Macro Creatine Kinase BB in Serum, and Some Data on Its Prevalence

Petter Urdal and Sverre Landaas

We report the case of a patient with persistently above-normal activity of creatine kinase (CK) in serum, a major fraction of which on electrophoresis moved as a band between the MM and MB isoenzymes and on anion-exchange column chromatography eluted in the MB fraction. Measurements in the presence of specific M or B subunit-inhibitory antibodies indicated that 93 % of the activity consisted of B-isomers. From these experiments we conclude that the abnormal CK is of BB nature. Gel filtration and immunoglobulin precipitation showed that the CK-BB was complexed with IgG. Normal CK-BB, when mixed with the patient's serum, was converted to macro CK-BB. In vitro stability at 37 °C of the abnormal enzyme was much greater than that of normal BB and MM isoenzymes. Following this finding, we then assessed 310 sera, received for enzyme assay by the clinical laboratory, for electrophoretically abnormally migrating CK isoenzymes. Of these, five (1.6 %) contained such enzymes, all being of BB nature. They were of increased molecular mass, and at least three of them were complexed with IgG.

Measurement of creatine kinase (CK, EC 2.7.3.2) isoenzymes in serum is now routine, particularly in the diagnosis of acute myocardial infarction. In addition to the MM, MB, and BB forms, several authors have reported the existence of other CK isoenzymes, which differ in their behavior on electrophoresis (1) or on ion-exchange column chromatography (2). Such isoenzymes have been found in 1–3% of all sera examined (1–3), but their occurrence is not yet satisfactorily explained. As these CK isoenzyme variants may be misinterpreted to be of MB nature, particularly in ion-exchange column chromatography (2) or the immunoinhibition technique (1), they may result in a false laboratory diagnosis of acute myocardial infarction.

Here we report studies of such an atypical CK isoenzyme, detected in a patient who had increased serum CK activity, most of which moved between the MM and MB isoenzymes on electrophoresis. We show the isoenzyme to be a complex between CK-BB and immunoglobulin G. We also describe studies of the prevalence of such complexes.

Materials and Methods

The CK activity in serum was determined according to the Scandinavian recommended method (4) with kit reagents from Boehringer, Mannheim, F.R.G. The CK reference range for women is 35–200 U/L. For measuring the low activities in eluates and supernates, the sample fraction was increased to 10-fold that used in the serum assay; other conditions were unaltered.

The B and M activities were estimated by the same method after incubating serum or other materials with antibody to CK-M or CK-B (anti-M or anti-B), respectively, as described by Gerhardt et al. (5). Anti-B lyophilisate was added in amounts three times that of anti-M lyophilisate. The antibodies were purchased from Merck, Darmstadt, F.R.G. The CK isoenzymes were separated by electrophoresis on agarose gel and made visible by their fluorescence in ultraviolet light (6); in some experiments the gel was incubated with anti-M before visualization (1). Alternatively, they were separated by ion-exchange column chromatography on DEAE-Sephadex, with discontinuous elution (7), with use of columns from Roche Diagnostics, Nutley, NJ 07110.

To estimate the molecular size of the CK isoenzymes, we fractionated 0.5 mL of serum on a Sephadex G-100 or G-200 column, 50 × 1.5 cm (Pharmacia, Uppsala, Sweden), using 150 mmol/L NaCl in Tris-HCl (50 mmol/L, pH 7.2) as buffer. We measured the content of IgG in the eluates nephelometrically with a Beckmann immunoochemistry analyzer 1 h after mixing 10 µL of eluate and 40 µL of anti-IgG solution (Dacopatt, Copenhagen, Denmark) in 600 µL of 150 mmol/L NaCl. Serum immunoglobulins were precipitated by mixing diluted serum (150 µL of a 10-fold dilution with 10 mmol/L imidazole acetate, pH 6.5) and antibody (100 µL of anti-IgM and anti-IgA or 500 µL of anti-IgG) from Dacopatt. Isotonic saline was added to give a final volume of 650 µL. After 2 h of incubation at 20 °C and centrifugation (1000 × g, 10 min), CK activity was measured in the supernatant fluid. A pectoral muscle biopsy sample, taken under lidocaine anesthesia, was homogenized in 0.25 mol/L sucrose. The mitochondria were removed, together with nuclei and cell debris, by centrifugation (10 000 × g, 10 min). Cisaleral fluid with high CK-BB activity was collected postmortem from a patient who had suffered from a non-cerebral disease. After centrifugation (10 000 × g, 10 min) the supernate contained CK-BB in an activity of
3700 U/L. Sera were obtained by venipuncture. All specimens were either used immediately or stored at -80 °C.

Case History

A 78-year-old woman was admitted to the hospital in May 1978 with moderately severe chest pain. Except for hysterectomy in 1934, owing to a tumor, and rheumatic fever in 1948, she had been in good health, both physically and mentally. Neither electrocardiography nor results of routine clinical analyses implied any acute myocardial infarction, but she showed atrial fibrillation and a slight edema in both legs. Activities of aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2), lactate dehydrogenase (EC 1.1.1.27), alkaline phosphatase (EC 3.1.3.1), and amylase (EC 3.2.1.1) in her serum were all within normal limits. Electrophoresis of serum proteins in agarose gel revealed no abnormalities. Concentrations of immunoglobulins IgG, IgM, and IgA were only slightly above the normal ranges.

The chest pain disappeared spontaneously and she left the hospital after four days. She was on no medication before admission and received only a short course of digitoxin during her stay in hospital. She received no intramuscular injections.

Studies on an Atypical CK Isoenzyme

Case Findings and Comments

At the time of admission to the hospital, the total CK activity of the patient’s serum was 598 U/L. During the four days in the hospital, the activity remained practically unaltered; four weeks after her discharge, the value was still high (680 U/L). Electrophoresis in agarose gel revealed a minor, normal MM band and a major, abnormal band between the MM and MB position, closer to MM than to MB (Figure 1A). Both bands were absent when creatine phosphate substrate was omitted from the incubation mixture. On ion-exchange column chromatography, 8% of the CK activity was found in the MM fraction, 92% in the MB fraction, and no activity in the BB fraction (Figure 1B). Measurements of CK activity in the presence of anti-M (Table 1) indicated that CK-BB was responsible for 93% of the total activity. Experiments with anti-B also support the conclusion that the major isoenzyme is BB in nature, allowing for the fact that this antibody inhibits only about 75% of normal BB activity (Table 1).

We examined the stability to incubation at 37 °C of CK in the patient’s serum. As shown in Table 2, this particular isoenzyme was much more stable than were the concurrently examined normal BB and MM isoenzymes.

From these experiments we conclude that the patient’s serum contains both a normal proportion of MM isoenzyme (30–50 U/L) and a high proportion of BB isoenzyme (400–600 U/L). The physical properties of the latter differ markedly in many respects from those of normal CK-BB. Gel filtration (Figure 2) indicated that the unusual isoenzyme had a higher relative molecular mass than the normal CK isoenzymes. Evidence that the enzyme was bound in complex with an immunoglobulin was obtained by measuring the enzyme activity before and after the removal of IgG, IgA, or IgM by adding specific precipitating antibodies followed by centrifugation. Two-thirds of the activity disappeared when IgG was removed, whereas removal of IgM and IgA did not affect the activity (Table 3).

When normal CK-BB was added to the patient’s serum, the activity of the MB fraction on ion-exchange chromatography increased markedly, about half of the BB added being eluted as MB (Table 4). There was no such change in chromato-

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### Table 1. Influence of Inhibiting Antibodies on CK Activity

<table>
<thead>
<tr>
<th>Material</th>
<th>Original activity, U/L</th>
<th>Per cent inhibition</th>
<th>Anti-M</th>
<th>Anti-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MM</td>
<td>895</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>CK-BB</td>
<td>1190</td>
<td>2</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>CK-BB</td>
<td>523</td>
<td>0</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Serum from patient (May 9th)</td>
<td>598</td>
<td>7</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Serum from patient (June 5th)</td>
<td>680</td>
<td>7</td>
<td>61</td>
<td></td>
</tr>
</tbody>
</table>

* Serum of high activity (CK-MM) and cisternal fluid (CK-BB) diluted in cerebrospinal fluid of low CK activity.

### Table 2. Stability of CK During Incubation at 37 °C

<table>
<thead>
<tr>
<th>Material</th>
<th>Initial activity, U/L</th>
<th>Per cent of initial activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-BB</td>
<td>590</td>
<td>3 h 19 h</td>
</tr>
<tr>
<td>Normal serum 1 (MM only)</td>
<td>200</td>
<td>97</td>
</tr>
<tr>
<td>Normal serum 2 (MM only)</td>
<td>180</td>
<td>97</td>
</tr>
<tr>
<td>Serum from patient</td>
<td>440</td>
<td>98</td>
</tr>
</tbody>
</table>

All the samples were drawn simultaneously just before the start of the experiment.

— Cisternal fluid was diluted fivefold normal serum 1 and the CK-B activity was measured in the presence of anti-M.
Table 3. Residual Total CK Activity after Removal of Immunoglobulins by Addition of Precipitating Antibodies

<table>
<thead>
<tr>
<th>Addition</th>
<th>Normal sera (n = 3)</th>
<th>Patient’s serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-IgG</td>
<td>98</td>
<td>33</td>
</tr>
<tr>
<td>Anti-IgM</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Anti-IgA</td>
<td>99</td>
<td>98</td>
</tr>
</tbody>
</table>

Table 4. CK Isoenzyme Pattern, as Revealed by Ion-exchange Chromatography, in a Mixture of Serum and Cisternal Fluid

<table>
<thead>
<tr>
<th>CK activity in eluate</th>
<th>MM (U/L)</th>
<th>MB (U/L)</th>
<th>BB (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum 1</td>
<td>33.6</td>
<td>0</td>
<td>17.4</td>
</tr>
<tr>
<td>Normal serum 2</td>
<td>2.5</td>
<td>0</td>
<td>17.9</td>
</tr>
<tr>
<td>Normal serum 3</td>
<td>4.5</td>
<td>0</td>
<td>18.5</td>
</tr>
<tr>
<td>Patient’s serum</td>
<td>2.6</td>
<td>22.9</td>
<td>8.0</td>
</tr>
<tr>
<td>Patient’s serum</td>
<td>1.2</td>
<td>15.0</td>
<td>0</td>
</tr>
</tbody>
</table>

(BB not added)

0.1 mL of cisternal fluid was added to 0.3 mL of serum.

greater in vitro stability at 37 °C of the BB-IgG complex as compared to the uncomplexed normal MM and BB isoenzymes might also reflect the situation in vivo.

Prevalence of the Macro CK

We next undertook to see how often such a complex may be found in serum from patients. Sera from a total of 310 patients were chosen without conscious bias from among those sera sent by the clinical departments for assay of enzymes. In addition to the tests requested, these sera were also examined for atypically migrating CK isoenzymes on electrophoresis. The detection limit for CK isoenzyme activity by our electrophoretic method was 20–25 U/L.

We found an abnormally moving electrophoretic CK-band in serum from five of the 310 patients. These bands were still present and of essentially the same strength in serum samples drawn a week later. The aberrant bands of four of the five sera (sera 1–4) migrated between the MM and MB positions (Figure 3). In addition, a normal MM band was also present. Serum 5 contained three bands, of which two moved as ordinary MM and BB isoenzymes, whereas the third moved slightly cathodic to the MM (Figure 3). No bands were visible when creatine phosphate was omitted from the incubation mixture. The total CK activity was within reference limits in all of these five sera (Figure 3).

The immunologic nature of the CK bands was examined by incubating the gel in the presence of specific CK-M-inhibitory antibody (anti-M). This did not seem to reduce the intensity

<table>
<thead>
<tr>
<th>Patient</th>
<th>Isoenzyme marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CK (U/L)</td>
<td>70</td>
</tr>
</tbody>
</table>

Electrophoresis

Immunoinhibition

Ion-exchange

Chromatography

<table>
<thead>
<tr>
<th>CK-B (U/L)</th>
<th>21</th>
<th>58</th>
<th>30</th>
<th>27</th>
<th>46</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MM (U/L)</td>
<td>47</td>
<td>73</td>
<td>86</td>
<td>46</td>
<td>46</td>
<td>1010</td>
</tr>
<tr>
<td>CK-NB (U/L)</td>
<td>14</td>
<td>43</td>
<td>22</td>
<td>18</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>CK-BB (U/L)</td>
<td>3</td>
<td>11</td>
<td>6</td>
<td>8</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 3. Abnormal CK isoenzymes in sera from five patients

The isoenzymes were assayed by electrophoretic, immunologic, and column-chromatographic techniques. The control patient had suffered an acute skeletal muscle injury.

graphic properties when CK-BB was added to normal sera. This shows that the enzyme-binding immunoglobulin in the patient’s serum could complex normal BB isoenzyme.

Electrophoresis of the muscle homogenate from the patient revealed only MM isoenzyme, and the CK activity was completely inhibited by anti-M.

The results with anti-M and anti-B immunoglobulins leave little doubt that the abnormal isoenzyme, which is responsible for about 90% of the total CK activity, is of BB type. The remaining activity is due to an MM isoenzyme, which behaves normally in all of the analytical systems applied. The MM activity is low, 30–50 U/L, but this is not extraordinary for a woman of this age. There is no reason to believe that the CK-BB produced in our patient is itself abnormal because the IgG in the patient’s serum complexed with normal CK-BB from other sources.

The BB isoenzyme is usually not detectable in serum by electrophoretic, immunoinhibiting, or column-chromatographic methods, and has only been described in connection with severe brain damage (8), labor (9), and a few other clinical conditions (10, 11). Our patient was mentally clear, despite her age, and showed no neurological symptoms. Lumbar puncture, for examination of CK in cerebrospinal fluid, was not performed because there were no other indications for this procedure. Another potential source of CK-BB, the uterus, had been removed many years previously, and there were no clinical or biochemical abnormalities indicating disease in organs with large amounts of smooth muscle, such as the gastrointestinal tract or the lungs. The skeletal muscles contained only normal MM isoenzyme. There is thus no reason to suspect any abnormal release of BB to the blood. Most likely, the high serum activity of CK-BB is caused by a decreased rate of inactivation rather than an increased release from tissues. The activity decay of CK is considered to be primarily temperature dependent (12), and the markedly

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of any of the aberrant bands or of the BB band in serum 5, whereas all the MM bands essentially disappeared. The quantitative immunoinhibition technique revealed that from 25 to 60% of the CK activity in the five sera was caused by the B-monomer (Figure 3). Thus it seems likely that all the abnormal CK isoenzymes were isoenzyme B in nature.

On ion-exchange column chromatography of sera 1–4 the activities that appeared in the MB plus BB fractions corresponded well with the CK-B activities determined by immunoinhibition technique (Figure 3). Some of the CK activity of serum 5 eluted in the BB fraction (cf. the BB band on electrophoresis), but most appeared in the MM fraction. However, only half of the latter activity could be inhibited by anti-M, suggesting that this fraction contained in addition the abnormal isoenzyme, which migrated cathodically on electrophoresis.

Gel filtration suggested that the abnormal isoenzymes in sera 1–5 all were of increased relative molecular mass (Figure 4). The findings were practically similar in sera 1–4. In the case of serum 5, about half of the CK-B activity appeared in the void volume, while the rest of it eluted together with normal CK-MM. The measurements in the presence of anti-M (Figure 4) add further proof to the suggestion that the macroenzymes all are of BB nature.

Removal of IgG by precipitation with specific antibody concomitantly removed most of the CK-B activity in sera 2–4, while there was little or no reduction in sera 1 and 5. (Table 5). Removal of IgM and IgA did not influence the CK-B activity in any of the sera. CK-M activity did not decrease in any of the sera after removal of immunoglobulins (results not shown). Thus, for three of the five sera evidence was obtained suggesting the presence of a specific CK-binding IgG molecule. However, the results do not exclude a CK-immunoglobulin complex also in the cases of sera 1 and 5. The antibody used to precipitate the immunoglobulins of the sera may have been unable to precipitate the CK-binding immunoglobulin, or the enzyme may have detached from the immunoglobulin during the precipitation.

Upon incubation at 37°C, BB activity normally declines five to six times faster than MM activity (12). After 6 h of incubation of serum 2, however, the activity decreased by 63 and 47% for CK-M and CK-B, respectively. The increased stability of CK-BB of this serum also supports our hypothesis that the high CK-BB activity in the sera with abnormal isoenzymes is due to a decreased rate of inactivation rather than to increased release from tissues.

Our five patients had no clinical disorder in common.

**Discussion**

The binding of enzyme to immunoglobulin has already been described for amylase (13), lactate dehydrogenase (14), alkaline phosphatase (15), and alanine aminotransferase (16). The total activity of the enzyme reportedly was increased in some cases (13, 14); in others it was within reference limits (17). For amylase, as for the CK described in our case, it has been found that the immunoglobulin can bind to a normal enzyme (18). It is not clear whether this is a specific antigen–antibody reaction or a nonspecific protein–immunoglobulin complex.

Macroamylasemia has been reported to occur in as many as 2% of sera from patients with various disorders (17). In the present study we found macro CK in serum of about 1.5% of the 310 patients examined.

The abnormal isoenzymes all appear to be BB in nature. Because apparently normal CK-MM is also present in the five sera, the binding substance evidently may be specific for CK-BB. In contrast, the binding substance in macroamylasemia has been found to complex both the pancreatic and the salivary isoenzymes of amylase (18).

CK isoenzymes that migrate between the MM and MB or cathodically to the MM position on electrophoresis have been reported previously (1–3, 19, 20). In light of our results, complex formation with serum immunoglobulins may well be the mechanism responsible for the occurrence of these isoenzyme variants. Sax et al. (2) claimed the isoenzyme to be of normal molecular mass, and Yuu et al. (19) could not demonstrate any binding to immunoglobulin. Their findings may be due to the detachment of enzyme from the complexing immunoglobulin during the in vitro treatment of the serum, as discussed for two of our sera.

In conclusion, our results demonstrate that increased activity of CK-BB of high molecular weight, probably due to a complexing with immunoglobulin, occurs in serum from a considerable fraction of patients. When using immunologic technique or ion-exchange column chromatography for the measurement of CK isoenzymes, these abnormal isoenzymes may be misinterpreted to be of MB nature.

**References**


