Abnormal Electrophoretic Mobility of a Creatine Kinase MM Isoenzyme

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We describe the abnormal electrophoretic mobility of an MM isoenzyme of serum creatine kinase on cellulose acetate, a change evidently attributable to complexing of the enzyme with serum β-lipoprotein. This complex formation also may lead to difficulty in interpretation of creatine kinase isoenzyme separation by ion-exchange column chromatography.

The routine determination of creatine kinase (EC 2.7.3.2) isoenzymes has recently gained acceptance as a diagnostic tool in laboratory medicine (1–3). Electrophoretically three distinct CK isoenzymes can be separated (4, 5). These dimeric bands have been identified throughout the literature as MM, MB, and BB, and originate mainly from skeletal muscle, heart, and brain tissues, respectively. We would like to report a patient with a serum CK MM isoenzyme, which, when mixed with other serum proteins, migrates on cellulose acetate with an electrophoretic mobility intermediate to that of the normal MM and MB.

Case History

B.B., a 76-year-old Caucasian woman was admitted to our hospital with a diagnosis of myalgia and depression, complaining of dull muscle aches during the six weeks before admission. Abnormal laboratory data include a serum CK of 300 U/liter (6) (Boehringer Mannheim Corp., New York, N. Y. 10017; normal 0–75 U/liter), erythrocyte sedimentation rate of 50 mm/h (7) (Westergren, normal 0–20 mm/h), blood urea nitrogen of 300 mg/liter (8), and a rheumatoid factor (9) positive 1:640 (Hyland Laboratories, Costa Mesa, Calif. 92629). Values for serum electrolytes, lactate dehydrogenase (EC 1.1.1.27), aspartate aminotransferase (EC 2.6.1.1), fructose diphosphate aldolase (EC 4.1.2.13), immunoglobulins, protein electrophoresis, and immunoelectrophoresis were within normal limits.

Muscle biopsy of the left thigh showed no primary muscle disease, but some focal Type II atrophy. Electrocardiogram displayed nothing to suggest the presence of acute pathology. Chest x-ray revealed arteriosclerotic disease with no evidence of congestive heart failure. Throughout nine days of hospitalization values for the patient’s serum CK activity remained abnormally high, about 300 U/liter.

Materials and Methods

CK electrophoresis on cellulose acetate, Mylar-backed, was performed with the "Zip Zone" fluorometric (10) system (Helena Laboratories, Beaumont, Tex. 77705). The CK isoenzymes were separated by a modification of Mercer’s (11) discontinuous buffer gradient elution ion-exchange method (12–14) (Worthington Biochemical Corp., Freehold, N. J. 07728). Immunoelectrophoresis was performed with the IEP system (15) (Hyland Laboratories). Unconjugated anti-sera was purchased from Hyland Laboratories.

A tissue specimen was obtained from a muscle biopsy of the left thigh. A portion of the specimen was immediately homogenized with cold 10 mmol/liter phosphate-buffered saline (pH 7.4), centrifuged (2100 rpm, 4° C, 10 min) and the supernatant fluid was diluted 100-fold with phosphate-buffered saline to yield a solution with a CK activity of 1700 U/liter.

Further Laboratory Results

In addition to a small MM band (24%), the initial serum CK electrophoresis showed the presence of an abnormal band (76%) migrating with a protein mobility between the MM and MB regions on the cellulose acetate (Figure 1a).

CK electrophoresis of the muscle extract showed normally migrating MM (79%) and MB (21%) isoenzymes (Figure 1b). The muscle extract diluted with an equal volume of normal serum (Figure 1c) then showed a normally migrating MM band (65%), the abnormal band (22.0%), and a normal migrating MB band (10.1%) by electrophoresis (Figure 1d).
The patient’s serum, 10 μl, was mixed with 200 μl of IEP antiserum and incubated overnight (16 h) at room temperature. Electrophoresis of this mixture showed an increase in the MM band to 46.7% and a decrease in the abnormal band to 53.3% as compared with original results for the serum. Another serum/antiserum mixture was similarly prepared by using beta-lipoprotein antiserum and the electrophoresis results were 54.4% MM band and 45.6% abnormal band of CK activity.

Immunoelectrophoresis on agarose was done on the patient’s serum vs. anti-whole human serum and against anti-human β-lipoprotein. After electrophoresis and diffusion, the plates were covered with CK reagent and incubated for 30 min at 45 °C. In both cases, the β-lipoprotein arc showed fluorescence. Normal serum treated in the same manner showed no fluorescence.

The patient’s isoenzymes were then separated by ion exchange, and MM, MB, and BB eluates were collected according to manufacturer’s instructions. CK activity was measured on these eluates; the results were 213 U/liter (65.9%) MM, 54 U/liter (16.7%) MB, and 56 U/liter (17.4%) BB. CK electrophoresis of a concentrate of each of these eluates showed a normally migrating band for the MM eluate, the abnormally migrating band for the MB eluate, and no detectable bands for the BB eluate.

Discussion

The data lead us to conclude that the unusual mobility of the isoenzyme in the serum of this patient is due to formation of a complex of an abnormal CK with a β-lipoprotein. Abnormal mobility is seen in the muscle-extract enzyme only when it is mixed with serum. This complex formation is analogous to the phenomenon of lactate dehydrogenase/immunoglobulin complexes (16–18).

The relative decrease in the amount of abnormally migrating enzyme and relative increase in enzyme of normal MM mobility when the abnormal serum is mixed with antiserum is probably the result of the enzyme/protein complex breaking while forming antigen/antibody complexes.

The two fractions obtained from ion-exchange column chromatography of the serum most likely represent CK from intra-column breaking of the complex and from intact complex. However, without subsequent cellulose acetate electrophoresis, the ion-exchange results could have been interpreted as showing the presence of MM and MB isoenzyme in the serum. Caution in interpreting results of ion-exchange separation is implicit.

References