related by post-translational modification.

The peptide sequence information provides an explanation for the observation that the (Y, but not (Y', reacts with an antipeptide raised against a synthetic peptide (EDLYDYDKYN-IEL). Only the (Y subunit contains this peptide sequence identical to that of the synthetic peptide (Fig. 5 and residues 252–265 in Fig. 6). By comparison, the corresponding peptide sequence from (Y' (DELYGYLK-YHIDL) differs from that of (Y at seven positions.

**DISCUSSION**

Taking advantage of the high activity of CKII in testis, we have developed a protocol for the rapid purification of milligram quantities of the enzyme from bovine testis. The enzyme is purified in high yield, has high specific activity and maintains that activity when stored for a year at 4 °C. The protocol may be useful for the purification of CKII from other sources, although its application to bovine lung gave a lower overall yield of enzyme. It is of interest to note that the bovine lung enzyme had the same subunit composition as the testis enzyme as judged by SDS-polyacrylamide gel electrophoresis (results not shown).

As isolated from bovine testis in the absence of phosphatase inhibitors, CKII contains approximately 2 equivalents of phosphate/mol of tetramer. It is likely that most of the phosphate is incorporated into the (Y subunit, which is normally the subunit that is autophosphorylated (Hathaway and Traugh, 1979; Meggio and Finn, 1984). However, the observation of different isoelectric variants of the (Y and (Y' subunits suggests that there may be additional phosphorylation of these subunits. Isoelectric variants have also been identified for the (Y and (Y' subunits of protein kinase NII (the nuclear form of CKII) isolated from rat liver nuclei (Qi et al., 1986).

Alkaline phosphatase treatment of that kinase altered the isoelectric pattern of the (Y and (Y' subunits, clearly implying that the (Y and (Y' subunits of CKII can be modified by phosphorylation. The protein kinase(s) responsible for this phosphorylation are not known, but it should be noted that the (Y subunits of CKII can be autophosphorylated under certain conditions (Meggio et al., 1983). To date, the functional consequences of phosphorylation, autophosphorylation, or dephosphorylation have not been conclusively identified.

However, CKII is activated by treatment of 3T3-L1 adipocytes or H4-IIE hepatoma cells with insulin or by treatment of A431 cells with epidermal growth factor, and phosphorylation may be involved (Sommercorn et al., 1987; Ackerman and Osheroff, 1989). Since the epidermal growth factor-activated enzyme was inactivated by treatment with immobilized alkaline phosphatase, phosphorylation is again indicated (Ackerman and Osheroff, 1989).

In this report, differences between the (Y and (Y' subunits of CKII have been observed in their electrophoretic behavior, immunological reactivity, peptide map patterns, and amino acid sequences. Taken together these results conclusively demonstrate that the two subunits of the bovine testis enzyme are distinct, but related, polypeptides. This conclusion extends the observations previously obtained by examination of CKII from calf thymus (Dahmus et al., 1984) and bovine lung (Takio et al., 1987). However, a number of reports have suggested that the (Y subunit is a proteolytic fragment of the (Y subunit (Hathaway and Traugh, 1982; Zandomeni et al., 1988). We have also observed proteolytic fragments of (Y that migrate very closely to the (Y' subunits or its fragments on SDS-polyacrylamide gels. Alternatively, some mammalian sources may contain CKII with a subunit composition similar to that of the Drosophila enzyme (Glover et al., 1983) with its single form of a subunit.

The amino acid sequence data obtained for the peptides derived from the (Y subunit were identical to that encoded by cDNA clones isolated from Hep G2 (Meisner et al., 1989) and from human T-cell cDNA libraries. Thus, it appears that these clones encode the (Y subunit (M, 45,000) of CKII. The alternative sequences of peptides derived from the (Y' subunit differed from the corresponding peptides from the (Y subunit at 12 positions out of the 70 residues from (Y' that were examined. All of these peptide sequences were observed during analysis of the mixture of (Y and (Y' subunits of the bovine lung enzyme. Moreover, sequence deduced from a cDNA isolated from a human T-cell library contains 11 of the amino acid substitutions observed for the (Y' derived peptide sequences. Taken together, these results suggest that the (Y and (Y' subunits are encoded by different gene products, or are derived by alternate splicing of the same gene. The former possibility is more likely since 31 loci of sequence heterogeneity for the mixture of (Y and (Y' subunits of the bovine lung enzyme (Takio et al., 1987) were detected throughout the sequence and did not seem to be restricted to one particular domain. The results also suggest virtual identity between the subunits of CKII from bovine lung and bovine testis and even near identity of the bovine enzyme with the human enzyme.

The demonstration that the (Y and (Y' subunits of CKII are distinct polypeptides raises a number of interesting questions. It is appealing to postulate that there may be differences in the functional properties of the two subunits. It should be interesting to compare the two subunits in their substrate specificities, nucleotide utilization, and sensitivity to polyions and polycations. Although the native molecular weight of CKII is consistent with an (Yβ2 holoenzyme structure, there are three possible arrangements of (Y and (Y' subunits that conform to this structure; (Yβ2, (Y'β2, and (Yαβ. Which of these possible structures exist in vivo has not been established. In any event, our results demonstrate that there are isozymic forms of CKII. Protein kinases, in particular protein kinase C (Nishizuka, 1988), and cAMP-dependent protein kinase are known to exist in different isozymic forms (Uhler et al., 1986; Showers and Maurer, 1986).

CKII has been detected in virtually all mammalian tissues examined and although the majority of activity can be recovered in the cytosolic fraction, CKII or similar activities have been identified in a number of cellular compartments. Proteins that have been identified as substrates for CKII are localized within, or are associated with, many of these cellular compartments. The tissue and subcellular distribution studies...
have relied on enzyme activity measurements; no indication of the subunit composition of the enzyme was obtained. As in our own study, attempts to characterize the cell types or cellular compartments from which the CKII was purified have generally not been made. Therefore, whether or not different forms of the enzyme are localized within different cellular compartments remains to be established. There may even be differential expression of the $\alpha$ and $\alpha'$ in different tissues or at various stages of development within the same tissue.

The development of reagents, in particular cDNA probes and antibodies, specific for the $\alpha$ and $\alpha'$ subunits of CKII will allow examination of some of these issues. Information derived from these analyses will undoubtedly yield important clues concerning the physiological functions of CKII.

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Subunit Structure of Casein Kinase II
Subunit structure of casein kinase II from bovine testis. Demonstration that the alpha and alpha' subunits are distinct polypeptides.
D W Litchfield, F J Lozeman, C Piening, J Sommercorn, K Takio, K A Walsh and E G Krebs

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