Heterogeneity of Serum Creatine Kinase Isoenzyme MM in Myocardial Infarction: Clinical Significance and Post-Synthetic Conversion of “Abnormal” Sub-Bands

John Williams, Katherine M. Williams, and Thomas Marshall

We investigated the “abnormal” sub-bands of serum creatine kinase (CK; EC 2.7.3.2) isoenzyme MM in acute myocardial infarction (AMI), using isoelectric focusing in polyacrylamide gels, and compared the patterns with those for non-AMI patients and for athletes with increased CK. The “abnormal” sub-bands 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), in common with “normal” sub-bands 1 (pI 6.91), 2 (pI 6.65), and 3 (pI 6.35), were less uniformly distributed in the AMI and demonstrated a faster rate of anodal conversion than in the non-AMI patients and athletes. “Abnormal” sub-bands a and b were detected only in the AMI patients. Incubation of myocardial CK-MM, predominantly CK-MM 1, with 2-mercaptoethanol, 15 mM/L, resulted in conversion to the “abnormal” sub-bands b and c. Incubation of myocardial CK-MM with normal serum of low total CK yielded CK-MM 3, which on further incubation with 2-mercaptoethanol, 15 mM/L, resulted in conversion to the “abnormal” sub-bands f and g. Comparable in vitro incubation of serum from AMI patients gave pattern changes consistent with conversion of CK-MM 1 to b, c; CK-MM 2 to d, e; and CK-MM 3 to f, g.

Additional Keywords: athletes compared • heart disease • effects of exercise • nomenclature for sub-bands

Creatine kinase (CK; ATP:creatinine N-phosphotransferase, EC 2.7.3.2), a dimer of subunits M and B, exhibits three isoenzymes (CK-MM, CK-MB, and CK-BB) with differential chemical, immunological, and electrophoretic properties (1, 2). CK-MB in serum indicates myocardial damage (1–7), as in acute myocardial infarction (AMI). Serum CK-MM is demonstrably heterogeneous (8–19) but the clinical significance of this in AMI is unclear. Fourteen CK-MM sub-bands have been detected in human serum (19), 21 in muscle extracts (20). This complexity is compounded by contradictions in serum CK-MM nomenclature (9, 11–14, 16, 19) and by the anodal shift in sub-band distribution (8, 11–14, 17, 19), which is induced by a “thermolable factor” in serum (10, 11, 13–15, 18, 20) now identified as carboxypeptidase N (EC 3.4.17.3) (18, 21–23). We recently proposed (19) a serum CK-MM nomenclature that distinguished the three “normal” and 11 “abnormal” sub-bands seen in AMI patients and was consistent with the anodal direction of CK-MM conversion (19). We now evaluate the clinical significance of these “abnormal” sub-bands and demonstrate their formation from the major “normal” sub-bands.

Materials and Methods

Reagents. Acrylamide and N,N’-methylenebisacrylamide (“Electran”) and pl calibration proteins were purchased from BDH Chemicals, Poole, Dorset, U.K. Ampholites (“Pharmalyte,” pH 5–8) were from Pharmacia, Uppsala, Sweden. All solutions were freshly prepared with de-ionized water (“Elgastat purification system).

Apparatus. For flat-bed isoelectric focusing (IEF) we used a power supply unit from LKB Instruments, Bromma, Sweden, and a Model 600 electrophoresis system from Shandon Southern Products Ltd., Runcorn, Cheshire, U.K.

Samples: Serum samples. Serum from venous blood, processed within 2 h of collection, was immediately assayed for total CK activity with a centrifugal analyzer (Cobas Bio; Hoffmann-La Roche & Co. Ltd., Basel, Switzerland). CK isoenzymes (CK-MB) were determined (sometimes after storage at −20 °C for as long as three days) with a Corning electrophoresis system as recommended by the manufacturer. Potassium EDTA (5 mmol/L) was added to stabilize the CK-MM sub-bands (12) and the sera were stored in liquid nitrogen (19) until IEF. Samples were obtained from:

• 74 AMI patients (45 men, 29 women; ages 42–87 y), diagnosed on the basis of clinical history, electrocardiography, and changes in serum lactate dehydrogenase/CK activity (24). The location of the infarction was inferred in 50% of the patients, anterior in 39%, and global (with inferior and anterior wall changes) in 11%; 23% had a history of AMI, 26% had angina, and 12% died within the next four months. None of these patients received thrombolytic therapy. Samples were routinely obtained on hospital admission and 12 and 36 h later but also at 6-h intervals for three days after admission (12 patients) or daily for nine days (10 patients). The time of infarct was judged to be from the onset of severe chest pain.

• 19 non-AMI patients (11 men, eight women; ages 41–84 y), hospitalized with chest pain but diagnosed as not having AMI by the above criteria. This group included a 62-year-old woman with polymyositis and patients with cardiac arrest, ischemic heart disease, papillary muscle dysfunction, atrial fibrillation, left bundle-branch block, acute pulmonary edema, hypertension, or seizures (post-ictal state). Samples were routinely collected at the time of hospital admission and 12 and 36 h later, but occasionally at 6-h or daily intervals as described above. Twelve of these patients had increased CK, and sequential sampling indicated its concentration to be increasing in five, decreasing in five, and static in two. The remaining seven patients (predominantly angina) had normal values for total CK.

• 14 athletes (13 men, one woman; ages 22–45 y), sampled 24–60 h before and 24 and 48 h after a triathlon (2 km swimming, 90 km bicycling, 21 km marathon running).

Tissue samples. Myocardial slices obtained at autopsy (24–48 h after death) and stored frozen were rinsed in water, dissected, and homogenized (1.3-g aliquots) in 10 mL of either (a) heat-inactivated (60 °C, 30 min) normal serum with low CK content, or (b) Tris HCl buffer (100 mmol/L, pH 8) containing 5 mmol of dithiothreitol per liter (21). We then
centrifuged (2000 × g, 10 min) the homogenates and diluted the supernatants with isotonic saline (NaCl, 150 mmol/L) to give a final CK activity of ≈ 1000 U/L.

In vitro incubations. We incubated the serum samples at room temperature for 15 min with an equal volume of Tris HCl buffer (100 mmol/L, pH 7.4) containing 50–15000 μmol of 2-mercaptoethanol per liter.

The myocardial extracts were also incubated at room temperature for 15 min with an equal volume of the Tris HCl buffer but containing 30 mmol of 2-mercaptoethanol per liter. Some aliquots had undergone prior incubation at 37 °C for 72 h with an equal volume of normal serum of low CK activity.

Isoelectric focusing. Polyacrylamide gels (130 × 110 × 0.48 mm; T = 5%, C = 3%) containing, per liter, 20 g of Pharmalyte pH 5–8 and 200 g of glycerol, were polymerized with N,N,N',N'-tetramethylethylenediamine (0.3 mL/L) and ammonium persulphate (0.4 mg/L) and focused at 200 V (10 W) for 10 min, then at 1400 V (10 W) for 20 min, and 1400 V (15 W) for 2 h, with 0.5 mol/L sodium hydroxide as catholyte and 40 mol/L DL-glutamic acid as anolyte. Fluorescence of CK-MM was viewed under ultraviolet light after incubation (37 °C, 20 min) with commercial CK reagent ("CK-Nac"; BCL, Lewes, Sussex, U.K.) and drying of the gel (60 °C, 20 min).

Results

Our serum CK-MM nomenclature is shown in Figure 1. The "abnormal" sub-bands c (pl 7.25), e (pl 6.85), and g (pl 6.50), and the diffuse zone comprising i (pl 6.28) and j (pl 6.20), were detected in all AMI patients; 26% (19 patients) demonstrated b (pl 7.35) and 4% (three patients) also showed a (pl 7.55). The minor "abnormal" sub-bands d (pl 7.05), f (pl 6.72), and h (pl 6.40) were detected only when sub-bands a and (or) b were prominent. The total serum CK (and % CK-MB) of patients demonstrating sub-band b was not atypical of the other AMI patients, but these patients were more likely to be smokers (79%) with a family history of ischemic heart disease (58%) and a personal history of AMI (37%) than were the other 55 AMI patients (respective values 60%, 19%, and 21%). Furthermore, 63% of these infarcts were inferior in location (26% anterior; 11% global), whereas only 28% of those lacking sub-band b were inferior (61% anterior; 11% global). The three patients demonstrating CK-MM sub-band a were smokers and two had diabetes mellitus; their prognosis was poor and two died. The changes in the serum CK-MM sub-band pattern after AMI are shown in Figures 1–3. An initial cathodal shift in sub-band distribution (CK-MM 1>2>3; c>e>g; Figures 2A, 3), with simultaneous appearance of a and (or) b if present, accompanied the increase in total serum CK between 3 and 12 h after infarction and was not detected unless patients were quickly hospitalized (Figure 1, 2B). This was followed, between 24 and 36 h after infarction, by the widely reported anodal shift (CK-MM 1<2<3; c<e<g), with simultaneous loss of a and (or) b if present, and diffuse appearance of sub-bands i, j, and sometimes k (Figures 1–3). Normalization of the pattern continued after the total serum CK had declined and stabilized. It occurred in a step-wise fashion; i.e., sub-bands 1 and c normalized within two days, followed by 2 and e (three days), then 3 and g (seven days), and finally j (nine days) (Figure 2B). The CK-MM sub-band patterns before

![Fig. 1. Serum CK-MM nomenclature as previously described (19), indicating the anodal shift in pattern distribution after AMI.](image)

![Fig. 2. Changes in serum CK-MM after AMI.](image)
and immediately after an AMI were identical, as indicated by the case of a patient hospitalized with angina who suffered a massive global AMI about 8 h after admission (Figure 3A). The effect of re-infarction is evident from Figure 3B; i.e., the anodal shift was reversed, with intensification of sub-bands 1 and c before further anodal conversion.

The serum CK-MM sub-band patterns of non-AMI patients with increased total serum CK (and %CK-MB) resembled those of AMI with prominent detection of CK-MM 1–3 and the "abnormal" sub-bands c, e, and g (Figure 4). However, a and b were not detected, nor were d, f, and h clearly distinguished (Figure 4), even when total serum CK exceeded 31 000 U/L (%CK-MB = 20%). The sub-band distribution pattern was generally more uniform (CK-MM 1 – 2 = 3; c = e = g = j) than in AMI and usually remained so upon sequential sampling; i.e., the anodal distribution changes, characteristic of AMI, were far less pronounced (Figure 4A) and "normalization" in the patient with polymyositis involved a gradual and uniform decrease in sub-band intensity (Figure 4B).

Figure 5 compares the serum CK-MM sub-band patterns of athletes sampled 24–60 h before and 24 and 48 h after a triathlon (2 km of swimming, 90 km of bicycling, 21 km of marathon running). The pre-race values for total serum CK (range 78-489 U/L; mean 230 U/L) exceeded those of 15 modestly active young individuals (range 43–167 U/L; mean 82 U/L; age 18–40 y) but were greatly increased 24 h (range 367–3619 U/L; mean 1600 U/L; CK-MB 1.5–8.9%; mean 4.8%) and 48 h (range 366–3658 U/L; mean 1030 U/L; CK-MB 0–3.5%; mean: 1.4%) after the race. Increased values for total serum CK were accompanied (in the woman as well as the men) by prominent detection of the "normal" sub-bands CK-MM 1–3 and the "abnormal" sub-bands c, e, g, and j (Figure 5). The patterns resembled those of the non-AMI, rather than the AMI, because (a) a and b were not detected nor were d, f, and h clearly distinguished; (b) the sub-band distribution was relatively uniform (CK-MM 1 = 2 = 3; c = e = g = j); and (c) the rate of anodal conversion in sequential samples was comparatively slow (Figure 5).

The tissue CK-MM sub-band of myocardium extracted in heat-inactivated normal serum consisted predominantly of CK-MM 1. This was anodally converted to CK-MM 3 by incubation (37 °C, 72 h) with "active" normal serum of low CK (15 U/L). (Incubation with heat-inactivated serum was without effect.) Incubation (room temperature, 15 min) of either (a) the extract (CK-MM 1), or (b) its serum product (CK-MM 3), with 15 mmol of 2-mercaptoethanol per liter yielded CK-MM patterns consisting predominantly of (a) "abnormal" sub-bands b and c (with loss of CK-MM 1), or (b) "abnormal" sub-bands f and g (with loss of CK-MM 3),
increased
MM
extracted
MiMi
consistencies
is
Discussion
"normal"
serum
(8,
(3.0)
The
triathion
or
temperature,
10-15,
5.
6.
145(0),
1-2,
tracks
8,
CK
Serum
(25)].
(100
mmol/L,
PH
(3670
sub-bands
1-3
of
15
serum
1-3
tracks
1)
serum
2-mercaptoethanol
1-3
(15,27),
with
other
sub-bands.
We
suspect
sub-band
CK-MM
(25)],
because
it
be
induced
from
other
CK-MM
isoforms
and
its
detection
in
AMI
is
associated
with
a
grave
prognosis.
Unlike
others
(20),
we
failed
to
detect
tissue
CK-MM
isoforms
(or
derivatives)
that
were
detected
in
serum.
In
the
light
of
these
findings
we
feel
justified
in
choosing
a
nomenclature
(19)
that
does
not
conform
to
standard
recommendations
because
(a)
the
cathodal
sub-band
CK-MM
(11-13,
16,
19)
is
the
predominant
isoform
in
serum
(15,27)(Figure
6).
The
use
of
CK-MM
3
(9,
14)
is
therefore
misleading
and
the
post-translational
conversion
is
numerically
inverted
(i.e.,
CK-MM
3
2
2
1).
(b)
The
"abnormal"
sub-bands
that
arise
by
a
secondary
conversion
are
differentially
translated
from
CK-MM
1-3.
Furthermore,
the
intermediate
sub-bands
are
equivalent
to
e
and
g
as
MM1B
and
MM2B
(16)
is
misleading,
because
they
are
detected
in
CK-MM
2
3.
respectively.

The
AMI
CK-MM
patterns
in
serum
(Figure
1-3)
confirm
an
early
increase
in
the
proportion
of
CK-MM
1
CK-MM
2
(figure
of
total
CK)
with
detection
of
cathodal
sub-bands
that
disappear
with
subsequent
conversion
of
CK-MM
1
2
3
(11, 14, 15, 27)
but
our
findings
are
more
comprehensive.
Chapelle
only
detected
cathodal
sub-bands
pI
7.1
(MM-0)
and
pI
7.29
(MM-1)
in
50% and
8%,
respectively,
of
AMI
(15,27),
except
in
patients
who
received
intravenous
perfusion
of
streptokinase
(27).
None
of
our
patients
received
thrombolytic
therapy,
and
yet
sub-bands
pI
7.25,
b
pI
7.35,
and
a
pI
7.55
were
detected
in
100%,
26%,
and
4% of
those
with
AMI.
Sub-bands
a
(probably
CK-MiMi)
and
d-g
have
not
been
detected
in
AMI
by
others
or
has
a
secondary
conversion
between
CK-MM
1-3
and
b-g
been
demonstrated.
Cathodal
sub-bands
have
been
assumed
to
be
natural
core
of
CK-MM
27)
but
in
vitro
conversion
of
(14,15,27).
We
have
demonstrated
re-intensification
of
CK-MM
1
and
c
(delayed
anodal
conversion)
upon
reinfarction
(Figure
3B)
and
have
demonstrated
that
the
CK-MM
\[\text{Fig. 5. Serum CK-MM in sequential samples collected from two male athletes (tracks 1–2, 5–6) and one female athlete (tracks 3–4) before the race (tracks 1, 3, 6) and 24 h (tracks 4, 7) and 48 h (tracks 2, 5, 8) after the race.}
\]
\[\text{The total serum CK activities, U/L (and percentage CK-MB activity), were (left to right: 165 (0), 362 (1.9), 161 (0), 887 (1.5), 366 (0), 216 (0), 999 (4.9), and 899 (3.0).}
\]

respectively
(Figure
6).
Pattern
a
(b
and
c,
with
little
CK-
MM
1)
was
detected
directly
when
the
myocardium
was
extracted
to
Tris
HCl
buffer
(100
mmol/L,
PH
8)
containing
5
mmol
of
dithiothreitol
per
liter.
Similar
cathodal
CK-
MM
sub-band
conversions
occurred
when
serum
of
high
total
CK
(3670
U/L,
CK-MB
23.4%) was
briefly
incubated
with
2-mercaptoethanol;
"abnormal" sub-bands
b–g
increased
as
CK-MM
1–3
and
(i, j)
decreased
(Figure
7).

Fig. 6. Tissue CK-MM and its 2-mercaptoethanol-induced cathodal conversion
Myocardium
extracted
in
heat-inactivated
serum
(track
1)
was
incubated
(room
temperature,
15
min)
with
an
equal
volume
of
Tris
HCl
buffer
(100
mmol/L,
PH
7.4)
containing
30
mmol
of
2-mercaptoethanol
per
liter
(tracks
2, 4),
without
track
4)
or
without
track
2
prior
incubation
(37
°C,
72
h)
with
"active"
normal
serum
of
low
CK
content
(track
3).
Track
5
is
cord-blood
serum
standard.

Fig. 7. Serum CK-MM and its 2-mercaptoethanol-induced cathodal conversion
Serum
with
high
total
CK
(3670
U/L,
MB
23.4%) was
incubated
(room
temperature,
15
min)
with
an
equal
volume
of
either
Tris
HCl
buffer
(100
mmol/L,
PH
7.4)
(track
1),
or
the
same
buffer
containing
0.05,
0.075,
0.38,
0.75,
3.8,
7.5,
or
15
mmol
of
2-mercaptoethanol
per
liter
(tracks
2–6, respectively).

sub-band normalization period in serum after AMI is longer (nine days) than was previously assumed. Thus, it offers a wider "time-window" (3 h to nine days) than other clinical tests.

Similarities between CK-MM sub-band patterns in serum in AMI and those of non-AMI patients and athletes (with increased total serum CK and %CK-MM) have been reported (11, 13, 16, 17, 30) but differences in detection of cathodal (and other "abnormal") sub-bands and rates of anodal conversion have not. The non-AMI groups may be characterized by continuous release of tissue CK and (or) lower serum carboxypeptidase N activity, with possible displacement of an equilibrium dictating conversion of CK-MM 1 and b, c; CK-MM 2 and d, e; and CK-MM 3 and f, g. In contrast to previous reports (16), we detected intermediate sub-bands in the female as well as male athletes.

The molecular basis of the conversion between CK-MM 1–3 and b–g is unknown, but it merits urgent investigation because the biochemical agents involved could govern AMI survival or even be indicative of AMI susceptibility in high-risk groups. The 2-mercaptoethanol effect suggests sequential conformational changes in the CK structure (perhaps involving monomer–dimer interchange) or sequential displacement of two molecules of an acidic thiol compound such as glutathione (glutamyl-cysteinyl-glycine).

We gratefully acknowledge the support of Dr. D. M. Collins and the staff and patients of the Coronary Care Unit, Sligo General Hospital, and of the organizers of the triathlon and its participating athletes.

References