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Expression of active octameric chicken cardiac mitochondrial creatine kinase in *Escherichia coli*

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Sarcomeric mitochondrial creatine kinase (Mi_b-CK) of chicken was expressed in *Escherichia coli* as a soluble enzyme by using an inducible phage-T7 promoter. Up to one third of the protein in *E. coli* extracts consisted of soluble recombinant Mi_b-CK in an enzymically active form. Approx. 20 mg of nearly-homogenous Mi_b-CK was isolated in a two-step isolation procedure starting with 1 litre of isopropyl β-D-thiogalactopyranoside-induced *E. coli* culture, whereas previous attempts to express other CK genes in *E. coli* have resulted in 20-fold lower yields and inclusion-body formation. Selection of the Mi_b-CK expression plasmid on media containing kanamycin rather than ampicillin extended the time period of maximal Mi_b-CK expression. Recombinant Mi_b-CK displayed an identical *N*-terminal amino acid sequence, identical K_m for phosphocreatine and $V_{max.}$ values, the same electrophoretic behaviour and the same immunological cross-reactivity as the native enzyme isolated from chicken heart mitochondria. The recombinant Mi_b-CK had the same molecular mass as native chicken Mi_b-CK in m.s. analysis, indicating that post-translational modification of the enzyme in chicken tissue does not occur. As judged by gel-permeation chromatography and electron microscopy, recombinant enzyme formed predominantly octameric oligomers with the same overall structure as the chicken heart enzyme. Furthermore, the enzymes isolated from both sources formed protein crystals of space group $P42_12$, when grown in the absence of ATP, with one Mi-CK octamer per asymmetric unit. The indistinguishable X-ray-diffraction patterns indicate identical structures for the native and recombinant proteins.

INTRODUCTION

Creatine kinase (CK; EC 2.7.3.2) reversibly transfers the phospho group of phosphocreatine to ADP, regenerating ATP. The enzyme exists in different isoforms, which are expressed tissue-specifically and localized subcellularly in an isoenzyme-specific manner (for a review, see Wallimann *et al.*, 1992). At least four genes located on different chromosomes encode the members of the CK family (Mariman *et al.*, 1987; Klein *et al.*, 1991). The 'cytosolic' M- and B-CK isoenzymes form homo- and hetero-dimers (Eppenberger *et al.*, 1983). Additional isoforms are restricted to the mitochondria (Jacobs *et al.*, 1964; Jacobus & Lehninger, 1973), where at least two tissue-specific isoforms exist. The basic 'sarcomeric' Mi_b-CK is found in heart and skeletal muscle, whereas the more acidic 'ubiquitous' Mi_a-CK is present in most other tissues (Hossle *et al.*, 1988; Haas & Strauss, 1990; Klein *et al.*, 1991; Payne *et al.*, 1991).

Many functional and structural studies have been carried out with the mitochondrial creatine kinase isoenzymes isolated from heart and brain tissue of chicken and other species [for reviews, see Wallimann *et al.*, 1989, 1992], and recently crystals of Mi_b-CK have been obtained (Schnyder *et al.*, 1990, 1991). Mi-CK is bound to the mitochondrial inner membrane (Scholte *et al.*, 1973) and is enriched in mitochondrial contact sites (Adams *et al.*, 1989). Despite the well-conserved amino-acid sequence between all four CK isoforms (Hossle *et al.*, 1988; Klein *et al.*, 1991), Mi-CK extracted from isolated mitochondria exists in an octameric form with a molecular mass of 340 kDa and a dimeric form, whereas the cytosolic isoenzymes form dimers only (Schlegel *et al.*, 1988*b*; Wyss *et al.*, 1990). As shown recently, octamers bind distinctly better to model membranes (Rojo *et al.*, 1991) and to mitoplasts (Schlegel *et al.*, 1990) and the octameric form was the only one found in isolated contact sites (Adams *et al.*, 1989). Thus it is likely that the functional form of Mi-CK, especially within the contact sites of mitochondria, is the octamer (Quemeneur *et al.*, 1988; Brdiczka, 1991; Wallimann *et al.*, 1992).

To probe further the structure and function of Mi_b-CK, we expressed the Mi_b-CK cDNA in *Escherichia coli* using the phage-T7 RNA polymerase system developed by Studier *et al.* (1990, and references cited therein). Large quantities of soluble enzyme were purified to near-homogeneity from *E. coli* extracts, and several biochemical properties of the recombinant protein were compared with native Mi_b-CK isolated from chicken heart mitochondria. The recombinant enzyme preparation was shown to be identical within experimental error with the native enzyme.

MATERIALS AND METHODS

E. coli strains, plasmids and DNA manipulations

E. coli strain BL21(DE3)pLysS and expression vector pET-3b have been described (Studier *et al.*, 1990). *E. coli* strain TG1, media and standard DNA manipulations have already been described (Sambrook *et al.*, 1989). In the Mi_b-CK cDNA (Hossle *et al.*, 1988; a gift from Dr. J. C. Perriard of this Institute), an *Nde*I site was inserted at the 5′-end of the sequence encoding the mature Mi_b-CK protein, creating a new initiating methionine codon; the *Bam*HI site within the coding sequence was destroyed, and a new *Bam*HI site was created in the 3′-flanking sequence of the gene. Plasmid pRF23 was constructed by insertion of the *Nde*I–*Bam*HI fragment, containing the complete sequence of the mature Mi_b-CK, between the *Nde*I and *Bam*HI site of vector pET-3b; additionally, the *Eco*RV/*Eco*RI fragments of pET-3b were deleted and an *Xho*I linker inserted at the *Pvu*II site of pET-

Abbreviations used: CK, creatine kinase; B-CK, brain-type; M-CK, muscle-type; Mi-CK, mitochondrial-type CK isoforms; Mi_b-CK, basic 'sarcomeric' mitochondrial CK isoform; Mi_a-CK, acidic 'ubiquitous' mitochondrial CK; IPTG, isopropy β-D-thiogalactopyranoside; PCr, phosphocreatine; native and recombinant Mi-CK refer to the enzyme isolated from chicken heart and from *E. coli* lysates respectively.

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3b. Insertion of a kanamycin-resistance gene (contained in a 1430 bp *Hpa*II fragment of transposon *Tn903*; Oka *et al.*, 1981) at the *Xho*I site of pRF23 by blunt-end ligation resulted in pRF72.

Expression and purification of Mi_b-CK

A 2 ml portion of 2XYT medium was inoculated with BL21(DE3)pLysS cells freshly transformed with pRF23 or pRF72. The two plasmids were selected on media containing ampicillin (100 μg/ml) or kanamycin (25 μg/ml) respectively. Cells were grown at 37 °C to an A_{590} of about 0.5 and then induced by the addition of 0.4 mM isopropyl β-D-thiogalactopyranoside (IPTG). Growth was continued for the time period indicated in the individual experiments. Cells were then harvested by centrifugation, washed in 0.9% NaCl, resuspended in lysis buffer [50 mM-Tris(pH 8.0)/10 mM-EDTA/100 mM-NaCl/15% (w/v) sucrose] and sonicated twice for 1 min. Cell debris was removed by centrifugation. For preparative purposes 1 litre of induced cells was used (about 2.5 g wet weight of cells). The cells were sonicated twice for 1 min in 20 ml of lysis buffer each time, then cell debris was removed by centrifugation at 17000 *g* for 15 min (crude extract). The supernatant was diluted with 1 vol. of Blue Sepharose loading buffer [50 mM-sodium phosphate (pH 6.5)/1 mM-$MgCl_2$/2 mM-2-mercaptoethanol/0.2 mM-EGTA] and the pH was adjusted to 6.5. The crude extract was absorbed at 45 ml/h to a 75 ml Blue Sepharose column equilibrated in loading buffer and extensively washed. Creatine kinase activity was eluted with elution buffer [50 mM-sodium phosphate (pH 8.0)/1 mM-$MgCl_2$/2 mM-2-mercaptoethanol/0.2 mM-EGTA) containing 10 mM-ADP. Pooled fractions were concentrated by ultrafiltration (Amicon Diaflo PM30; Amicon Corp., Lexington, MA, U.S.A.) and dialysed against Mono S buffer [25 mM-sodium phosphate (pH 7.0)/1 mM-2-mercaptoethanol/0.2 mM-EGTA] (Blue Sepharose fraction). For the final purification step, 10 mg of protein were absorbed on a Mono S HR5/5 column (Pharmacia), equilibrated in Mono S buffer, and CK activity was eluted with a NaCl gradient from 50 to 450 mM. Fractions containing the highest creatine kinase activity were pooled (Mono S fraction). Mi_b-CK isolation from chicken heart mitochondria has been described (Schlegel *et al.*, 1988*a*). Protein concentrations were determined with the Bio-Rad protein assay (Bradford, 1976), with BSA as standard.

Characterization of Mi_b-CK

Mi-CK activities were determined by measurement of the H^+ release in the reaction phosphocreatine + ADP → creatine + ATP, as described (Wallimann *et al.*, 1984). Characterization of the Mi_b-CK oligomer pattern by FPLC Superose 12 HR 10/30 gel-permeation chromatography and cellulose polyacetate electrophoresis has been described (Schlegel *et al.*, 1988*b*). Dimerization of octameric Mi_b-CK by transition-state analogues (4 mM-ADP/5 mM-$MgCl_2$/20 mM-creatine/50 mM-KNO_3, pH 7.2) has been described (Schlegel *et al.*, 1988*b*). *N*-Terminal protein-sequence analysis was done with an ABI model 470A automated sequenator with on-line detection of phenylthiohydantoin derivatives (James *et al.*, 1990). Electrospray mass spectra were generated using a Finnigan MAT model 710 triple quadruple mass spectrometer (Finnigan, MAT; San José, CA, U.S.A.) with a 20 kV conversion dynode and 400 m/z mass range; data was routinely acquired with a mass accuracy of ± 0.01%. Mi_b-CK crystallization and sample preparation for electron microscopy were performed as described by Schnyder *et al.* (1988, 1990). Kinetic measurements using 3.0 μg of Mi_b-CK enzyme after fractionation on Mono S were done in a pH-stat by varying the phosphocreatine concentration at constant ADP and $MgCl_2$ concentration of 4 mM and 10 mM respectively. The values for the K_m for phosphocreatine and $V_{max.}$ were determined by linear regression in an Eadie–Hofstee plot of four series of independent measurements.

RESULTS AND DISCUSSION

Expression and purification of the chicken mitochondrial creatine kinase

The coding sequence of the mature Mi_b-CK (Hossle *et al.*, 1988) was fused to the T7-phage promoter in the expression vector pET-3b (Studier *et al.*, 1990; see the Materials and methods section) to create Mi_b-CK expression plasmid pRF23. To express and purify mature Mi_b-CK, a 1 litre culture of BL21(DE3)pLysS cells containing plasmid pRF23 was grown under the inducing conditions for 4.5 h, 2.5 g cells (wet weight) were harvested and lysed. Mi_b-CK activity was found solely in the supernatant of the *E. coli* lysate at a specific activity of 27 units/mg protein (Table 1). Chromatography on Blue Sepharose, followed by FPLC Mono S chromatography (see the Materials and methods section) resulted in a 3-fold enrichment of Mi_b-CK, which was judged by PAGE to be a nearly homogeneous protein preparation (Fig. 1). The maximal specific activity of Mono S peak fraction was around 115 units/mg. Pooled Mono S fractions had a somewhat lower specific activity of 55–80 units/mg, depending on the isolation and the length of storage at 4 °C. Minor low-molecular-mass contaminants were detected on overloaded SDS/polyacrylamide gels (results not shown). A further purification by gel-permeation chromatography could be included to remove these impurities. *E. coli* itself does not contain any detectable CK activity. Cross-contamination with other CK isoenzymes, as it might occur when purifying Mi-CK from animal tissue, is therefore not a problem. Isolation of 20 mg of Mi_b-CK from chicken heart mitochondria requires at least 0.5 kg of heart tissue and longer purification times.

So far, two other CK proteins have been expressed in *E. coli*, namely rabbit M-CK (Chen *et al.*, 1991) and CK from the electric organ of *Torpedo californica* (Babbitt *et al.*, 1990). However, both proteins could only be expressed and isolated using special precautions, since cultures containing the expression plasmids grown at 37 °C yielded mainly insoluble protein aggregates (inclusion bodies). In addition, the yield of purified enzyme from these expression systems was in the range of only 1 mg/litre of *E. coli* culture, around 20-fold lower than the yield of Mi_b-CK. For these two CKs, protein-folding pathways may favour inclusion-body formation. We observed that deletion of as little as 30 amino acids (including six positively and five negatively charged residues) from the *C*-terminus of the Mi_b-CK

Table 1. Purification of Mi_b-CK from *E. coli* strain BL21(DE3)pLysS transformed with expression plasmid pRF23

The Table shows a representative purification protocol indicating purification steps, amount of protein material, total CK activity, increase in specific activity and Mi_b-CK yield. Total protein content was determined by Bio-Rad protein assay, using BSA as standard. A unit of enzyme activity is equal to 1 μmol of phosphocreatine transphosphorylated/min at pH 7.0 and 25 °C.

Purification step	Protein (mg)	Total CK activity (units)	Specific CK activity (units/mg)	Yield (%)
Crude *E. coli* lysate	149.6	4010	26.8	100
Blue Sepharose fraction	52.8	3190	60.4	80
Mono S fraction	28.0	2052	73.3	51

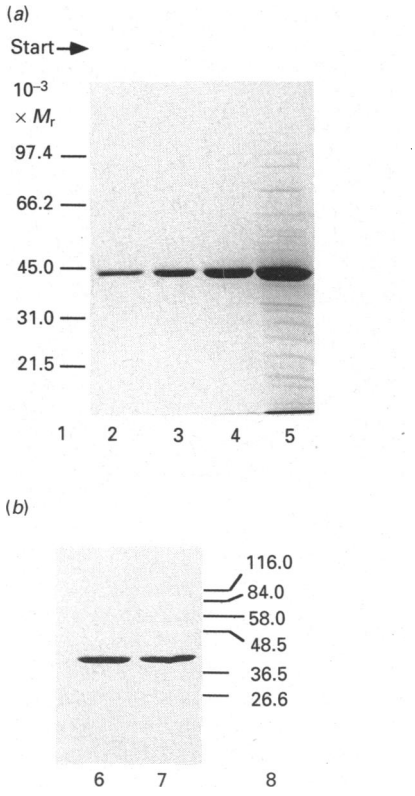

Fig. 1. SDS/PAGE and immunoreactivity of native and recombinant Mi_b-CK

Individual fractions of a Mi_b-CK purification were separated on a 10% polyacrylamide/SDS gel and stained with Coomassie Brilliant Blue R-250 (lanes 2–5). Lane 1, positions of molecular-size markers (Bio-Rad, low-M_r standards); lane 2, purified Mi_b-CK from chicken heart mitochondria (Mono S fraction); lane 3, purified recombinant Mi_b-CK isolated from strain BL21(DE3)pLysS transformed with pRF23 (Mono S fraction); lane 4, Blue Sepharose fraction of the recombinant Mi_b-CK; lane 5, crude lysate of *E. coli* strain BL21(DE3)pLysS transformed with pRF23 used to isolate recombinant Mi_b-CK. (*b*) Immunological detection of Mi_b-CK in an *E. coli* lysate and a lysate from chicken heart mitochondria, separated on a 4–12.5% gradient polyacrylamide/SDS gel and shown after electrophoretic transfer to nitrocellulose and immunoperoxidase staining with a monoclonal anti-(chicken cardiac Mi-CK)antibody (mAb 30; Schlegel *et al.*, 1988*b*). Lane 6, Mi_b-CK isolated from *E. coli* strain BL21(DE3)pLysS transformed with pRF23 (Mono S fraction); lane 7, Mi_b-CK isolated from chicken heart mitochondria (Mono S fraction); lane 8, positions of molecular-size markers (Sigma 7B; prestained standards).

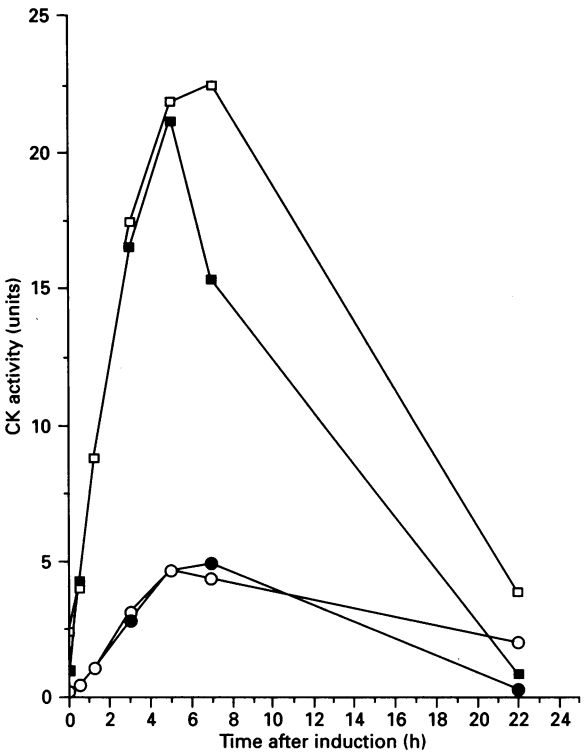

Fig. 2. Mi_b-CK expression under different selection conditions

The Mi_b-CK expression plasmid pRF72 was grown on media containing either ampicillin (100 μg/ml; ■, ●) or kanamycin (25 μg/ml; □, ○). At zero time IPTG was added to induce Mi_b-CK expression. CK activity in aliquots of the cultures was determined after the time intervals indicated. Shown in the figure is one representative experiment. ■, Specific activity (units/mg) of an extract from a culture grown on medium containing ampicillin. □, Specific activity (units/mg) of an extract from a culture grown on medium containing kanamycin. ●, Total yield of CK activity (units)/ml of culture of the ampicillin-selected culture. ○, Total yield of CK activity (units)/ml of culture of the kanamycin-selected culture.

resulted in a highly expressed, but totally insoluble, protein (results not shown). Identification of regions in the primary structure that differ between insoluble rabbit M-CK (Chen *et al.*, 1991), the truncated insoluble Mi_b-CK, soluble Mi_b-CK and soluble chicken B-CK (R. Furter, unpublished work) might point to important factors governing the formation of inclusion bodies for creatine kinases expressed in *E. coli*.

Stability of Mi_b-CK expression plasmid pRF72 in *E. coli*.

Selection for ampicillin resistance to maintain plasmids in *E. coli* cultures is effective only during initial stages of culture growth, since the β-lactamase, the enzyme conferring ampicillin resistance, is secreted into the medium and the ampicillin is degraded rather quickly. Plasmids can be lost at a particularly high rate if the expressed foreign gene product is toxic for *E. coli* (Studier *et al.*, 1990). To test whether selection for kanamycin can improve plasmid stability and the amount of Mi_b-CK expressed from the Mi_b-CK expression plasmid, a kanamycin gene was added to plasmid pRF23 to form pRF72 (see the Materials and methods section). A small amount of Mi_b-CK is produced under non-inducing conditions (results not shown). Under non-inducing conditions all the plasmids tested (pBR322, pET-3b, pRF23 and pRF72) were stably maintained in strain BL21(DE3)pLysS during 18 h of growth under ampicillin or kanamycin selection (results not shown), indicating that Mi_b-CK *per se* is not highly toxic for the host cell. During the first 3 h after induction with IPTG, however, viable cell counts dropped sharply and all plasmids harbouring a phage-T7 promoter, i.e. pET-3b, pRF23 and pRF72, were rapidly lost. When selecting for ampicillin resistance under inducing conditions, more than 99% of the cells lost plasmid pRF72 after 5 h. Only 0.1% of the cells remained ampicillin-resistant after overnight growth of the culture. After growth for 5 h in medium containing kanamycin and IPTG, maximally 95–97% of cells lost plasmid pRF72. However, when growth was continued overnight, the percentage of plasmid-containing colonies increased with time up to 100%, indicating that cells that maintained their plasmids during the first 5 h were selectively propagated during longer growth periods. These kanamycin-resistant cells could be newly induced to express normally high amounts of Mi_b-CK (results not shown), showing that neither the plasmids nor the chromosomally encoded T7 RNA polymerase gene were inactivated by deletion or other

Table 2. Comparison of kinetic parameters of Mi_b-CK isolated from chicken heart mitochondria and *E. coli*

Kinetic parameters K_m and $V_{max.}$ were calculated from sets of four independent measurements, the phosphocreatine concentration being varied between 0.2 and 30 mM at a constant ADP concentration of 4 mM, an $MgCl_2$ concentration of 10 mM and with 3.0 μg of Mi_b-CK. Specific activities under standard conditions were determined at 10 mM-phosphocreatine, 4 mM-ADP and 10 mM-$MgCl_2$. A unit of enzyme activity is defined in Table 1.

CK source	K_m for phosphocreatine (mM)	Specific activity (units/mg)	$V_{max.}$ (units/mg)
Native Mi_b-CK	1.41±0.04	52.3±1.5	66.6±0.7
Recombinant Mi_b-CK	1.41±0.07	56.1±0.9	63.0±1.3

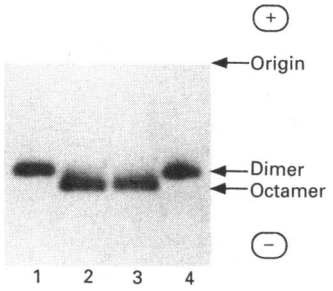

Fig. 3. Cellulose-polyacetate-gel electrophoresis for detection of dimeric and octameric Mi_b-CK

Mi_b-CK was separated electrophoretically on cellulose polyacetate strips under non-denaturing conditions and stained for enzyme activity *in situ*. Addition of $MgCl_2$, creatine, ADP and KNO_3 was used to convert octamers into dimers (see the Materials and methods section). Mi_b-CK octamers and dimers, although both migrating towards the cathode, can be separated by this method, owing to their slightly different pI values (pI of Mi_b-CK octamer and dimer are pH 9.5 and pH 9.3 respectively; Schlegel *et al.*, 1990). Lane 1, Mono S fraction of Mi_b-CK isolated from *E. coli* (see Fig. 2, lane 3), after conversion into dimers; lane 2, Mono S fraction of Mi_b-CK isolated from *E. coli*; lane 3, Mono S fraction of Mi_b-CK isolated from chicken heart mitochondria, representing Mi_b-CK octamers (see Fig. 2, lane 2); lane 4, Mono S fraction of Mi_b-CK isolated from chicken heart mitochondria after conversion into dimers, representing Mi_b-CK dimers. The polarity of the electric field is indicated (+/−).

mutations. Hence, using kanamycin selection, it is also possible to subculture and induce cells repeatedly without losing the expression plasmid.

To test the effect of selection on Mi_b-CK yield, plasmid pRF72 was expressed in BL21(DE3)pLysS under ampicillin or kanamycin selection. Aliquots of cells were harvested at different times, lysed, and creatine kinase activity was determined in the 10000 *g* supernatant of the lysate. Total activity and specific activity increased during the first 4–5 h after induction (Fig. 2). Maximally, Mi_b-CK constituted about one-third of all soluble protein in the *E. coli* lysate, independent of the selection chosen. However, the specific activity of Mi_b-CK in kanamycin-selected cultures increased for a longer period of time, and specific and total activity also decreased more slowly than the activity of Mi_b-CK in ampicillin-selected cultures, probably reflecting a somewhat slower plasmid loss when cultures were grown in kanamycin-containing media. This finding can be exploited by using kanamycin rather than ampicillin selection in order to extend the time window for obtaining maximal yields of Mi_b-CK and probably other proteins with good stability in *E. coli* cells.

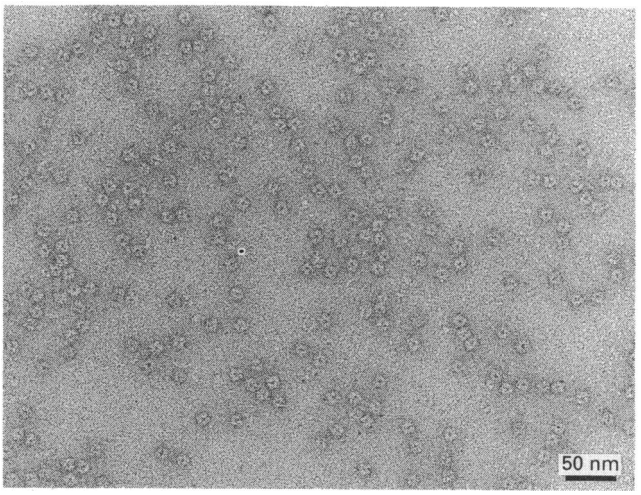

Fig. 4. Electron micrograph of negatively stained Mi_b-CK octamers

Recombinant Mi_b-CK octamers revealed with 2% uranyl acetate exhibit the 4-fold molecular symmetry with the central, stain-filled, indentation. The lack of side views indicates that the absorption properties (of the recombinant molecule to the substrate) are preserved as in the native Mi_b-CK from chicken heart tissue.

Characterization of recombinant Mi_b-CK

Several biochemical properties of the purified recombinant protein were compared with 'native' Mi_b-CK isolated from chicken heart mitochondria to ensure that the two proteins are identical. In electrophoretic migration under denaturing conditions, both proteins showed a subunit M_r of 42000 (Fig. 1*a*; compare lanes 2 and 3). Additionally, both proteins cross-reacted with a polyclonal (result not shown) and a monoclonal antibody raised against chicken heart Mi_b-CK (Fig. 1*b*; lanes 6 and 7). The native Mi_b-CK protein initially contains an *N*-terminal mitochondrial targeting sequence which is cleaved off to give a mature protein with an *N*-terminus beginning Thr-Val-... The expression of the mature moiety of the Mi_b-CK cDNA required the addition of an initiator methionine at its *N*-terminus. However, this methionine is removed quantitatively during expression, since protein sequencing showed that the recombinant protein had an *N*-terminus identical with the mature Mi_b-CK isolated from chicken heart mitochondria. Direct mass determination of chicken heart Mi_b-CK and recombinant Mi_b-CK showed molecular masses of 43194 and 43209 respectively. This agrees well with the calculated mass of 43195 and suggests the absence of post-translational modifications in the native Mi_b-CK isolated from chicken heart mitochondria. Rabbit M-CK was also shown to lack post-translational modifications (Chen *et al.*, 1991).

The specific activities of Mi_b-CK prepared from *E. coli* or chicken heart, measured under standard conditions, were very similar (Table 2), though somewhat dependent on the individual preparation used. The K_m values and $V_{max.}$ of the native Mi_b-CK and recombinant Mi_b-CKs are also very similar (Table 2), indicating that the recombinant protein is folded correctly to give full enzymic activity.

Oligomerization of the recombinant Mi_b-CK

The dimeric form of all mitochondrial creatine kinases studied so far can associate to form octamers (Schlegel *et al.*, 1988*b*; Schnyder *et al.*, 1988; Quemeneur *et al.*, 1988; Wyss *et al.*, 1990; Belousova *et al.*, 1991). This feature distinguishes the mitochondrial isoenzymes clearly from the cytosolic isoforms which are only found as dimers. A detailed analysis of the

oligomeric structure of the recombinant Mi_b-CK showed that the enzyme isolated from *E. coli* cells is identical with the native enzyme. The purified recombinant enzyme was analysed by cellulose polyacetate electrophoresis under non-denaturing conditions (Fig. 3). Most of the recombinant CK activity co-migrated with the octameric species of the native chicken Mi_b-CK. Addition of compounds known to induce a transition-state analogue complex (Schlegel *et al.*, 1988*b*; see the Materials and methods section) converted octamers quantitatively into dimers (Fig. 3). These findings are supported by analysis of the oligomers by gel-permeation chromatography. Purified, recombinant Mi_b-CK showed an elution pattern on an FPLC Superose-12 column fully compatible with a predominantly octameric form (with an octamer/dimer ratio > 9; result not shown). Addition of transition-state-analogue complex-inducing compounds converted octamers quantitatively into dimers; after removal of the reagents, 70–80% of the dimers re-oligomerized to octamers within a 16 h period (result not shown).

Octamers could also be observed directly by microscopy. At the resolution of about 2 nm (20 Å), no differences were detected between the shape of single Mi_b-CK molecules isolated from chicken heart mitochondria and those expressed in *E. coli* (Fig. 4). The negatively stained molecules clearly showed the typical 4-fold symmetry and the dark-stained cavity in the middle of the molecule, diagnostic of the octamer (Schnyder *et al.*, 1988). Recombinant Mi_b-CK formed crystals under the same conditions as described for the chicken heart enzyme (Schnyder *et al.*, 1991). Crystals grown from recombinant Mi_b-CK were indistinguishable in morphology from those obtained from chicken heart protein and diffract equally well to about 0.3 nm (3 Å) resolution (results not shown). X-ray diffraction showed that, in the absence of ATP, the recombinant protein also crystallized in the space group $P42_12$ with one octamer per asymmetric unit as it was shown previously for the native protein (Schnyder *et al.*, 1990). This finding supports strongly the notion that native and recombinant protein have the same structure.

We have developed a system for the efficient expression of mature octameric chicken mitochondrial creatine kinase. For all the biochemical parameters tested the recombinant protein has the same structural and functional properties as the mature native enzyme isolated from chicken heart mitochondria. In particular, direct comparison of the molecular mass strongly suggested that the native Mi_b-CK is not post-translationally modified. Furthermore, we found that enzymic activity and the oligomeric structure of recombinant and native enzymes are indistinguishable. It is of particular interest that the enzyme can be isolated in its octameric form from *E. coli*. Several recent investigations support the hypothesis that the octamer is the physiologically active state of the protein. The highly efficient expression system presented here will be useful for obtaining altered forms of chicken Mi_b-CK and will aid in designing experiments to find further evidence for the central role of the octameric mitochondrial creatine kinases in the 'phosphocreatine shuttle' model (Bessman & Geiger, 1981; Saks *et al.*, 1985; Wallimann *et al.*, 1989, 1992).

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