THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 259, No. 14, Issue of July 25, pp. 9001-9006, 1984
Printed in U.S.A.

Similarities in Structure and Function of Calf Thymus and *Drosophila* Casein Kinase II*

(Received for publication, March 5, 1984)

Grace K. Dahmus‡, Claiborne V. C. Glover§¶, Douglas L. Brutlag¶‖, and Michael E. Dahmus‡**

From the ‡Department of Biochemistry and Biophysics, University of California, Davis, California 95616 and the ¶Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Both calf and *Drosophila* contain a type II casein kinase with similar molecular structure and catalytic activity. Purified calf thymus casein kinase II is composed of three subunits of M_r = 44,000 (α), 40,000 (α'), and 26,000 (β) (Dahmus, M. E. (1981) *J. Biol. Chem.* 256, 3319–3325), whereas the *Drosophila* enzyme is composed of two subunits of M_r = 36,700 (α) and 28,200 (β) (Glover, C. V. C., Shelton, E. R., and Brutlag, D. L. (1983) *J. Biol. Chem.* 258, 3258–3265). The native form of the enzyme is an $\alpha_2\beta_2$ tetramer. Polyclonal antibodies prepared against each enzyme react with both the α and β subunits of the homologous enzyme and cross-react with both subunits of the heterologous enzyme. Reaction of polyclonal antibodies with proteins resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis establishes that no significant difference in subunit molecular weight exists between the purified enzymes and the enzyme present in initial cell extracts. Each antibody effectively inhibits the *in vitro* activity of the homologous enzyme and causes a slight inhibition in the activity of the heterologous enzyme. Peptide maps derived from purified subunits indicate that the α and β subunits are unique and that there is extensive primary sequence homology between the corresponding subunits of the calf and *Drosophila* enzyme. Casein kinase II from both sources phosphorylates the same subunits of calf thymus RNA polymerase II and an identical set of proteins in a complex mixture of acid-soluble proteins from *Drosophila* tissue culture cells. The striking similarity in molecular structure and catalytic activity between the calf and *Drosophila* enzyme suggests that casein kinase II has been highly conserved in evolution.

Phosphorylation of protein substrates has proven to be extremely common in eukaryotic cells, and it is now well recognized that this modification plays a major role in regulating the activity of both enzymatic and structural proteins (Krebs and Beavo, 1979; Cohen, 1982). A variety of enzymes that catalyze the phosphorylation of protein substrates have been purified to homogeneity and characterized in detail (Flockhart and Corbin, 1982). Although the physiological role of some of these protein kinases (*e.g.* cAMP-dependent kinase) is well understood, the function of others remains obscure.

Casein kinases are cyclic nucleotide-independent protein kinases that phosphorylate casein and phosvitin *in vitro* but not histones or protamines (Hathaway and Traugh, 1982). Casein kinases that have been purified to date are of two types. The type I enzyme is composed of a single subunit of M_r = 30,000–40,000 and exists in solution as a monomer; the type II enzyme is composed of two dissimilar subunits, α (M_r = 37,000–44,000) and β ($M_r \simeq$ 26,000), and exists in solution as an $\alpha_2\beta_2$ tetramer. Both enzymes appear to be ubiquitous in eukaryotic organisms, and each phosphorylates a broad spectrum of endogenous substrates including translational initiation factors, membrane proteins, proteins associated with mRNP particles, glycogen synthase, and nonhistone nuclear proteins. They are not localized to any particular tissue and can be isolated from a number of subcellular compartments including nuclei, cytosol, membranes, and ribosomes. Although the function of casein kinases is unknown, their broad substrate specificity and wide distribution suggest that they play an important role in the regulation of cellular activity. They are not related to the enzyme that functions in the phosphorylation of casein *in vivo* (Hathaway and Traugh, 1982).

Casein kinase II has been purified from a variety of mammalian and avian species (Hathaway and Traugh, 1982), from *Drosophila* (Glover *et al.*, 1983), and from yeast (Bell *et al.*, 1977; Rigobello *et al.*, 1982). The enzymes prepared from these various sources are generally found to be remarkably similar in their properties. These properties include a native $M_r \sim$ 130,000–140,000, autophosphorylation of the β subunit, self-aggregation at low ionic strength, ability to use GTP in place of ATP as nucleotide substrate, and extreme sensitivity to heparin. In spite of the similarities, however, actual homology between any of these enzymes has not yet been demonstrated at the level of primary structure. Furthermore, although the subunit structure is invariably found to be a tetramer consisting of two dissimilar subunits, considerable variation has been observed in the α subunit. The enzyme purified from most mammalian and avian species contains α and α' subunits (Hathaway and Traugh, 1982) which, in the case of the calf thymus enzyme, have been shown to be related in primary structure (Dahmus, 1981a). In contrast, purified casein kinase II from yeast (Rigobello *et al.*, 1982), *Drosophila* (Glover *et al.*, 1983), and calf brain (Walinder, 1973) appears to contain a single α subunit. The significance of this variation is not completely understood, although proteolysis *in vitro* appears to account for at least some of it (Hathaway and Traugh, 1982).

We have undertaken a detailed comparison of casein kinase II from calf thymus and *Drosophila*. These two sources were

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Present address, Department of Biochemistry, the University of Georgia, Athens, GA 30602.

‖ Recipient of National Institutes of Health Grant GM28079.
** Recipient of National Science Foundation Grant PCM8217065.
To whom reprint requests should be addressed.

chosen because of the relatively large phylogenetic distance separating the two organisms and because of the differences reported in the subunit structure of their corresponding enzymes. We report here that casein kinases II from calf and *Drosophila* are related at the level of primary structure, as determined by immunological cross-reactivity and by two-dimensional peptide mapping, that the subunit structure of the enzyme in rapidly prepared whole cell extracts resembles that of the purified proteins, and that the two enzymes have indistinguishable protein substrate specificities. These results imply that casein kinase II has been highly conserved both structurally and functionally during evolution.

EXPERIMENTAL PROCEDURES

Materials

Unlabeled ATP was obtained from P-L Biochemicals; [γ-^{32}P]ATP (3000 Ci/mmol) was from Amersham. Carrier-free Na^{125}I was purchased from New England Nuclear.

Casein, obtained from Nutritional Biochemicals, was treated as described by Reimann *et al.* (1971). Marker proteins, phosphorylase *b*, albumin, ovalbumin, carboic anhydrase, trypsin inhibitor, and α-lactalbumin were purchased from Pharmacia. Trypsin (L-1-tosylamido-2-phenylethyl chloromethyl ketone) was obtained from Worthington. Thermolysin was obtanied from Boehringer Mannheim.

Iodogen (1,3,4,6-tetrachloro-3*a*,6*a*-diphenylglycoluride) was purchased from Pierce Chemical Co. TLC cellulose sheets, layer thickness of 0.1 mm, were obtained from EM Reagents. Phenylmethylsulfonyl fluoride was obtained from Sigma.

Methods

Protein Kinase Assay—The standard reaction mixture contained 50 mM Tris-HCl, pH 7.9, 100 mM NaCl, 10 mM $MgCl_2$, 0.25 mM EDTA, 0.25 M dithiothreitol, 5% glycerol, 0.025% Triton X-100, 1 μM [γ-^{32}P]ATP (1 μCi/reaction), 1 mg/ml casein, and enzyme in a final volume of 28 μl. Serum and enzyme samples were diluted in 20 mM Tris-HCl, pH 7.9, 0.5 mM EDTA, 200 mM NaCl, 0.5 mM dithiothreitol, 10% glycerol, and 0.05% Triton X-100 and preincubated at 25 °C for 30 min in a final volume of 14 μl. Salts, nucleotide, and protein substrate were added and the incubation continued for 15 min at 25 °C. Reactions were stopped by the addition of 10 μl of 1 mM ATP and 1 ml of 20% trichloroacetic acid. Precipitates were collected on nitrocellulose filters (Millipore HAWP 02400). The filters were washed three times with 10-ml aliquots of 3% trichloroacetic acid, dried, and counted in 2,5-diphenyloxazole toluene scintillant.

Gel Electrophoresis—Electrophoresis in 10 or 12% polyacrylamide-SDS[1] gels was as described by Laemmli (1970). Acid-urea polyacrylamide gels (12% acrylamide, 0.08% *N,N'*-methylene bisacrylamide containing 6 M urea and 5% acetic acid) were made as described by Panyim and Chalkley (1969) and run as previously described (Glover, 1982).

Preparation of Polyclonal Antibodies—Antibodies to calf thymus casein kinase II were prepared as previously described (Dahmus, 1981c). Antibodies to *Drosophila* casein kinase II (isolated from 6–18-h embryos as described (Glover *et al.*, 1983)) were prepared as follows. A female New Zealand White rabbit was injected intramuscularly at approximately six sites in the back with 800 μg of purified enzyme emulsified with an equal volume of complete Freund's adjuvant. The rabbit was boosted at 4- to 8-week intervals (five boosts total) with 250 μg of enzyme emulsified with an equal volume of incomplete Freund's adjuvant. Serum was collected at 1-week intervals beginning 1 week after the first boost and stored in aliquots at −80 °C. A maximum titer was reached after the third boost (50% inhibition of enzyme activity at a 1:100 serum dilution) and maintained thereafter.

Protein Transfer—Protein was transferred from SDS-polyacrylamide gels to either DPT paper (Figs. 3 and 4) or nitrocellulose (Fig. 5). DPT paper was prepared as described by Seed (1982) using Whatman 540 paper. Proteins were transferred and reacted with serum and ^{125}I-protein A as previously described (Dahmus, 1981c) or as described by Burnette (1981).

Protein Iodination—Casein kinase II subunits were purified by SDS-polyacrylamide gel electrophoresis according to the procedure of Laemmli (1970). Subunits were extracted from the gel, reduced, alkylated with 2 mM iodoacetamide for 30 min at 25 °C, and labeled with ^{125}I as described by Tolan *et al.* (1980) and Dahmus (1983).

Proteolytic Digestion and Peptide Mapping—^{125}I-labeled subunits were dissolved in 0.05 M ammonium bicarbonate, pH 8.0, and digested with trypsin or thermolysin as previously described (Dahmus, 1983). Peptides were resolved on TLC cellulose sheets by electrophoresis in the first dimension and chromatography in the second dimension (Dahmus, 1983). TLC plates were dried and exposed to Kodak X-Omat AR x-ray film with a DuPont Cronex Lightning Plus intensifying screen at −80 °C.

RESULTS

Subunit Composition of Purified Enzymes—Calf thymus casein kinase II is composed of three subunits, α, α', and β, having M_r = 44,000, 40,000, and 26,000, respectively (Dahmus, 1981a). Purified *Drosophila* casein kinase II is composed of two subunits, α and β, having M_r = 36,700 and 28,200, respectively (Glover *et al.*, 1983). A comparison of the electrophoretic mobility of calf thymus and *Drosophila* casein kinase II subunits in an SDS-polyacrylamide gel is shown in Fig. 1. The mobility of all of the subunits is consistent with previous molecular weight assignments, confirming that the *Drosophila* α subunit has a molecular weight lower than that of the α' subunit of calf thymus casein kinase II. The *Drosophila* β subunit has a mobility slightly less than that of the corresponding calf thymus subunit and is somewhat more diffuse.

Immunological Cross-reactivity between Calf Thymus and Drosophila Casein Kinase II—Polyclonal antibodies were prepared against calf and *Drosophila* casein kinase II by immu-

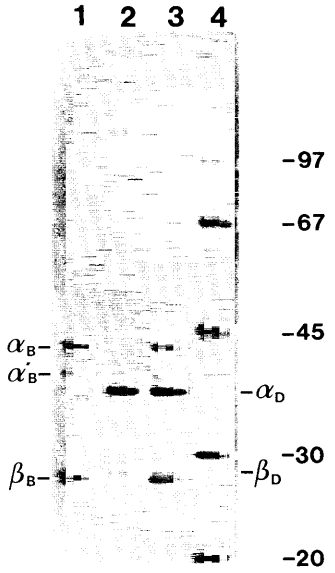

FIG. 1. **Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of calf thymus and *Drosophila* casein kinase II.** Electrophoresis was carried out in a 10% polyacrylamide gel according to the procedure of Laemmli (1970) and stained with Coomassie blue. *Lane 1*, 2.5 μg of calf thymus casein kinase II; *lane 2*, 2.5 μg of *Drosophila* casein kinase II; *lane 3*, 2.5 μg each of calf thymus and *Drosophila* kinase; *lane 4*, molecular weight markers: phosphorylase *b* (97,000), albumin (67,000), ovalbumin (45,000), carbonic anhydrase (30,000), and trypsin inhibitor (20,100). The mobility of the α, α', and β subunits of calf thymus casein kinase II (*subscript B* for bovine) is indicated at the *left*. The mobility of the α and β subunits of *Drosophila* casein kinase II is indicated at the *right*. The molecular mass (in kilodaltons) of standard proteins is also indicated at the *right*.

[1] The abbreviations used are: SDS, sodium dodecyl sulfate; DPT, diazophenylthioether paper.

nization of rabbits with purified native protein. The effect of increasing concentrations of immune serum on the activity of calf thymus and *Drosophila* casein kinase II is shown in Fig. 2. Each serum is an effective inhibitor of its homologous casein kinase in a standard reaction. There is a slight but reproducible inhibition of enzymatic activity with the heterologous sera.

The subunits of calf thymus and *Drosophila* casein kinase II were resolved by polyacrylamide gel electrophoresis in the presence of SDS and transferred to DPT paper as described under "Methods." The transfers were reacted with serum and reactive subunits were visulaized by subsequent treatment with ^{125}I-protein A and autoradiography. The results presented in Fig. 3, *B–D* (*lane 1*) show that antibody prepared against calf thymus casein kinase II reacts with the α, α', and β subunits of the calf enzyme. Although the sera from three different rabbits all react with each subunit, quantitative differences in reactivity are apparent. Similarly, antibodies prepared against *Drosophila* casein kinase II react with both the α and β subunits of *Drosophila* casein kinase II (Fig. 3*E*, *lane 2*).

Antibody prepared against calf thymus casein kinase II cross-reacts with both the α and β subunit of *Drosophila* casein kinase II (Fig. 3, *B–D*, *lane 2*). Quantitative differences in the reactivity of serum from different rabbits is again apparent. Antibodies prepared against *Drosophila* casein kinase II cross-react with comparable intensity with the β subunit of the calf enzyme and to a lesser extent with the α and α' subunits (Fig. 3*E*, *lane 1*). None of the sera cross-reacts with any of the standard proteins used (Fig. 3, *lane 3*). These results indicate a high degree of conservation in the determinants recognized by these antibodies.

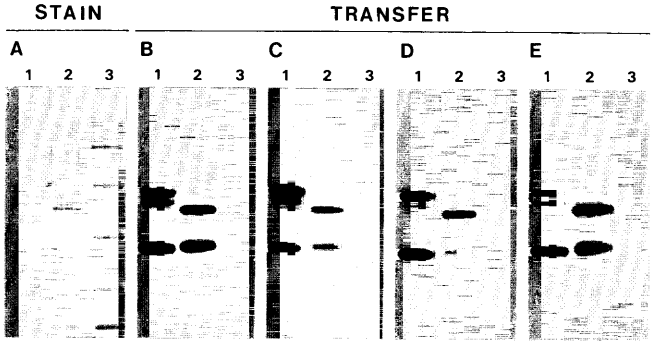

FIG. 3. **Reaction of antibody prepared against calf thymus and *Drosophila* casein kinase II with purified casein kinase subunits.** The subunits of calf thymus and *Drosophila* casein kinase II were resolved by SDS-polyacrylamide gel electrophoresis on a 12% gel. Following electrophoresis, the gel was cut into three sections, each equivalent and containing one lane each of calf thymus casein kinase II, *Drosophila* casein kinase II, and molecular weight markers. One section (*A*) was stained with Coomassie blue. The remaining two sections were transferred to DPT paper, incubated with antibody (1:50 dilution of antisera), and visualized by reaction with ^{125}I-protein A and autoradiography as described under "Methods." One of the two transfers was reacted with calf thymus casein kinase II antiserum (*B*), the other with *Drosophila* casein kinase II antiserum (*E*). The first transfer (from *B*) was then stripped, checked for residual radioactivity, and reacted with a second calf thymus casein kinase II antiserum (*C*). This transfer was then stripped again and reacted with a third calf antiserum (*D*). *Lane 1* contains 2.5 μg of calf thymus casein kinase II; *lane 2*, 2.5 μg of *Drosophila* casein kinase II; *lane 3*, molecular weight markers as indicated in Fig. 1.

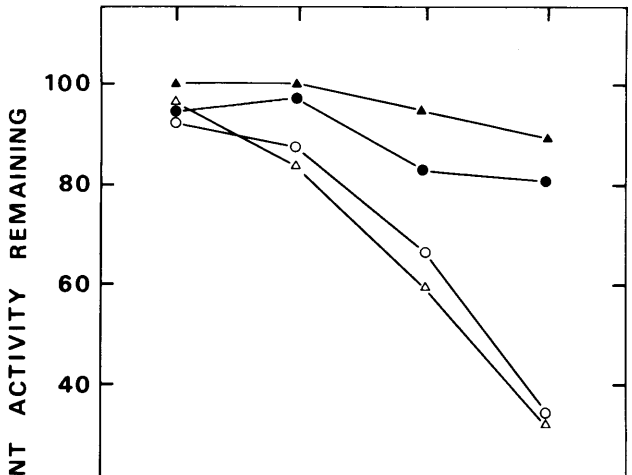

FIG. 2. **Inhibition of protein kinase activity with immune serum.** Each reaction contained the equivalent of 0.3 μl of serum made up of varying proportions of control and immune serum. The per cent enzymatic activity remaining, relative to the activity in the presence of 0.3 μl of control serum, is plotted as a function of the amount of immune serum present. Protein kinase and antibody were preincubated and the reaction initiated by the addition of ATP and casein as described under "Methods." Reactions contained about 0.02 unit of enzyme. *Open symbols* represent reactions with homologous serum and *filled symbols* represent reactions with heterologous serum. Effect of antibody prepared against calf thymus casein kinase II on calf (○) and *Drosophila* (●) enzyme. Effect of antibody prepared against *Drosophila* casein kinase II on *Drosophila* (△) and calf (▲) enzyme.

Polypeptide Composition of Casein Kinase II in Vivo—The data of Fig. 1 confirm that the calf and *Drosophila* enzymes have significantly different molecular structures with respect to the α subunit. This result raises the question of whether or not the molecular structure of the purified enzymes is an accurate reflection of their *in vivo* structure, particularly since limited proteolysis has been reported to give rise to the α' subunit of casein kinase II in rabbit reticulocytes (Hathaway and Traugh, 1982). This question was addressed by using the casein kinase II-specific antisera to visualize the enzyme subunits in crude fractions and initial homogenates.

Aliquots containing equal units of calf thymus casein kinase II, at various stages of purification, were denatured in the presence of SDS and resolved by polyacrylamide gel electrophoresis. Proteins were transferred to DPT paper and reacted with antibody and ^{125}I-protein A as described under "Methods." Fig. 4 shows that the molecular composition of the enzyme in the initial cell extract (*lane 1*) is identical to that of the purified enzyme (*lane 9*). The α' subunit is present in all stages and the α:α' ratio does not appear to change significantly during the course of purification. Identical results were obtained when calf thymus tissue was homogenized directly in sample application buffer containing SDS and β-mercaptoethanol, denatured at 85 °C, and loaded onto the polyacrylamide gel within minutes after cell disruption.

In a similar set of experiments, antibodies prepared against *Drosophila* casein kinase II were reacted with whole cell extracts from either intact embryos or exponentially growing tissue culture cells (Fig. 5). The mobility of the α and β subunits in extracts from both whole embryos (*lane 1*) and tissue culture cells (*lane 2*) is identical to that of the purified enzyme (*lane 3*). The α:β ratio is also the same in the whole cell homogenates as in the purified enzyme, and no bands are

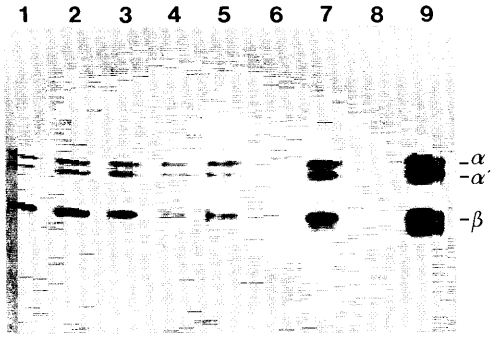

FIG. 4. **Reaction of antibody prepared against calf thymus casein kinase II with partially purified enzyme.** Samples of partially purified casein kinase II were resolved by SDS-polyacrylamide gel electrophoresis on a 12% slab gel and electrophoretically transferred to DPT paper. The paper was reacted with a 1:50 dilution of calf thymus casein kinase II antisera and ^{125}I-protein A as described under "Methods." *Lanes 1–7* contain aliquots of casein kinases I and II at various stages of purification (Dahmus, 1981a). Loadings in *lanes 1–7* were adjusted so that each lane contained approximately 2 units of casein kinase II except *lane 1* which contained 1 unit and *lane 6* which contained 2 units of casein kinase I. *Lane 1*, whole cell extract, 105 μg of protein; *lane 2*, ammonium sulfate precipitate, 120 μg; *lane 3*, protamine sulfate supernatant, 72 μg; *lane 4*, DEAE-cellulose flow-through, 26 μg; *lane 5*, phosphocellulose P1, 14 μg; *lane 6*, casein kinase I phosphocellulose P11, 3 μg; *lane 7*, casein kinase II phosphocellulose P11, 0.6 μg; *lane 8*, purified casein kinase I, 2 μg; *lane 9*, purified casein kinase II, 2 μg. The mobility of the α, α', and β subunits of calf thymus casein kinase II is indicated at the *right*.

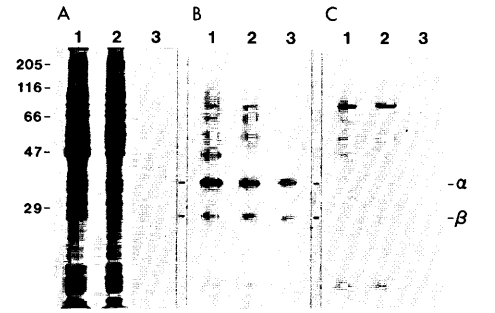

FIG. 5. **Reaction of antibody prepared against *Drosophila* casein kinase II with *Drosophila* cell extracts.** Three identical sets of protein samples were analyzed in parallel by SDS gel electrophoresis in a 12% polyacrylamide gel. One set (*A*) was stained with Coomassie blue. The remaining two sets were electrophoretically transferred to nitrocellulose paper and reacted with either a 1:50 dilution of antiserum prepared against *Drosophila* casein kinase II (*B*) or a 1:50 dilution of preimmune serum from the same rabbit (*C*). Bound antibodies were visualized by reaction with ^{125}I-protein A and autoradiography (Burnette, 1981). All lanes contained approximately the same amount of casein kinase II activity. *Lane 1*, whole *Drosophila* proteins from intact embryos, 50 μg/lane; *lane 2*, whole *Drosophila* proteins from exponentially growing tissue culture cells (line K_c), 30 μg/lane; *lane 3*, purified *Drosophila* casein kinase II, 0.05 μg/lane. For whole protein samples, the fresh tissue was either lysed (K_c cells) or homogenized (embryos) directly in sample application buffer (Laemmli, 1970) heated to 100 °C for 5 min, quick frozen, and stored at −20 °C until used. The mobility and molecular mass (in kilodaltons) of standard proteins electrophoresed in the same gel are indicated at the *left*. The mobility of the α and β subunits of *Drosophila* casein kinase II is indicated at the *right*.

apparent in the region corresponding to proteins of M_r = 40,000–44,000. The prominent *band* in *lane 1* of Fig. 5*B*, with M_r = 47,000, corresponds to yolk protein. Some nonspecific reaction with yolk protein as well as with other unidentified peptides is observed for both immune and preimmune serum.

Analysis of Tryptic and Thermolytic Peptides of Calf and Drosophila Casein Kinase II—As an alternate means of assessing the relationship between calf and *Drosophila* casein kinase II, the α and β subunits were analyzed by peptide mapping. Subunits were purified by SDS-polyacrylamide gel electrophoresis, eluted from the gel, and labeled with ^{125}I. Electrophoresis of the ^{125}I-labeled subunits established that the subunits were intact and free of contamination. The iodinated subunits were digested with trypsin or thermolysin and the peptides were resolved by electrophoresis in the first dimension and by chromatography in the second dimension. Resolution of the thermolytic peptides for the various subunits is shown in Fig. 6. The map of the calf thymus α' subunit closely resembles that of the α subunit and is not included in this figure. For each enzyme, the map of the α subunit is distinct from that of the β subunit indicating that the α and β subunits are not related in primary sequence. There is, however, extensive similarity between the peptide maps of the α subunits from the two sources (Fig. 6, *A* and *C*). The *map* in *B* represents a 1:1 mixture of peptides from the calf and *Drosophila* α subunit and confirms that these subunits give rise to numerous peptides of identical mobility. Similarly, the thermolytic peptide map of the calf thymus β subunit is strikingly similar to the β subunit of *Drosophila* casein kinase II (Fig. 6, *D* and *F*). The *composite map* (Fig. 6*E*) confirms that peptides derived from the two sources have identical mobilities. The tryptic peptides (data not shown) were analyzed in the same way and also support the conclusion that there is extensive primary sequence homology between corresponding subunits in the calf and *Drosophila* enzymes.

Substrate Specificity of Calf Thymus and Drosophila Casein Kinase II—Reactions containing either calf thymus casein kinase II, *Drosophila* casein kinase II, or the catalytic subunit of bovine brain cAMP-dependent protein kinase were incubated with acid-soluble proteins from *Drosophila* K_c tissue culture cells in the presence of [γ-^{32}P]ATP. The ^{32}P-labeled proteins were than analyzed by acid-urea-polyacrylamide gel electrophoresis. The results presented in Fig. 7 show that the

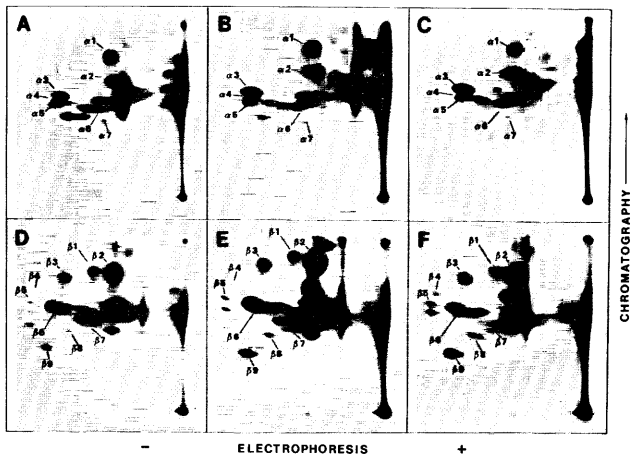

FIG. 6. **Analysis of thermolytic peptides from calf thymus and *Drosophila* casein kinase II.** ^{125}I-labeled subunits were prepared and digested with thermolysin as described under "Methods." Samples were subjected to electrophoresis toward the cathode at pH 4.4. Chromatography in the second dimension was as described under "Methods." The origin is at the *lower right* of each *map*. *A–C* are peptide maps of the α subunit. *A*, calf thymus α subunit (2.4×10^4 cpm); *B*, a 1:1 mixture of calf thymus and *Drosophila* α subunits (4.8×10^4 cpm); *C*, *Drosophila* α subunit 2.4×10^4 cpm). *D–F* are peptide maps of the β subunit. *D*, calf thymus β subunit (2.5×10^4 cpm); *E*, a 1:1 mixture of calf thymus and *Drosophila* β subunits (2.5×10^4 cpm); *F*, *Drosophila* β subunit (2.5×10^4 cpm). Peptides common to a specific subunit are *numbered*. Autoradiograms were exposed for 2 days for *A–C* and 4, 6, and 8 days for *D–F*, respectively.

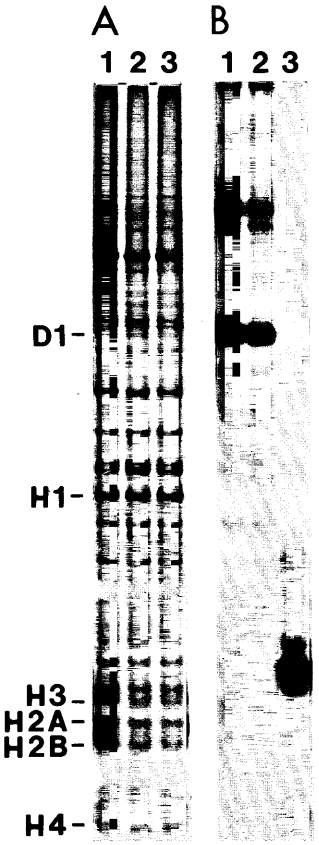

FIG. 7. ***In vitro* phosphorylation of acid-soluble proteins from *Drosophila* by purified protein kinases.** Fresh *Drosophila* tissue culture cells (line K_c) were cooled to 0 °C, harvested by centrifugation at 3,000 × *g* for 5 min, washed once with 25 mM EDTA, pH 6.8, 75 mM NaCl, and then extracted with 0.2 M H_2SO_4 for 3 h. The extract was clarified by centrifugation at 12,000 × *g* for 10 min and precipitated with 20% trichloroacetic acid for 40 min at 0 °C. the precipitate was pelleted, washed once with 0.02% HCl in acetone, once in acetone, dried under reduced pressure, and then dissolved in H_2O and stored at −20 °C until used. Aliquots of 40 μg were mixed with calf thymus casein kinase II (*lane 1*), *Drosophila* casein kinase II (*lane 2*), or the catalytic subunit of bovine brain cAMP-dependent kinase (*lane 3*) and incubated for 15 min at 25 °C in 50 mM Tris, pH 8.5, 10 mM $MgCl_2$, 125 mM NaCl, 0.05% Triton X-100, 10 μM ATP (4,000 cpm/pmol). Reactions were stopped by precipitation with 10% trichloroacetic acid. Precipitates were processed as shown and analyzed by acid-urea gel electrophoresis in a 12% polyacrylamide slab gel. The gel was stained with Coomassie blue (*A*) and autoradiographed (*B*). The mobilities of chromosomal protein D1 and of histones H1, H2A, H2B, H3, and H4 are indicated.

pattern of proteins phosphorylated by casein kinase II from calf (*lane 1*) is indistinguishable, quantitatively as well as qualitatively, from that of *Drosophila* casein kinase II (*lane 2*). In particular, protein D1 (Alfageme *et al.*, 1980) is a prominent substrate for casein kinase II, as noted previously (Glover *et al.*, 1983). Identical patterns were also obtained when acid-soluble extracts from either K_c cell nuclei or whole HeLa cells were used as substrate (not shown). In each case,the catalytic subunit of bovine brain cAMP-dependent kinase phosphorylated a distinct set of proteins (*e.g.* Fig. 7*B*, *lane 3*).

Calf thymus casein kinase II has been previously reported to phosphorylate the IIa subunit of RNA polymerase II in addition to a small subunit of M_r = 20,500 (Dahmus, 1981b). These same subunits of calf thymus RNA polymerase II are phosphorylated by *Drosophila* casein kinase II (data not shown).

DISCUSSION

The molecular structure of purified calf thymus casein kinase II appears to be an accurate reflection of the structure of this enzyme *in vivo*. This is indicated by the fact that cell extracts prepared in the presence of protease inhibitors and denatured within minutes of cell disruption contain both α and α^r subunits in the same molar ratio as the purified enzyme. Therefore, in spite of the fact that α and α′ are related in primary sequence, α′ does not appear to arise from α by proteolytic processing during enzyme purification. This is also supported by the observation that HeLa cells, lysed in Laemmli sample application buffer and analyzed by similar procedures, contain both α and α′ (data not shown). Two possibilities that cannot be distinguished at this time are that there are two distinct but related α genes, one for α and one for α′, or that α and α′ are related by a physiologically relevant processing step either at the level of transcription or translation. In either case, one might expect the molar ratio of α:α′ to be dependent on the tissue type, developmental stage, and physiological state. This is consistent with the variability in casein kinase II structure observed in various tissues of different organisms (Walinder, 1973; Dahmus and Natzle, 1977; Thornburg and Lindell, 1977; Hathaway and Traugh, 1979; Dahmus, 1981a; Rigobello *et al.*, 1982; Huang *et al.*, 1982; Ahmad *et al.*, 1982; Glover *et al.*, 1983).

Hathaway and Traugh (1982) have reported that casein kinase II from rabbit reticulocytes contains both α and α′ subunits when purified in the absence of protease inhibitors but only an α subunit when purified in the presence of inhibitors. Furthermore, they have observed complete conversion of α into α′ *in vitro* during prolonged storage of the enzyme at 4 °C. These results indicate that only one α subunit is expressed in rabbit reticulocytes and that the presence of α′ is an artifact for this particular tissue. At first glance, it appears surprising that a polypeptide of the same molecular weight should be a legitimate subunit of casein kinase II in the case of calf thymus but an artifact produced *in vitro* in the case of rabbit reticulocytes. However, if the α polypeptide is physiologically processed to α′ *in vivo* in at least some tissue, then it is possible that small amounts of the responsible protease are present in reticulocytes and effect the same cleavage *in vitro* during purification or long term storage of the enzyme. Alternatively, if there are two genes or two different mRNAs derived from a single gene, then perhaps α and α′ differ by an additional small domain (perhaps encoded by a separate exon) which is sensitive to attack *in vitro* by nonspecific proteases.

Purified *Drosophila* casein kinase II consists of a single α and β subunit. The α subunit is unusually small, suggesting that it may correspond to the α′ subunit of calf casein kinase II. The immunological transfer experiments indicate that the subunit composition of the purified enzyme is an accurate reflection of the structure of the enzyme *in vivo* in both embryos and tissue culture cells. In particular, there is no indication of heterogeneity to subunit α. The results suggest that there is only a single α gene expressed in *Drosophila* and that the product is not processed physiologically. While it is possible that a second α subunit exists but is not expressed in 6–18-h embryos (or in tissue culture cells), this seems unlikely in view of the number of tissue types present at this stage. A more complete analysis of the relationship between the α and α′ subunits of calf thymus and the α subunit of *Drosophila* casein kinase II will require primary sequence analysis of these subunits as well as a characterization of the genes that code for these subunits.

The results presented here establish that *Drosophila* casein

kinase II is homologous to casein kinase II from mammalian sources. Antibodies prepared against the calf and *Drosophila* enzyme show extensive cross-reactivity in protein transfers. Furthermore, a slight but reproducible inhibition of enzymatic activity was observed when antibody was reacted with the heterologous enzyme. The enzymes from the two sources also show extensive structural similarities. Native casein kinase II from calf thymus and *Drosophila* have M_r = 132,000–140,000 and 130,000, respectively and are composed of polypeptides of approximately the same size (Dahmus, 1981a; Glover *et al.*, 1983). Peptide maps derived from purified subunits establish that homologous subunits from the two enzymes share extensive primary sequence homology. The catalytic activity of the two enzymes is also comparable in that each enzyme can utilize either ATP or GTP as nucleotide substrate and is inhibited by heparin (Dahmus, 1981a; Glover *et al.*, 1983).[2] The protein substrate specificity of calf thymus and *Drosophila* casein kinase II also appears to be identical. This is true even when the enzymes are challenged with complex mixtures of potential substrates. Such striking similarities in structure and function imply a high degree of conservation of casein kinase II since the divergence of these organisms.

Acknowledgments—We gratefully acknowledge the technical assistance of Mark Warren in the protein iodination and peptide mapping. We also wish to thank Dr. Howard Schulman for generously providing the catalytic subunit of bovine brain cAMP-dependent kinase.

REFERENCES

Ahmad, Z., DePaoli-Roach, A. A., and Roach, P. J. (1982) *J. Biol. Chem.* **257,** 5873–5876

Alfageme, C. R., Rudkin, G. T., and Cohen, L. H. (1980) *Chromosoma* **78,** 1–31

Bell, G. I., Valenzuela, P., and Rutter, W. J. (1977) *J. Biol. Chem.* **252,** 3082–3091

Burnett, W. N. (1981) *Anal. Biochem.* **112,** 195–203

Cohen, P. (1982) *Nature (Lond.)* **296,** 613–620

Dahmus, M. E. (1981a) *J. Biol. Chem.* **256,** 3319–3325

Dahmus, M. E. (1981b) *J. Biol. Chem.* **256,** 3332–3339

Dahmus, M. E. (1981c) *J. Biol. Chem.* **256,** 11239–11243

Dahmus, M. E. (1983) *J. Biol. Chem.* **258,** 3956–3960

Dahmus, M. E., and Natzle, J. (1977) *Biochemistry* **16,** 1901–1980

Flockhart, D. A., and Corbin, J. D. (1982) *CRC Crit. Rev. Biochem.* **12,** 133–186

Glover, C. V. C. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 1781–1785

Glover, C. V. C., Shelton, E. R., and Brutlag, D. L. (1983) *J. Biol. Chem.* **258,** 3258–3265

Hathaway, G. M., and Traugh, J. A. (1979) *J. Biol. Chem.* **254,** 762–768

Hathaway, G. M., and Traugh, J. A. (1982) *Curr. Top. Cell. Regul.* **21,** 101–127

Huang, K. P., Itarte, E., Singh, T. J., and Akalsuka, A. (1982) *J. Biol. Chem.* **257,** 3236–3242

Krebs, E. G., and Beavo, J. A. (1979) *Annu. Rev. Biochem.* **48,** 923–959

Laemmli, U. K. (1970) *Nature (Lond.)* **227,** 680–685

Panyim, S., and Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130,** 337–346

Reimann, E. M., Walsh, D. A., and Krebs, E. G. (1971) *J. Biol. Chem.* **246,** 1986–1995

Rigobello, M. P., Jori, E., Carignami, G., and Pinna, L. A. (1982) *FEBS Lett.* **144,** 354–358

Seed, B. (1982) *Nucleic Acids Res.* **10,** 1799–1810

Thornburg, W., and Lindell, T. J. (1977) *J. Biol. Chem.* **252,** 6660–6665

Tolan, D. R., Lambert, J. M., Boileau, G., Fanning, T. G., Kenny, J. W., Vassos, A., and Traut, R. R. (1980) *Anal. Biochem.* **103,** 101–109

Walinder, O. (1973) *Biochim. Biophys. Acta* **293,** 140–149

[2] M. E. Dahmus, unpublished results.