Apparent Mitochondrial Creatine Kinase in the Serum of a Patient with Metastatic Cancer to the Liver

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Creatine kinase (CK, EC 2.7.3.2) activity in the serum of a patient with metastatic carcinoma migrated as two distinct bands cathodal to the origin and to CK-3 on agarose gel electrophoresis. The more cathodal isoenzyme (CKm-2) is of high molecular mass, is precipitated by ammonium sulfate at 30% of saturation, and is not retarded by Sephadex G-100. Treatment with urea at a concentration of 6 mol/L caused CKm-2 to elute with proteins of lower molecular mass on a G-100 column and shifted the electrophoretic migration to a position just cathodal to the origin (CKm-1). Antibody to CK-1 and CK-3 did not affect the activity of either CKm-1 or CKm-2. Similarities between these cathodal bands of CK activity and mitochondrial CK suggest the mitochondrial origin of these isoenzymes. These cathodal CK isoenzymes react unpredictably with different commercial reagent systems for determination of CK activity in serum or in agarose gel.

Serum creatine kinase (CK)³ that migrates cathodal to the origin and to CK-3 on electrophoresis has been an infrquent finding and has been attributed to mitochondrial CK (CKm) or to CK-1 binding to immunoglobulins (1-4). Ordinarily, mitochondrial CK is bound to the exterior surface of the inner mitochondrial membrane and is considered to be particulate in nature (5). Isolated and purified CKm exists in two active forms: (a) CKm-1 is a dimer (M r 64,000) of two equal subunits; it migrates electrophoretically just cathodal to the origin in agarose gel. (b) A larger aggregate (CKm-2), Mr 180,000 to 190,000, migrates electrophoretically to a more cathodal position than CKm-1 (6, 7).

A patient with metastatic carcinoma to the liver developed Gram-negative septicemia, pulmonary edema, and hypotension. In initial specimens submitted for determination of total CK activity and CK isoenzymes, to evaluate the possibility of a myocardial infarction, a large proportion of CK-1 was demonstrable. Increasing CK activity, electrophoretically migrating in two bands cathodal to the origin and to CK-3, was found in subsequent specimens (Figure 1). We investigated the characteristics of the cathodal CK activity to determine the identity of the enzyme and its possible clinical significance.

Case History

A 59-year-old white woman was admitted with diffuse, persistent abdominal pain. The patient had a history of hypertension for the past six years, diabetes mellitus controlled by diet for the past three or four years, and diverticulitis, which had been diagnosed one year earlier. Previous surgery included an appendectomy and a cholecystectomy.

A liver scintiscan demonstrated an enlarged liver with a mottled appearance consistent with multiple small metastases or severe hepatitis. A liver biopsy showed metastatic carcinoma with a pattern suggestive of melanoma. Despite full clinical evaluation, no primary site of the carcinoma could be located.

While the diagnosis was being made, the patient became febrile with Gram-negative septicemia complicated by hypotension and pulmonary edema. A cardiac enzyme profile, requested to rule out a myocardial infarction (Table 1), showed no CK-2 (MB) in any of the serum specimens.

Materials and Methods

Activities of some enzymes in serum were determined by published methods (8, 9) at 25 °C with use of an ENI-GEM-SABEC centrifugal analyzer (Electro-Nucleonics Inc., Fairfield, NJ 07006). Serum total CK activity was routinely estimated at 25 °C with Sigma reagents (Sigma Chemical Co., St. Louis, MO 63178) (10). Also, activity of CK in serum, serum fractions, and chromatographic column fractions was estimated at 37 °C with CK-NAC reagents (cat. no. 128349; Biodynamics/bmc, Indianapolis, IN 46260) according to the modifications of Szasz et al. (11).

We electrophoretically separated and fluorometrically detected CK isoenzymes in agarose gel with the Cornig-ACI system according to the manufacturer's instructions (Cornig, Palo Alto, CA 94306). Electrophoresis was also performed with the use of Cornig Special-Purpose Electrophoresis Film Agarose (cat. no. 470104) with 4-morpholinopropanesulfonic acid buffer (50 mmol/L, pH 7.8 at 25 °C, containing 3 mmol of sodium azide per liter as preservative) in a Model 310 electrophoresis chamber and power supply (G. K. Turner Associates, Inc., Palo Alto, CA 94303) run at 15 mA for 20 min. The gels were overlaid with 1 mL of CK-NAC reagent, incubated in a moist chamber at 37 °C for 20 min, and dried at 50 °C for 20 min. Fluorescence elicited by ultraviolet light was quantitated with a Cornig 720 densitometer.

Gel exclusion column chromatography was performed with Sephadex G-10, G-25 fine, and G-100 (Pharmacia Fine Chemicals, Piscataway, NJ 08854). Columns of G-10 (0.6 x 37 cm), G-25 fine (1.5 x 29 cm), and G-100 (1.2 x 44 cm), were poured with an eluting buffer containing, per liter, 50 mmol of tris(hydroxymethyl)methylamine acetate, pH 7.5 (25 °C),

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The column effluent was monitored at 280 nm and 0.8-mL fractions were collected. Pooled column fractions were concentrated by ultrafiltration with a 10-PA cell and a PM 30 filter (30,000 M<sub>r</sub> cut-off; Amicon, Lexington, MA 02173).

Samples from the patient that had CK activity in cathodal bands were electrophoresed in duplicate. One set was overlaid with the complete substrate mixture and the duplicate set with the substrate mixture without phosphocreatine. The substrate was prepared according to the recommendations of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (12).

Ammonium sulfate was added to serum samples that had cathodal CK isoenzymes to give a final concentration equivalent to 30% saturation (172 g/L) (13). The precipitate was isolated by centrifugation at 8000 × g for 30 min at 4°C. The supernatant fluid was desalted by passage through Sephadex G-10 or G-25 columns. The precipitate was dissolved in the column-eluting buffer. Portions of the dissolved ammonium sulfate precipitate had NaCl added to a concentration of 4 mol/L, or urea added to a concentration of 6 mol/L, or were untreated. The treated and untreated portions were chromatographed on the Sephadex G-100 column. One drop of a 250 g/L solution of human albumin (Albuspan; Parke-Davis, Detroit, MI 48232) was added to each of the column fractions to stabilize the eluted enzyme. Column fractions of interest were assayed for total CK activity, and CK isoenzymes were determined by electrophoresis.

We injected CK activity with precipitating antibody to CK by adding 20 µL of either sheep anti-human CK-1 or sheep anti-human CK-3 (E. Merck, Darmstadt, F.R.G.) to 100 µL of serum, serum fraction, or concentrated fractions from the column. The antibody sample mixture was incubated at room temperature for 20 min and assayed for CK activity with the CK-NAC reagents. Samples containing CK-1 or CK-3, or both, were used as controls to determine the effectiveness of the antibody as an inhibitor of CK activity.

**Results**

That the bands seen on routine electrophoretic analysis of

![Fig. 1. Representative agarose gel electrophoretic pattern of CK isoenzymes present in this patient’s serum](image)

10 mmol of 2-mercaptoethanol, and 3 mmol of sodium azide. This patient’s serum actually represented CK activity was confirmed by their absence in the absence of phosphocreatine.

The most cathodal electrophoretic band of CK activity (CKm-2) precipitated with ammonium sulfate at 30% of saturation. The less-cathodal band (CKm-1), CK-3, and CK-1 remained in the supernatant fluid. The CKm-2 eluted in the void volume of the G-100 column, and treatment with 4 mol/L NaCl did not alter the elution pattern. Denaturation of CKm-2 with 6 mol/L urea shifted the eluting CK activity on the G-100 column to coincide with fractions containing proteins of lower molecular mass (Figure 2). After urea treatment, the CK activity eluting from the G-100 column also changed electrophoretic mobilities from the position of CKm-2 to the position of CKm-1. To determine whether the complex of high molecular mass reformed after column elution, we reapplied concentrated CK activity from the G-100 fractions, after urea treatment, to the G-100 column; the elution pattern at the lower molecular mass was duplicated.

Anti-human CK-1 inhibited CK-1 in samples containing electrophoretically demonstrable CK-1. Inhibition with anti-human CK-3 demonstrated only a small proportion of CK-3 in the patients’ samples. The CK isoenzyme in the ammonium sulfate precipitate before and after urea treatment was not inhibited by either anti-CK-1 or anti-CK-3.

Total CK activity was determined by both CK methods (Sigma and CK-NAC, Biodynamics/bmc) in a series of serum specimens submitted for routine enzyme analysis and in a
Table 2. Comparison of Total CK Activity in Patient Samples Using Two Different Reagent Systems

<table>
<thead>
<tr>
<th>Sample collected</th>
<th>CK Sigma units/mL</th>
<th>CK (CK-NAC), U/L</th>
<th>Ratio (CK-NAC/Sigma)</th>
<th>CK isoenzymes present</th>
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<td>20</td>
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<td>2</td>
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Specimens assayed 3/11/80 after storage at 4 °C.
Ratio (CK-NAC/Sigma) for a series of routinely submitted specimens for total CK activity = 15.7 (SD 1.6), range 11.8–18.6, n = 17; isoenzymes assumed to be of cytosol origin only; activity range: Sigma: 4–36; CK-NAC: 35–571.

series of specimens from the case presented. We calculated the ratio of apparent total CK activity, CK-NAC/Sigma, to compare an apparent difference in the reactivity of the reagent systems to the presence of CKm-1 and CKm-2 (Table 2). The CK-NAC reagent system appears to have a greater reactivity in the presence of CKm-1 and CKm-2 than the Sigma reagent system. A difference in reactivity between CK-NAC reagents and the Corning reagents for detection of CK activity in electrophoretic gels was also reflected in the difference in percent of total CK activity for CK-1.

Discussion

This patient was brought to our special attention by the extremely large proportion of CK-1 in her serum and the increasing CK activity present in the electrophoretically cathodal bands. Our first hypothesis was that CK-1 was being rapidly released from some tissue source and bound to serum proteins to form complexes of high molecular mass and decreased negative charge.

Treatment of CKm-2 with urea altered the Sephadex G-100 elution profile so that it resembled the elution profile of CK-3. However, the urea-treated CKm-2 continued to migrate cathodally on electrophoresis. Furthermore, antibodies to CK-1 and CK-3 failed to inhibit the CK activity present in the CKm-2 fraction both before and after treatment with NaCl or urea. The CK activity of this fraction thus appears to be distinct and separate from that of CK-1 and CK-3 isoenzymes.

As mentioned earlier, mitochondrial CK exists in two forms, both of which migrate toward the cathode on electrophoresis in agarose gel at pH 7.8. The cathodal CK activity found in this patient thus closely resembles the known characteristics of CKm. Unfortunately, specific antibodies to CKm are not available for evaluation of specific isoenzyme inhibition, and the amino acid composition of the patient's cathodal CK is not known (6, 14).

The origin of the cathodal CK in this patient is uncertain. The increase in enzyme activity followed needle biopsy of the liver and was associated with an increase in other enzymes associated with liver damage. Normal liver contains very little CK activity (15), which suggests that the CK may have been released from the metastatic carcinoma. No tissue was available to evaluate the CK isoenzymes present in the cancer cells.

Release from other tissues cannot be ruled out, especially in view of the septicemia and hypotension.

The different reagent systems showed a difference in sensitivity to the presence of the cathodal CK. The reagents of Biodynamics/bmc appeared to be more sensitive than either the Sigma or Corning systems. This difference in sensitivity to reagents has not been reported previously and its mechanism is unknown. The presence of CKm in serum may lead to a misinterpretation of CK activity and isoenzyme content when certain reagent systems are used.

Creatine kinase isoenzymes are not routinely determined in patients with disease not associated with cardiac or skeletal muscle. Thus, it is difficult to associate abnormal CK isoenzymes with pathology of other organ systems or disease. Increasing reference to CK-1 and cancer in the literature is suggestive of a possible relationship but the data are not conclusive. The presence of CKm in serum suggests tissue damage great enough to disrupt the mitochondrial membranes, or a high rate of cell turnover and breakdown, or both.

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References


10. Technical Bulletin No. 45UV, revised November 1978. Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.


