KINETIC PROPERTIES AND THE FUNCTIONAL ROLE OF PARTICULATE MM-ISOENZYME OF CREATINE PHOSPHOKINASE BOUND TO HEART MUSCLE MYOFIBRILS

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1. Introduction

Fractional extractions of heart muscle have revealed that about 30% of cellular creatine phosphokinase activity are located in mitochondria and about 20% are bound to myofibrils [1,2]. The presence of significant creatine phosphokinase activity in heart and skeletal muscle myofibrils has been demonstrated also in studies of isolated myofibrillar preparations [1,3,4]. This particulate enzyme has been found to be electrophoretically identical to MM-isoenzyme of CPK* [1–4]. However, no detailed information concerning its properties including kinetic parameters is available. Comprehensive kinetic analyses have been made only for soluble isoenzymes from skeletal muscle [5–7], brain [5,8] as well as for the mitochondrial isoenzyme from heart [9,10]. It has been shown recently that creatine phosphokinase isoenzymes may play an active role in intracellular energy transport [2,9,10]. In this situation any information of functional properties of myofibrillar CPK becomes to be of special importance.

The purpose of this study was to determine the kinetic parameters of myofibrillar CPK from heart muscle and to compare them with the kinetic parameters of the myofibrillar ATPase reaction and mitochondrial CPK.

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* *Abbreviations:* CPK, creatine phosphokinase (EC 2.7.3.2); CP, creatine phosphate; Cr, creatine; EGTA, ethylene glycol-bis (β-aminoethylether)-*N,N'*-tetraacetic acid; IU - international units of enzyme activity (μmoles/min).

2. Materials and methods

The hearts of random-bred white rats (males) were used in the experiments. The hearts were removed after opening the thoracic cages of anaesthetized animals.

2.1. *Isolation of myofibrils*

Purified preparations of myofibrils were isolated by a modified procedure suggested by Solaro et al. [11]. Three hearts were homogenized in a 'Virtis-45' homogenizer for 30 s at 7000 rev/min in a buffer containing 0.3 M sucrose and 30 mM imidazole, pH 7.2. Homogenates were centrifuged for 20 min at 1000 *g*. The precipitate was treated in the same way three more times. The resulting pellet was used for isolation of purified myofibrillar fractions in the following way.

Step A. The pellet was again homogenized in glass—Teflon homogenizer with clearence 0.1–0.2 mm for 3 min at 3000 rev/min in 5 volumes of 0.1 M potassium phosphate, pH 7.2, containing 2 mM glutathione and centrifuged for 15 min at 15 000 *g*. The last treatment was repeated four times.

Step B. The myofibrillar pellet from step A was homogenized in 'Virtis-45' homogenizer in 0.1 M potassium phosphate buffer, pH 7.2, containing 0.1 M KCl for 15 s at 2000 rev/min and centrifuged for 15 min at 15 000 *g*. This treatment was repeated once in a buffer containing 60 mM KCl, 2 mM MgCl$_2$, 2 mM EGTA, 30 mM imidazole, pH 7.2.

Step C. The myofibrillar pellet was suspended in a buffer made 60 mM in KCl, 2 mM MgCl$_2$, 30 mM

imidazole, pH 7.2 and 2% in Triton X-100 and homo-
genized for 2 min in Teflon—glass homogenizer with
clearence 0.8—0.9 mm at 1000 rev/min. The suspen-
sion was centrifuged for 15 min at 15 000 g and the
treatment with Triton X-100 was repeated. The
myofibrillar pellet obtained was washed twice in
imidazole buffer to remove Triton X-100.

ATPase activity of myofibrils obtained was sensi-
tive to inhibition by EGTA (1.6 mM) and not sensi-
tive to inhibition by sodium azide (10 mM) and
ouabain (0.16 mM). Cytochromes aa_3, b and cc_1
were present only in trace amounts (lower than 1—2
nmol per g of protein).

2.2. Extraction of CPK from myofibrils

The pellet of myofibrils was homogenized 5 min
at 3000 rev/min in Teflon—glass homogenizer
(clearence 0.2 mm) in a solution containing 0.6 M
KCl, 0.1 M potassium phosphate, pH 7.2 and 10 mM
glutathione. The extraction procedure was continued
for 30 min by stirring. After 15 min of extraction
4 volumes of 0.1 M potassium phosphate were added
to precipitate both actine and myosin. After centrifu-
gation 30 min at 15 000 g the supernatant obtained
was dialyzed 24 h against 0.1 M potassium phosphate,
pH 7.2 with 0.5 mM glutathione. Final CPK solutions
were concentrated at Amicon filters and used in
experiments.

2.3. Assays

Determinations of kinetic parameters were carried
out with coupled enzyme systems as described earlier
[9]. Electrophoretic analyses were performed as
indicated in [2]. ATPase activity of myofibrils was
measured according to Solaro [11]. Myofibrillar
protein concentrations were determined by a biuret
method [12].

Spectrophotometric measurements were carried
out on Perkin-Elmer Model 402 and DW-2 UV VIS
(Aminco-Travenol) spectrophotometers.

3. Results

In fig.1 the results of electrophoretic analysis of
CPK extracted from purified mitochondrial and
myofibrillar preparations are shown. It is evident
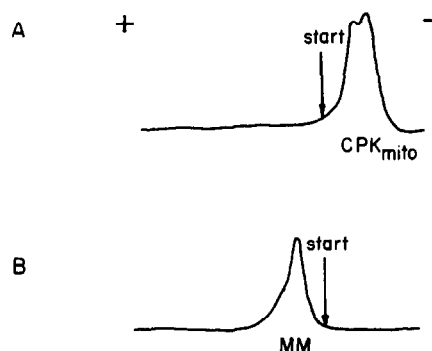that these two cellular structures contain electropho-

Fig.1. CPK electrophoretograms of phosphate extract of
purified mitochondrial preparation (A) and KCl-extract of
purified myofibrillar preparation (B). Extraction and elec-
trophoresis on Cellogel strips were carried out as indicated
in Materials and methods and in [2]. CPK isoenzyme pattern
was visualized by activity staining.

retically different isoenzymes of CPK, the myofibrillar
one being very close or identical to MM-isoenzyme
[2—4].

As one can see from table 1 myofibrillar and mito-
chondrial CPK are very close to each other kinetically.
Some differences are observed only in K_M values for
creatine and creatine phosphate: those are about 3
times higher for myofibrillar CPK.

CPK can be easily extracted from myofibrils only
by treatment with 0.6 M KCl which results in parallel

Table 1
Kinetic constants for myofibrillar and mitochondrial
creatine phosphokinase from rat heart

Constant	CPK isoenzyme (M ± SD, n=5)	
	myofibrillar	mitochondrial[a]
$K_a = K_M^{MgATP}$ (mM)	0.95 ± 0.25	0.73 ± 0.13
$K_b = K_M^{Cr}$ (mM)	15.5 ± 2.5	4.9 ± 0.1
$K_c = K_M^{MgADP}$ (mM)	0.077 ± 0.031	0.051 ± 0.008
$K_d = K_M^{CP}$ (mM)	1.67 ± 0.04	0.5 ± 0.02
V_{-1}/V_1	4.26 ± 1.5	4.2 ± 0.2

[a] Determined in [9].
V_1, the maximal rate of the forward reaction,
V_{-1}, the maximal rate of the reverse reaction,
V_1 and V_{-1} for myofibrillar CPK were determined for the
same solution of the enzyme.

extraction of actomyosin [13]. CPK activity of puri-
fied myofibrils was equal to 44 ± 8 mIU per mg of
protein thus being still comparable (after agressive
treatment during isolation) with ATPase activity of
myofibrils (see below). Thus, CPK extracted from
myofibrils is likely to be a component of native CPK—
myofibrillar complexes. It was of interest therefore
to compare the kinetic parameters of CPK with those
of the ATPase reaction of myofibrillar myosin. From
these experiments the following values of the kinetic
constants have been obtained which are similar to
those reported earlier [11,14]: K_M for MgATP is
equal to 0.16 ± 0.04 mM and V is equal to 100 ± 10
nmol per min per mg of protein (the mean values ±
standard deviations are given for n=4). It is remark-
able that K_M for MgATP in the ATPase reaction is
close to K_M for MgADP in the CPK reaction and
about an order lower than K_M for MgATP in the
latter reaction (see Discussion).

4. Discussion

The results obtained show that the particulate
isoenzymes of CPK in heart cells, both the mitochon-
drial one and that bound to myofibrils which are
different electrophoretically have very similar kinetic
properties. Both of them have much higher affinity
toward MgADP⁻ and CP than toward MgATP²⁻ and
creatine. The ratios of maximal velocities V_{-1}/V_1
are the same and equal to 4.2 ± 1.5 (M ± m). An
analysis of the literature shows that the relationship
between kinetic parameters observed is characteristic
for many types of CPK [5—8] and is obviously
related to low value of the equilibrium constant of
the CPK reaction connected with kinetic parameters
by Haldane relationship [5,8,9,15]:

$$K_{eq} = \frac{V_1 \, K_{ic} \, K_d}{V_{-1} \, K_{ia} \, K_b} = 1.6 \times 10^{-3}.$$

It follows from these data that differences in kinetic
parameters of separate CPK isoenzymes can not be
considered to be a basic regulatory mechanism deter-
mining their different functional roles in the cell. It
is evident that for all of them the reverse reaction
(ATP formation) is kinetically preferable.

It has been shown recently that in heart mito-
chondria the kinetic properties of CPK are compen-

sated for by the functional coupling between CPK
and ADP—ATP translocase [9,16]. On the other hand,
there is a reason to believe that the CPK reaction is
tightly coupled to the myosin ATPase reaction in
myofibrils. Two lines of evidences support this conclu-
sion. (1) The myofibrillar component which CPK is
bound to is likely to be myosin. In fact, Yagy and
Mase as well as Botts and Stone have shown that
myosin is able to form strong complexes with CPK,
myosin—CPK complexes having a dissociation constant
equal to 7 × 10⁻⁸ M [17—19]. Recently Turner et al.
as well as Houk and Putnam have also demonstrated
that CPK and myosin may be physically related in
myofibrils [4,20]. (2) As one can see from the
results of this study the kinetic parameters of myosin
and CPK are fairly well fitted to each other to allow
the tight functional coupling. It is evident that the
ATPase reaction catalyzed by myosin can run at
significant rate (in the presence of Ca^{2+}) at concen-
trations of MgATP about 0.2—0.3 mM (higher than
the value of K_M for MgATP) giving the same amount
of MgADP. The latter will be effectively trapped by
CPK due to its high affinity toward this substrate
(K_M is equal to 0.08 mM, see table 1) and will be
rephosphorylated into MgATP if CP is present at
concentration about 2—3 mM or higher. As the result
of about six-fold differences in K_M for MgATP in the
CPK reaction (0.95 mM) and in the ATPase reaction
(0.16 mM) MgATP formed will be rapidly redistri-
buted from CPK to myosin to start a new cycle
described.

The high content of CPK activity in myofibrils
(about 200 IU per g of wet weight of heart tissue [1,2])
makes this system of the coupled reactions able to
insure rapid and effective rephosphorylation of ADP
formed during contraction. One of the features of
this coupled system is that the rate of ADP rephos-
phorylation (and thus the rate of energy supply to
the contractile apparatus) may depend, under definite
conditions, upon CP concentration or upon CP/Cr
ratio due to a substrate regulation of CPK [9]. This
should be the case if CP concentration changes in a
range comparable with K_M value for this substrate.

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