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.

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 clearance 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 2 mM glutathione and centrifuged for 15 min at 15 000 g. This treatment was repeated four times.

Step C. The myofibrillar pellet was suspended in a buffer made 60 mM KCl, 2 mM MgCl2, 2 mM EGTA, 30 mM imidazole, pH 7.2.

*Abbreviations: CPK, creatine phosphokinase (EC 2.7.3.2); CP, creatine phosphate; Cr, creatine; EGTA, ethylene glycol-bis (p-aminoethylether)-N,N'-tetraacetic acid; IU - international units of enzyme activity (μmoles/min).
imidazole, pH 7.2 and 2% in Triton X-100 and homogenized for 2 min in Teflon–glass homogenizer with clearance 0.8–0.9 mm at 1000 rev/min. The suspension 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 sensitive to inhibition by EGTA (1.6 mM) and not sensitive to inhibition by sodium azide (10 mM) and ouabain (0.16 mM). Cytochromes aa₃, b and c₁ 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 (clearance 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 centrifugation 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

Determination 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 electrophoretically 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 mitochondrial 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>
<thead>
<tr>
<th>Constant</th>
<th>myofibrillar</th>
<th>mitochondrial⁹</th>
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<tbody>
<tr>
<td>$K_a = K_{M,cAMP}$ (mM)</td>
<td>0.95 ± 0.25</td>
<td>0.73 ± 0.13</td>
</tr>
<tr>
<td>$K_b = K_{M,cG}$ (mM)</td>
<td>15.5 ± 2.5</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>$K_c = K_{M,cADP}$ (mM)</td>
<td>0.077 ± 0.031</td>
<td>0.051 ± 0.008</td>
</tr>
<tr>
<td>$K_d = K_{M,cP}$ (mM)</td>
<td>1.67 ± 0.04</td>
<td>0.5 ± 0.02</td>
</tr>
<tr>
<td>$V_{-1}/V_1$</td>
<td>4.26 ± 1.5</td>
<td>4.2 ± 0.2</td>
</tr>
</tbody>
</table>

⁹ 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 purified myofibrils was equal to 44 ± 8 mIU per mg of protein thus being still comparable (after aggressive 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 remarkable 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 mitochondrial 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 MgATP$^2-$ 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]:

$$K_{eq} = \frac{V_1}{V_{-1}} \frac{K_{c}}{K_{a}}.$$

It follows from these data that differences in kinetic parameters of separate CPK isoenzymes can not be considered to be a basic regulatory mechanism determining 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 mitochondria the kinetic properties of CPK are compensated 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 conclusion. (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 $7 \times 10^{-6}$ 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 concentrations 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 redistributed 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 rephosphorylation (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.

References