Biochimica et Biophysica Acta 890 (1987) 233–241
Elsevier

BBA 41944

Only one of the two interconvertible forms of mitochondrial creatine kinase binds to heart mitoplasts

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(Received 23 June 1986)

Key words: Creatine kinase; Mitochondrion; Isozyme; (Pig, Rabbit heart)

When analyzed by cellulose acetate electrophoresis, solubilized pig or rabbit heart mitochondrial creatine kinase is shown to exist under two distinct forms. The less cathodic one (form 1) is a dimer and the other having a higher cathodic mobility (form 2) has a molecular weight of about 350000. The latter form can be converted into the former by incubation at alkaline pH or when the enzyme forms a reactive or an abortive complex with its substrates. This conversion is a reversible phenomenon and is not due to proteolysis. When rabbit heart mitoplasts are treated with the creatine kinase releasing agents, the enzyme is always solubilized as its form 2 and conversion to form 1, when it occurs, always take place after solubilization. Form 2 is also the only form which can be bound to pig or rabbit mitoplasts. Thus form 2 may be the actual form associated with heart mitochondria in vivo.

Introduction

Creatine kinase (EC 2.7.3.2) catalyses the reversible transfer of a phosphoryl group from ATP to creatine to synthetize ADP and phosphocreatine. Three cytoplasmic isoenzymes have been described: MM composed of two M subunits (muscle type), BB composed of two B subunits (brain type) and MB which is a hybrid between M and B subunits [1]. Jacobs et al. [2] have reported the existence of a fourth isoenzyme localized in the mitochondrial intermembrane space, associated with the external face of the inner membrane. It has been suggested that these isoenzymes are involved in a creatine phosphate shuttle [3–5], the role of mitochondrial creatine kinase located near the adenine nucleotide translocase being to synthetize creatine phosphate from mitochondrial ATP. All these creatine kinase species can be separated by cellulose acetate electrophoresis [6]. When mammalian heart extracts are analyzed by this technique 3–4 forms of creatine kinase are revealed. The most anodic form corresponds to the MB isozyme, whereas the major form of creatine kinase in heart, MM, migrates slightly towards the cathode. The one or two additional cathodic bands which can also be seen correspond to the mitochondrial forms of the enzyme [7–9]. One of these forms is a dimer, the subunits of which do not hybridize with the M or B subunits [10,11]. According to Hall et al. [8,12] the other form is a polymer formed by reversible inter-

Abbreviations: PCMB, *para*-chloromercuribenzoate; Hepes, 4-(20-hydroxyethyl)-1-piperazineethanesulfonic acid; M and MM, enzymes composed of one or two M subunits of the muscle type, respectively; B and BB, idem for the brain type; MB, hybrid between M and B subunits.

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molecular disulfide cross-linkages, whereas for Roberts [13] this higher molecular form is an artifact.

In the present paper we have studied the abilities of the two forms solubilized from either pig or rabbit mitochondria to reassociate with heart mitoplasts; form 2 is the only one which can bind to membranes. We also give evidence for the first time that the reversible conversion of one form to another can be induced either by alkaline pH or by incubation of the enzyme with its substrates.

Experimental procedures

Pig heart mitochondria were prepared as previously described [14] and rabbit heart mitochondria were isolated by a modification of the method of Hansford et al. [15] not using a proteolytic enzyme. Usually mitochondrial creatine kinase was obtained by incubating mitochondria (2–10 mg/ml) in 20 mM sodium phosphate (pH 7.4) at 10°C for 15 min; the medium was then centrifuged at $15000 \times g$ for 15 min. The supernatant was used as the source of the mitochondrial kinase with no further purification steps. In some cases mitochondria (2.5 mg/ml) were firstly submitted to an hypotonic treatment in distilled water at 0°C for 5 min; after centrifugation at $15000 \times g$ for 5 min the pellet was suspended in 20 mM phosphate and treated as described above. Mitochondrial preparations, the external membranes of which have been broken by either phosphate or water treatments, are called mitoplasts.

Cellulose acetate electrophoresis. Unless otherwise indicated, the electrophoresis was conducted in 60 mM Tris/10 mM barbital/50 mM sodium barbital/1 mM EGTA/1 mM 2-mercaptoethanol (pH 8.8) (ionic strength, approx. 0,05). Cellulose acetate strips (CELLOGEL Chemetron Milano) were soaked in the buffer for at least 20 min and then blotted free from excess buffer with filter paper. The strips were mounted in a SEBIA electrophoresis chamber and the samples were applied on the anodic side. Since the intensity of staining may not be linearly related to the amount of enzyme care has been taken to apply constant amounts of activity (i.e., 5–8 mIU in 2–4 μl) to compare electrophoretic patterns with similar activities. The electrophoresis was performed in a cold room for 15–90 min with a 5 mA current per strip. Following electrophoretic separation the isoenzymes were visualized by incubating the strips on a tray containing 1% agarose, 0.03% nitro blue tetrazolium soaked for 10 min in 100 mM triethanolamine, 20 mM glucose, 10 mM magnesium acetate, 1 mM ADP, 0.6 mM NADP, 10 mM AMP, 35 mM phosphocreatine, 5.6 IU hexokinase, 2.8 IU glucose-6-phosphate dehydrogenase, 80 μM phenazine methosulfate (pH 7).

Molecular-weight estimation by gel filtration. Sephadex G-200 equilibrated in 100 mM sodium phosphate (pH 7.4) containing 1 mM dithiothreitol was packed in a 100×1.6 cm column (Pharmacia K 16/100) at 4°C. Fractions of 1.4 ml were collected and creatine kinase activity was measured by the pH stat method [14]. Cytochrome *c*, chymotrypsinogen A, ovalbumin, bovine serum albumin, aldolase, catalase, ferritine and CK MM were used as molecular-weight markers; a standard curve was obtained by plotting the logarithm of molecular weights against the elution volume.

Results

When subjected to cellulose acetate electrophoresis in Tris-barbital buffer (pH 8.8), which is the most widely used buffer for separating creatine kinase isozymes, tissue extracts or mitochondrial extracts show different patterns according to the duration of the electrophoretic run.

After a 90 min run (Fig. 1A) two cathodic enzyme forms can be seen in tissue extracts: the less migrating one, which has the same mobility in pig and rabbit represents MM isozyme. The other form which migrates more cathodically in rabbit (lane 2) than in pig (lane 1) whole tissue extracts, is the mitochondrial isozyme of creatine kinase, since these spots have the same mobility as those found in phosphate extracts of rabbit (lane 4) or pig (lane 3) mitochondria. The anodic bands which are present in tissue extracts are due to the MB isozyme. We have called form 2 the fast-moving form present in rabbit mitochondria and form 1 the slow-moving form present in pig mitochondria. When the electrophoretic duration is shortened to 20 min (Fig. 1B) a third cathodic form analogous to form 2 can be seen in pig whole heart and mitochondria extracts. If the Tris-barbital buffer

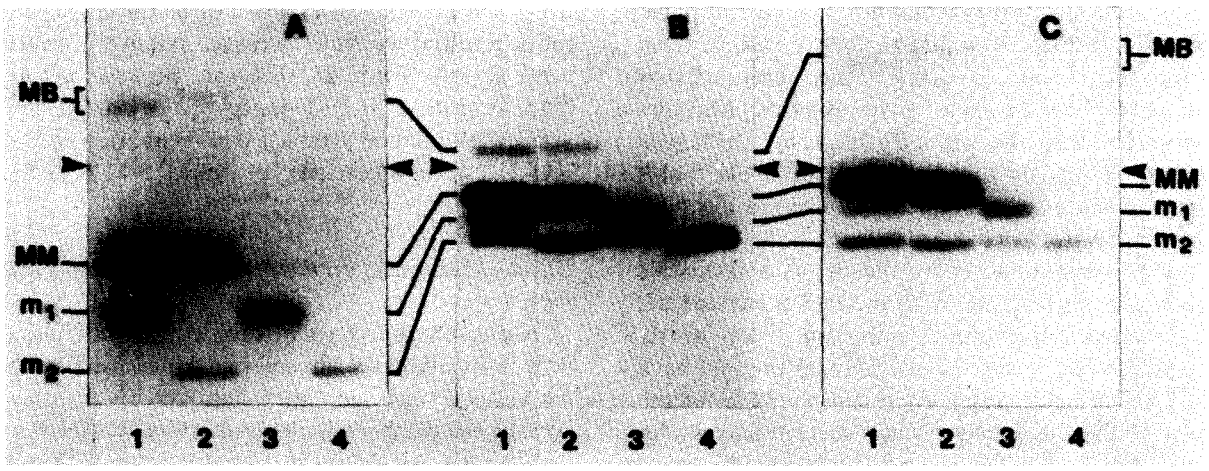

Fig. 1. Electrophoretic patterns of tissue and mitochondrial isozymes of creatine kinase. The electrophoretic conditions were as follows: (A) Tris-barbital buffer (pH 8.8), 90 min; (B) Tris-barbital buffer (pH 8.8), 20 min; (C) 40 mM sodium phosphate buffer (pH 7.4), 90 min. Lanes 1 and 2: whole myocardium extracts of pig and rabbit hearts, respectively. Lanes 3 and 4: 20 mM phosphate extracts of pig and rabbit mitochondria respectively. Arrows indicate the origin.

is replaced by 40 mM sodium phosphate (pH 7.4) (Fig. 1C) the pattern obtained after 90 min is similar to that obtained after a 20 min run in Tris-barbital buffer, i.e., two mitochondrial forms are present in pig-heart whole tissue and mitochondrial extracts, in contrast with that observed after a 90 min run in Tris-barbital buffer.

The disappearance of form 2 in the latter case is certainly not due to an inactivation of this form, since we have never observed a decrease of the total creatine kinase activity in Tris-barbital buffer for such a short period. It may rather be attributed to its transformation into a form of the form 1 type during the electrophoretic run.

Role of the pH in the conversion of form 2 into form 1

In order to make clear which of the pH or the nature of the buffer induces the conversion of form 2 into form 1, the enzyme has been extracted from rabbit heart mitochondria by treatment with 20 mM phosphate (pH 7.4) and diluted either in 20 mM phosphate (pH 7.4 or 8.8), or in Tris-barbital (pH 7.4 or 8.8). Rabbit enzyme has been chosen because it is always obtained as form 2 only and because it does not transform into form 1 during a 90 min electrophoretic run at pH 8.8 as does the pig enzyme (Fig. 1). The diluted extracts have been kept at 4°C during 3 days and the mobility of the mitochondrial enzyme has been checked every day (Fig. 2). No inhibition of enzyme activity was observed throughout the entire procedure. At a pH of 7.4 the form which is present immediately after solubilization, i.e., form 2, was not modified during the incubation period whatever was the nature of the medium, phosphate or Tris-barbital. On the contrary, when the pH of the medium was 8.8, the initial form 2 was nearly completely converted to form 1 after a 24 h

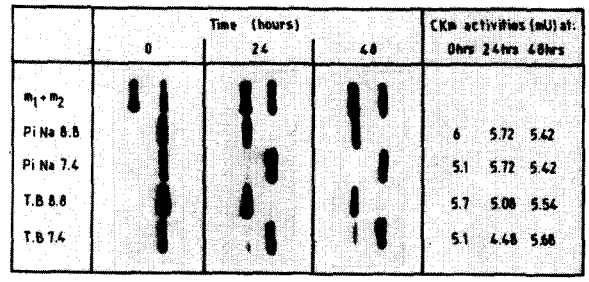

Fig. 2. Conversion by pH of form 2 into form 1. Freshly solubilized rabbit heart mitochondrial creatine kinase (CK_m) was 5-fold diluted at zero time in either 20 mM sodium phosphate (pH 7.4 (P_i 7.4) or pH 8.8 (P_i 8.8)) or in Tris-barbital buffer (pH 7.4 (TB 7.4) or pH 8.8 (TB 8.8)). 2 µl of each solution were loaded onto cellulose acetate strips at 24-h intervals and electrophoresis was performed in Tris-barbital buffer (pH 8.8) for 90 min at 4 mA per strip (in order to get an optimal separation of isozymes). $m_1 + m_2$ is a mixture of mitochondrial enzymes of pig and rabbit.

incubation in Tris-barbital as well as in phosphate. It is then clear that the conversion of form 2 into form 1 can also occur in the rabbit and that the pH is the main factor governing this phenomenon. However, the pig enzyme seems to have a greater sensitivity to alkaline pH than the rabbit enzyme, since it is completely converted during a 90 min electrophoretic run (Fig. 1).

The transformation by the pH is a reversible phenomenon (Fig. 3). With the rabbit enzyme the transformation of form 2 into form 1 was nearly quantitatively reversed if the pH was brought back to 7.4. The same observation can be made for the part of the pig enzyme which was initially under form 2; however, the part which was solubilized as form 1 was not converted back to form 2. The conversion of rabbit form 2 into form 1 after a 24 h incubation in Tris-barbital buffer (pH 8.8) (Fig. 2) is completely prevented by the addition of 2.5 mM *p*-aminobenzamidine in the incubation medium. The same effect is found with the pig enzyme: p-aminobenzamidine blocks the conversion of form 2 occurring after a 1 hour incubation in pH 8.8 Tris-barbital buffer. We have no indication about the mechanism of *p*-aminobenzamidine action. Aminocaproic acid, another inhibitor of proteolytic enzymes, has no effect, though.

The transformation of form 2 into form 1 can also be obtained by incubation of the enzyme in

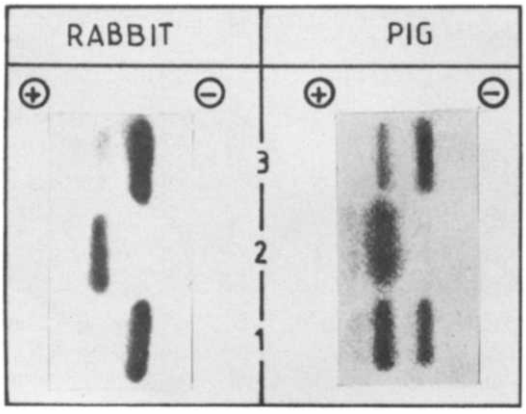

Fig. 3. Reversibility of the conversion by pH. Rabbit or pig mitochondrial enzyme was solubilized in 20 mM sodium phosphate pH 7.4 (1). The pH was adjusted to 8.8 and the solution kept at 4°C for 24 h (2). The pH was then brought back to 7.4 and the solution kept another 24 h at 4°C (3). The duration of the electrophoretic run in Tris-barbital buffer was 20 min.

urea or KCl. 1 M urea, which does not inhibit the mitochondrial enzyme activity, readily converts form 2 into form 1; this can be reversed by dialysis against 20 mM phosphate. A partial conversion is obtained after a 1 day incubation in 1 M KCl/10 mM Hepes (pH 7.4), but 0.5 M KCl has no effect.

Effect of substrates on the conversion of form 2 into form 1

A phosphate extract of rabbit heart mitochondria is incubated with creatine kinase substrates for various times and then analyzed by cellulose acetate electrophoresis in tris-barbital buffer during 20 min. As shown in Fig. 4A, after a 15 min incubation period, form 2 is completely transformed in form 1 when the enzyme exists as the dead-end complex creatine kinase-ADPMg-creatine-nitrate; it is only partially transformed when present as the reactive complex creatine kinase-ATPMg-creatine. But the conversion does not take place when the enzyme is incubated with only one of the ligands. This is also the case after a 60 min incubation period (Fig. 4B); however, the conversion of the enzyme in the reactive complex is more complete. For longer durations the creatine kinase-ATP-creatine complex also undergoes some degree of transformation into form 1. This conversion by the substrates is strongly inhibited by the addition of 2.5 mM *p*-aminobenzamidine in the incubation medium (Fig. 4C). These results, namely transformation by the substrates and inhibition by *p*-aminobenzamidine, also hold for the part of the pig mitochondrial isozyme under form 2. As for the pH effect the transformation by the substrates is reversible. Form 1 obtained after incubation with substrates is converted back to form 2 by dialysis against 20 mM phosphate buffer, 1 mM dithiothreitol (Fig. 5).

What is the form associated with the mitochondria?

When rabbit heart mitoplasts are incubated with the mitochondrial creatine kinase eluting agents (adenine nucleotides, phosphate, KCl, PCMB, Tris-barbital buffer (pH 8.8), deoxycholate) the enzyme is always solubilized as form 2 no matter which agent is used. In the presence of its substrates (ATP + Mg^{2+} + creatine) creatine kinase is quickly solubilized as form 2, with a

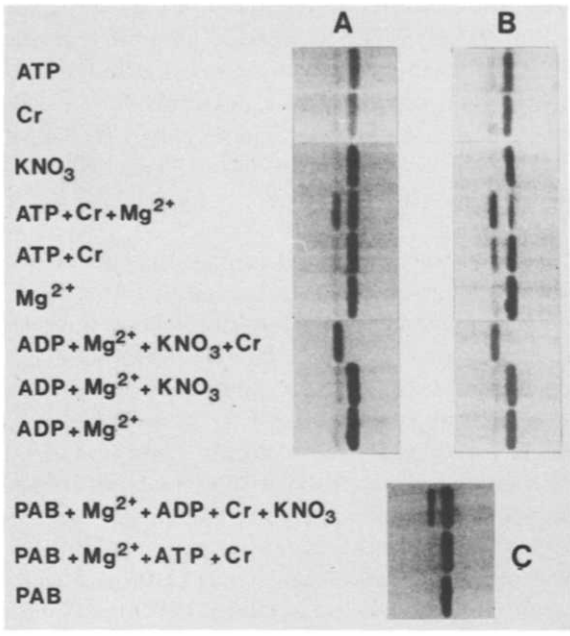

Fig. 4. Transformation of form 2 by its substrates. Solubilized rabbit heart mitochondrial enzyme was incubated for either 15 min (A) or 60 min (B, C) at 0°C in 20 mM phosphate containing the indicated substrates which final concentrations were: 2 mM ATP or ADP/2.5 mM $MgCl_2$/20 mM creatine/50 mM potassium nitrate. When present *p*-aminobenzamidine (PAB) was 2.5 mM. Electrophoresis was performed in Tris-barbital buffer (pH 8.8) for 20 min.

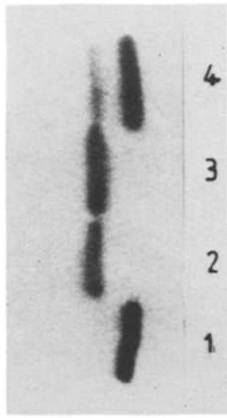

Fig. 5. Reversibility of the transformation of form 2 by its substrates. Rabbit heart enzyme was solubilized with 20 mM sodium phosphate (pH 7.4) (1) then incubated with its substrates as in Fig. 4 (2). An aliquot was dialysed overnight against 20 mM sodium phosphate, 1 mM dithiothreitol (pH 7.4) (4). Note that the electrophoretic migrations of the enzyme transformed either by its substrates (2) or by alkaline pH (3) are the same.

trace of form 1 (table I); the soluble form 2 is then converted into form 1 by the substrates. If *p*-aminobenzamidine is added before the substrates it does not prevent creatine kinase solubilization, but inhibits the conversion of form 2 into form 1. So it seems that solubilization occurs before conversion happens. The situation is not as clearcut with pig mitochondria where the two forms are always released; however, form 1 is usually the dominant one.

Reassociation experiments

We have studied the reassociation of forms 1

TABLE I

EVOLUTION OF THE ELECTROPHORETIC FORM OF THE MITOCHONDRIAL CREATINE KINASE SOLUBILIZED BY ITS SUBSTRATES

2.5 mg/ml water-treated mitochondria were incubated in 0.25 M sucrose 10 mM Hepes-K^+, 1 mM dithiothreitol (pH 7.4) for 2 min at 30°C. Solubilized enzyme activity was assayed after a 2 min centrifugation at 15000 × *g*. The electrophoretic form of the soluble enzyme was identified immediately, after 45, 90 min and then, 1 day later. m_1, m_2: mitochondrial forms 1 and 2.

Additions	% Solubilization	Electrophoretic form after			
		0 min	45 min	90 min	24 h
0.4 mM ATP	76.3	m_2	m_2	m_2	m_2
0.4 mM ATP + 2.5 mM $MgCl_2$ + 20 mM creatine	91.7	m_2 (trace of m_1)	$m_1 > m_2$	$m_1 > m_2$	m_1
0.4 mM ATP + 2.5 mM $MgCl_2$ + 20 mM creatine + 2.5 mM PAB	91.7	m_2	m_2	m_2	m_2
2.5 mM PAB	9.5	m_2	m_2	m_2	m_2

TABLE II

REASSOCIATION OF THE TWO FORMS OF PIG MITOCHONDRIAL CREATINE KINASE WITH PIG HEART MITOCHONDRIA

2.5 mg/ml pig-heart mitochondria are incubated at 10°C for 15 min in either 10 mM Hepes-K^+ (pH 7.4) or Tris-HCl (pH 8.8) containing $6.25 \cdot 10^{-5}$ M PCMB. The suspension is centrifuged for 5 min at $15000 \times g$ and 2 mM dithiothreitol is then added to the supernatant. The percentage of solubilized enzyme and its electrophoretic form are determined. The pellet is then rehomogenized in its supernatant, incubated for 10 min at 10°C. After centrifugation the percentage of enzyme remaining in the supernatant and its electrophoretic form are determined.

Solubilisation pH	% Reassociation	Electrophoretic form of the soluble activity	
		before reassociation	after reassociation
7.4	53	$m_2 + m_1$	m_1
8.8	0	m_1	m_1

and 2 with either pig of rabbit heart mitoplasts. As previously stated the enzyme solubilized from pig mitochondria can be very rapidly and completely converted in form 1 in an alkaline medium. We have estimated in Table II the percentage of reassociation of the enzyme solubilized from pig heart mitochondria either in a pH 7.4 or pH 8.8 medium. At pH 7.4 a mixture of both enzyme forms is obtained, a partial reassociation is observed and form 2 completely disappears from the supernatant. At pH 8.8, when form 1 is the single form, no reassociation can be observed, therefore it is clear that form 2 is the only form which binds to the inner membrane.

In order to confirm and extend these results we have conducted cross-reassociation experiments between pig or rabbit mitoplasts and rabbit or pig creatine kinase. Table III shows that rebinding is nearly completely when form 2 is the single form present in the supernatant, it takes place only partially with a mixture of forms 1 and 2, and no reassociation at all can be observed with only form 1 present in the extract. Whatever was the origin of the mitochondria and of the solubilized enzyme, reassociation occurs only in those experiments, where form 2 was present.

Molecular-weight determinations

20 mM phosphate extracts of pig or rabbit mitochondria were used to determine the apparent molecular weights of the two mitochondrial creatine kinase forms by Sephadex G-200 gel filtration. Pig or rabbit form 1 of the enzyme were obtained by alkaline pH treatment (incubation in

TABLE III

PERCENTAGE OF REASSOCIATION OF PIG OR RABBIT HEART MITOCHONDRIAL CREATINE KINASE WITH MITOPLASTS DEPLETED OF THEIR ENDOGENOUS MITOCHONDRIAL CREATINE KINASE ACTIVITY

The binding of mitochondrial enzyme with mitoplasts was studied by incubating mitochondrial pellets depleted of their endogenous enzyme with soluble creatine kinase during 10 min at 10°C in 10 mM Hepes (pH 7.4). After centrifugation the amount of reassociated enzyme was calculated and the electrophoretic form of the remaining soluble activity determined. To obtain soluble enzyme, mitochondria are suspended at 12.5 mg/ml in either 10 mM Hepes (pH 7.4) or 10 mM Tris (pH 8.8) containing 0.25 mM PCMB as a solubilizing agent. After incubation for 15 min at 10°C and centrifugation, 1 mM dithiothreitol was added to the supernatant to reactivate solubilized mitochondrial creatine kinase (7.5–12 IU/ml) and the electrophoretic form was identified. Mitochondrial pellets depleted of their endogenous mitochondrial creatine kinase activity were obtained by treating mitochondria (2.5 mg/ml) with 20 mM sodium phosphate (pH 7.4) during 15 min at 10°C; after centrifugation the extent of creatine kinase solubilization was checked.

	pig enzyme		rabbit enzyme	
	pH 7.4	pH 8.8	pH 7.4	pH 8.8
Electrophoretic form of the solubilized enzyme	$m_1 + m_2$	m_1	m_2	$m_1 + m_2$
Pig-heart mitoplasts	56%	0	82%	30%
Rabbit-heart mitoplasts	58%	0	82%	28.5%
Electrophoretic form of the non-reassociated enzyme	m_1	m_1	–	m_1

TABLE IV

APPARENT MOLECULAR WEIGHTS OF PIG AND RABBIT FORMS

Apparent molecular weights as obtained by gel filtration on Sephadex G-200: mean ± S.E.M. of (n) determinations.

Electrophoretic form	Rabbit	Pig
Form 2	357000 ± 6000 (8)	342000 ± 7000 (3)
Form 1	81000 ± 5000 (3)	75000 ± 1000 (4)

Tris-barbital buffer, pH 8.8); untreated extracts were used as the source of form 2. The correlation between electrophoretic forms and apparent molecular weights is shown in Table IV. This table clearly shows that the solubilized mitochondrial enzyme is an oligomer with a relatively high molecular weight of about 350 000 in the pig heart as well as in the rabbit heart. It also shows that alkaline treatment induces a dissociation of this oligomer into a form having an apparent molecular weight of 75 000–80 000, that is to say a dimer.

Discussion

Two forms of mitochondrial creatine kinase have been observed by cellulose acetate or agarose gel electrophoresis in various species [7–9]. The experimental results presented in the this study show for the first time that, in pig and rabbit heart, these two forms can convert from one form to another under the influence of pH or of the substrates of the enzyme. When creatine kinase is solubilized from rabbit heart mitochondria it is always under the fastest migrating form, form 2; when it is solubilized from pig heart, it occurs as a mixture of forms 1 and 2.

Solubilized form 2 is converted into form 1 by alkaline pH. At pH 8.8 the transformation of pig form 2 is much more rapid than that of rabbit form 2, since it is completed during a 90 min electrophoretic run. This effect may explain why some investigators [11,16] never found more than a single form of mitochondrial creatine kinase: their experimental protocols usually include steps occurring at alkaline pH either during elution of the enzyme from mitochondria or during purification. It has been described by clinical biochemists that the MM isozyme of creatine kinase after release into the plasma can give at least three molecular forms resulting from proteolytic degradation by a 'creatine kinase conversion factor' which might well be a carboxypeptidase [17–21]. A similar degradation can be ruled out in the present case, since the conversion of form 2 into form 1 is a completely reversible phenomenon in the rabbit as shown by cellulose acetate electrophoresis. However, the partial irreversibility observed with the pig enzyme could be accounted for by some degree of proteolysis occurring before the hearts could be taken off in the slaughterhouse.

The nature of bonds involved in the formation of both form 1 and form 2 forms of the enzyme is at present unknown. The fact that form 2 can be transformed into form 1 by alkaline pH and to a much lesser extent by 1 M KCl suggests that electrostatic interactions are involved. However, form 2 is also transformed by 1 M urea which is usually considered as an hydrogen bond breaker. Higher urea concentrations inhibit the mitochondrial enzyme activity probably by inducing dissociation of form 1 subunits as it has been shown for cytoplasmic isozymes [22,23]. According to Deluca's group the formation of aggregates could be due to the formation of disulfur linkages between dimers [8,12]. It should be noted here that the electrophoretic mobility of form 1 extracted from bovine mitochondria is slightly more cathodic than that extracted from pig or rabbit (not shown). In these three species we have never been able to convert form 2 into form 1 by reducing agents such as 2-mercaptoethanol or dithiothreitol so this hypothesis is probably not the correct one.

Our results provide evidence that the formation of reactive or abortive enzyme-substrate complexes induces the conversion of form 2 into form 1. This effect also is reversible: upon elimination of the substrates by dialysis, form 1 is transformed back into form 2. The effect of substrates may be ascribed to conformational changes. Biochemical and biophysical data are indeed consistent with large substrate-induced conformational changes occurring in many kinases. This has been shown to be the case for the MM isozyme of creatine kinase (see Ref. 24 for a review), the largest effect being due to the formation of the abortive com-

plex creatine kinase-MgADP-creatine-NO_3^-. Since this change seems to be a general feature of many kinases so far studied [25], it can be assumed that it is also the case for mitochondrial creatine kinase and that form 2-to-form 1 conversion originates in such a conformational change. One has to keep in mind however, the difference between the time course of the form 2-to-form 1 conversion and that of the catalytic events.

The prevention by *p*-aminobenzamidine of either the pH effect or the substrates effect is difficult to explain; in our experimental conditions this compound is neither a substrate nor an inhibitor for the enzyme.

We have shown by SDS-polyacrylamide gel electrophoresis that these two mitochondrial forms of creatine kinase are made of very similar subunits having very similar molecular masses of about 42 kDa [26]. Gel filtration data prove that in pigs as well as in rabbits form 1 is a dimer. It is more difficult to draw a clear-cut conclusion from these data for form 2 they seem to be made out of four or five dimers. Other techniques should be used to determine their precise number. It should be stressed that the occurrence of high molecular forms of bovine mitochondrial enzyme has been reported in the past [8,27,28] and that very recently, Walliman et al. [29] suggested that the chicken enzyme was an octamer. After submission of this manuscript we have been aware of the description by Lipskaya et al. [30] of the interconversion of the bovine enzyme studied by centrifugation on sucrose density gradient providing results essentially similar to ours. In contrast with Roberts' assumption [13] which regards these high molecular forms as artifacts, we have found that form 2 is the form eluted from rabbit mitoplasts by all the effectors so far studied. When this form is converted to form 1, for instance in the presence of substrates, conversion always occurs after solubilization. Furthermore, we have shown that reassociation of the creatine kinase is directly dependent upon the proportion of form 2 to form 1. Form 2, whatever is its origin, i.e., pig or rabbit, is the only form which is able to bind to pig or to rabbit mitoplasts. Thus, if these results hold when the outer membrane of mitochondria is intact, this means that form 2 may be the actual form associated with mitochondria in physiological conditions. Further studies are needed to evaluate the implications of this proposal on the efficiency of creatine phosphate synthesis by heart mitochondria.

Acknowledgments

The authors wish to thank Professor D.C. Gautheron for helpful discussions during the course of this study and for reading the manuscript. We are also indebted to C. Van Herrewege for the art work.

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