Heterogeneity of Serum Creatine Kinase Isoenzyme MM in Myocardial Infarction: Standardization of Patterns by Use of Cord-Blood Serum

John Williams1 and Thomas Marshall2

We investigated serum creatine kinase (CK; EC 2.7.3.2) isoenzyme MM in myocardial infarction, using isoelectric focusing in polyacrylamide gels. As many as 14 sub-bands were detected, and sequential sampling revealed a progressive anodal shift in their distribution. A nomenclature for sub-band typing is proposed. It denotes the major sub-bands as 1 (pI 6.91), 2 (pI 6.65), and 3 (pI 6.35), which are usually detected in sera of normal CK range. Abnormal sub-bands (associated with increases in the concentrations of CK) are denoted as a (pI 7.55), b (pI 7.35), c (pI 7.25), d (pI 7.05), e (pI 6.85), f (pI 6.72), g (pI 6.50), h (pI 6.40), i (pI 6.28), j (pI 6.20), and k (pI 6.15). Cord-blood sera gave highly reproducible CK-MM patterns characterized by prominent detection of sub-bands 1–3 and faint detection of e, f, g, h, i, and j. It is recommended as a standard for CK-MM sub-band typing.

Creatine kinase (CK; ATP:creatine N-phosphotransferase, EC 2.7.3.2) is a dimer of two subunits, M and B, giving three isoenzymes: CK-MM, CK-BB, and CK-MB (1–3). CK-MM and CK-BB predominate in skeletal muscle and brain tissue, respectively (2, 3). CK-MB is found almost exclusively in myocardium, and its release into serum is indicative of myocardial damage (2–5).

Serum CK-MM is heterogeneous (6–17), as indicated by (a) electrophoresis in cellulose acetate (12, 17), agarose (6–8, 11, 14) and polyacrylamide (9, 13, 15), or (b) isoelectric focusing (IEF) in agarose (13, 14, 17) and polyacrylamide (9, 10, 12, 13, 16). The CK-MM "sub-bands" are prominent after myocardial infarction and, upon sequential sampling, display a characteristic distribution and interconversion (7, 9, 10, 12, 14, 17). This has been correlated with in vitro conversion (towards the anode) of cathodal tissue CK-MM sub-bands during incubation with human serum—an activity attributed to a serum "thermolabile factor" (8, 9, 11–15, 18). The serum CK-MM sub-bands may be of diagnostic value in myocardial infarction (3, 9, 11, 12, 17), but the interpretation of their IEF patterns is complicated by inconsistencies in the reported number of sub-bands, nomenclature, and pl values. We report improved detection of serum CK-MM in myocardial infarction and propose a nomenclature for sub-band typing based on cord blood as a standard.

Materials and Methods

Reagents. Acrylamide, N,N'-methylenebisacrylamide (both of "Electran" grade), and pl calibration proteins were from BDH Chemicals, Poole, Dorset, U.K. Ampholytes ("Pharmalyte"), pH range 5–8, were from Pharmacia, Uppsala, Sweden; "Ultragrade" ammonium persulfate and TEMED (N,N,N',N'-tetramethylethlenediamine) from LKB, Bromma, Sweden. All solutions were prepared with deionized water ("Elgastat" purification system).

Apparatus. Flat-bed IEF was carried out with a Model 600 electrophoresis system (Shandon Southern Products Ltd., Runcorn, Cheshire, U.K.) and an LKB 2000 Power Supply Unit.

Samples. Serum was recovered within 2 h of blood collection from hospitalized patients (at admission and appropriate time intervals thereafter) in the coronary care unit of Sligo General Hospital. CK activity was determined immediately, and serum aliquots with added potassium EDTA (final concentration 5 mmol/L) (10) were stored in liquid nitrogen. Activity was unaffected after six months of storage.

Isoelectric focusing. The polyacrylamide gels (130 × 110 × 0.4 mm; T = 5%, C = 3%) containing 20 mL of Pharmalyte pH 5–8 and 200 mL of glycerol per liter were prepared in glass cassettes comprising two glass plates (the lower sarnized in acetone and the upper coated with 20 g/L repel silane (19)) separated by four layers of Parafilm (20). The gel mixture was extensively degassed, then polymerized with TEMED and ammonium persulfate (final concentration 0.3 mL/L and 0.4 mg/L, respectively). IEF was performed the following day with fresh degassed 0.5 mol/L sodium hydroxide as catholyte and 40 mmol/L L-glutamic acid (Sigma Chemical Co., St. Louis, MO) as anolyte. The samples (5 mL of serum in 2 × 5 mm strips of Whatman 41 filter paper) were loaded 20 mm from the anode, and electrode wicks (LKB electrofocusing strips, equilibrated in electrolyte) were applied to the gel surface. The gels were focused, without a pre-run, at 200 V (10 W) for 10 min and 1400 V (10 W) for 20 min; the sample applicators were then removed before further focusing at 1400 V (15 W) for 2 h. After the IEF, we monitored the pH gradients with a surface pH electrode (Orion Research, Inc., Cambridge, MA) and visualized the CK bands by fluorescence under ultraviolet light after having evenly applied 1 mL of commercial CK reagent ("CK-Nac"; BCL, Lewes, Sussex, U.K.) to the gel surface and incubating at 37 °C for 20 min. The colored pl calibration proteins (BDH) were detected as dull nonfluorescent bands.

Results

Figure 1 demonstrates the CK-MM sub-bands of sequential serum samples, with above-normal CK content (449–3056 U/L), collected from four hospitalized patients with a positive diagnosis of acute myocardial infarction (MI). Sub-bands 1, 2, and 3 (pI 6.91, 6.65, and 6.35, respectively) were faintly visible in adult serum having a "normal" total CK range (20–120 U/L). Abnormal MI sub-bands denoted a, b, and d (of higher pl: 7.55, 7.35, and 7.05, respectively) were only rarely detected, while sub-band e (pI 7.25) was always present. Increased total CK in serum was usually accompanied by sub-bands e (pI 6.85) and g (pI 6.50) and sometimes
by faint sub-bands f (pI 6.72) and h (pI 6.40). Sub-bands e/f and g/h were intermediate in position to the major "normal" MM sub-bands 1–2 and 2–3, respectively (Figure 1).

The sequential serum samples from MI patients usually demonstrated a characteristic anodal shift in the CK-MM sub-band distribution profile: sub-band c disappeared, the intensity of sub-bands 1 and e decreased, and the intensity of sub-bands g and 3 increased; moreover, abnormal sub-bands i (pI 6.28) and j (pI 6.20) appeared (diffusely). An additional anodal sub-band denoted k (pI 6.15) was sometimes detected.

The serum CK-MM sub-band patterns of the MI patients were of unprecedented complexity. Establishing the position of sub-bands 1–3 and avoiding misidentification, particularly of b and c, 1 and e, and 2 and g required careful calibration of the pH gradients. Co-electrophoresis of cord-blood serum proved suitable for this purpose, with different samples giving surprisingly consistent CK-MM sub-band patterns characterized by (a) prominent "normal" sub-bands 1–3; (b) faint sub-bands c, e, and g; and (c) detectable sub-bands f, h, i, and j (Figure 2). The relative intensity of these sub-bands (e.g., 1 > e > f) was characteristic of cord-blood sera (Figure 2) and was inherent, though variably present, in sera with increased concentrations of total CK (Figure 1). The repetitive nature of this pattern and the range and relative intensity of CK-MM sub-bands in sera from normal subjects, MI patients, and cord blood are summarized in composite form in Figure 3.

**Discussion**

The analysis of serum CK-MM sub-bands may be of diagnostic value in myocardial infarction (3, 9) for assessing the extent and time of onset of necrosis (11, 12), the effect of medication, likely prognosis (17), and occurrence of reinfarction (12). Analysis will require reproducible detection of all sub-band species without in vitro modification by the "thermolabile factor" in human serum. The complexity of the patterns also demands a simple method of standardizing/identifying the individual sub-bands and a sensible nomenclature for accurate reporting of results and interlaboratory comparison.

In the nomenclature we propose:
- the numbers 1–3 are assigned to the three "normal" (and usually predominant) CK-MM sub-bands, in order of increasing anodal mobility. This is consistent with previous reports (9, 10, 12, 16) and reflects the anodal direction of conversion after MI or after in vitro incubation with "thermolabile factor."
- the lower case letters a–k are assigned to the "abnormal" (and often minor) CK-MM sub-bands in order of increasing anodal mobility. This avoids the unproven implication that these sub-bands are derived from the major components and also avoids confusion with the M,B nomenclature; e.g., MM1B and MM2B (16) implies derivation from MM1 and MM2, respectively, and the use of capital B duplicates the standard BB, MM, MB isoenzyme nomenclature.

A comparison of our nomenclature with previous reports is summarized in Table 1. Much confusion exists. However, our nomenclature (although contrary to the recommendation in ref. 1) is consistent with most previous reports and simplifies clinical CK-MM sub-band typing by identifying abnormal sub-bands with letters (and normal sub-bands with numbers) in a sequence consistent with the direction of interconversion.

Co-electrophoresis of cord-blood serum proved a surprisingly reproducible and simple method for standardization/identification of CK-MM sub-bands in clinical samples. Its sub-band pattern, which has not previously been reported, was remarkably consistent and clearly detectable. This reflects its characteristic two- to threefold increase of total CK activity relative to "normal" adult sera (21). The latter was not a suitable standard because the CK-MM sub-bands
either were not detected or were present in variable proportions in different samples.

In contrast to previous reports, we have invariably detected, with a high degree of sensitivity, a wide range of abnormal CK-MM sub-bands in MI. We attribute this to the following:

(a) Sample collection and storage: cathodal CK-MM sub-bands are prone to serum "thermolabile factor" (8–10, 12–14) but can be stabilized by EDTA (10). We recommend rapid recovery of serum from blood, addition of EDTA (final concentration, 5 mmol/L) [and intermediate analysis or storage in liquid nitrogen followed by slow thawing at room temperature]. Less stringent protocols (including freezing and thawing at −20 °C to −70 °C) can lead to loss of sub-band c (pl 7.25) and some change in the overall pattern. This may explain the absence and lower frequency/sensitivity of detection of cathodal components (e.g., pl 7.25) in previous reports (7, 9, 12, 16).

(b) The electrophoresis method: IEF gives better resolution of CK-MM sub-bands than does electrophoresis (9) and more sub-bands are detected with polyacrylamide than agarose (13). We recommend cleaning the platinum electrodes after each run and using 0.5 mol/L rather than 1 mol/L sodium hydroxide as a catholyte (electrode wicks are more manageable at this concentration). Use of gels containing 200 mL/L (rather than 100 mL/L) glycerol minimizes protein precipitation at the site of sample application. Narrow-range ampholites give better resolution of CK-MM sub-bands but should be closely monitored because any slight impairment in resolution (as indicated by pl marker proteins) results in loss of sub-bands e, f, g, and h.

The detection limit and linearity of the method were determined by serial dilution (17) and analysis of 5 μL of sample. The response varied linearly with concentration up to 1000 U of total CK per liter and the minimum detectable amount of an individual CK-MM sub-band was equivalent to 2 U/L.

Gulisits and Jacobs (13) recently reported 21 CK-MM sub-bands in human poas muscle extract and monitored the response of the sub-bands to autolysis and in vitro incubation with normal human serum. The major CK-MM sub-bands detected after autolysis (denoted MM17, MM12, and MM7) may correspond to serum CK-MM sub-bands a, c, and d, respectively, while those prominently detected after in vitro incubation with human serum (MM12, MM6, MM1, and MM3) may correspond to serum CK-MM sub-bands c, 1, 2, and 3, respectively—those prominently detected in MI (Table 1, Figure 1). This suggests a possible sequence of molecular events in MI: a generally rapid anodal conversion of cathodal tissue CK-MM sub-bands to yield the serum CK-MM pattern (Figures 1 and 3), followed by further anodal conversion with generation of additional sub-bands (Figures 1 and 3). The proteolytic conversion of extracted tissue CK-MM (pl 7.58) to sub-bands of pl 7.43 and 7.30 by carboxypeptidases (15, 22) is consistent with the autolytic conversion of tissue CK-MM reported by Gulits and Jacobs (13) but is not consistent with the serum CK-MM sub-band conversion (over the pl 7.25–6.20) characteristic of serum "thermolabile factor" (8, 9, 11–15, 18). We believe these proteolytic activities are either inherent in normal serum or derived from tissue damage (e.g., as a consequence of exercise (16) or MI (7, 9, 11, 12, 14, 17) and are possibly associated with clotting/fibrinolysis—a hypothesis we are currently investigating.

Table 1. Comparison of Nomenclatures for the CK-MM Sub-Band

<table>
<thead>
<tr>
<th>This report:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>7: S</td>
<td>8, 10: S; H, M</td>
<td>12: S; H, M</td>
<td>14: S; H, M</td>
<td>16: P; M</td>
<td>13: M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>7.55 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>7.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>7.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>7.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.91</td>
<td>MM3, 6.86</td>
<td>MM1, 6.88</td>
<td>MM1, 6.90</td>
<td>MM3, 6.90</td>
<td>MM1, 6.90</td>
<td>MM6, 6.90 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>6.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>6.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.65</td>
<td>MM2, 6.49</td>
<td>MM2, 6.70</td>
<td>MM1, 6.49</td>
<td>MM2, 6.67</td>
<td>MM2, 6.62</td>
<td>MM1, 6.60 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>6.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>6.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6.35</td>
<td>MM1, 6.24</td>
<td>MM3, 6.45</td>
<td>MM3, 6.36</td>
<td>MM1, 6.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i</td>
<td>6.28</td>
<td>X, 6.07</td>
<td>MM4, 6.25</td>
<td>MMIV, 6.20</td>
<td>X, 6.23</td>
<td>MM4, 6.20</td>
<td>MM4, 6.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>j</td>
<td>6.15</td>
<td>Y, 6.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* S denotes serum; P, plasma; H, heart/myocardium extract; and M, skeletal muscle extract. a The adopted nomenclature is followed by the quoted pl values.

References

Spermidine Oxidase Activity in Serum of Normal and Schizophrenic Subjects

Khewala A. Flayeh

Spermidine oxidase activity in human serum is distributed over a relatively wide range, with a highly significant difference between normal and schizophrenic subjects. The enzyme activity showed no age- or sex-related differences. It is largely inhibited by quinacrine and chloroquine.

Additional Keyphrases: polyamine metabolism - schizophrenia - sex- and age-related differences

The two major polyamines, spermidine and spermine, occur ubiquitously in tissues (1). They may participate in one or more phases of nucleic acid metabolism (2-3). Polyamine concentrations are altered in some clinical conditions such as cancer (4), sickle cell anemia (5), and chronic renal failure (6). Schizophrenia is another disease in which polyamine concentrations are altered (7-8), and the polyamine, spermidine, may be involved (9).

To my knowledge, few data on distribution of polyamine oxidase (PAO) activity in human serum and tissues have been reported (10-11). Because of the possible significance of a polyamine-polyamine oxidase interaction that mediates polyamine catabolism, with formation of active metabolites, I estimated polyamine oxidase activity in the serum of a representative sample of healthy subjects, with attention to sex- and age-related differences. A comparison of spermidine oxidase activity in healthy subjects with that in schizophrenics is also reported here.

Materials and Methods

Samples

Blood was sampled from unselected normal (volunteer) men and non-pregnant women, with the help of the Blood Bank Institute, Mosul.

Blood samples from schizophrenic patients were obtained from Mosul Hospital. These patients manifested delusions, hallucinations, thought disorder, and inappropriate affect, but they were free of known organic disease. Their classification according to DSM-II (12) and their daily drug dosages, at the time of sampling were as follows: schizophrenia chronic, thioridazine, 600 mg, and trihexyphenidyl, 4 mg; schizophrenia chronic halluciatory, trifluoperazine, 20 mg, and maprotiline, 75 mg; schizophrenia chronic acute episode, thioridazine, 600 mg, and nitrazepram, 5 mg; schizophrenia paranoid, chlorpromazine, 300 mg; schizophrenia acute, trifluoperazine, 20 mg; schizophrenia acute nondifferentiated, trifluoperazine, 20 mg; schizophrenia acute cata-tonic, chlorpromazine, 75 mg; schizophrenia acute paretic, chlorpromazine, 75 mg, and trihexyphenidyl, 4 mg; and schizophrenia, thioridazine, 300 mg.

The blood was sampled by venepuncture into sterile disposable syringes, transferred into glass tubes, and allowed to clot for 5 min at 37°C. Sera were separated by centrifugation and assayed on the day of collection. Data on sex and age were recorded.

Procedures

Standard spermidine oxidase assay. I used the assay of Tabor and Kellogg (13) for determination of spermidine oxidase activity in untreated (native) human serum: the activity was assayed spectrophotometrically in a Beckman recording spectrophotometer by following the decrease in