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Crystallization and preliminary X-ray analysis of human muscle creatine kinase

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Creatine kinase is a key enzyme in the energy homeostasis of cells and tissues with high and fluctuating energy demands. Human muscle MM creatine kinase is a dimeric protein with a molecular weight of ~43 kDa for each subunit. It has been crystallized by the hanging-drop vapor-diffusion method using 2-methyl-2,4-pentanediol as precipitant. The crystals belong to the enantiomorphous space group $P6_222$ or $P6_422$ with cell parameters of $a = b = 89.11$ and $c = 403.97$ Å. The asymmetric unit of the crystal contains two subunits. A data set at 3.3 Å resolution has been collected using synchrotron radiation.

1. Introduction

Creatine kinase (E.C. 2.7.3.2) catalyzes the reversible transfer of a phosphate group from phosphocreatine to ADP to regenerate ATP in the presence of magnesium ions (for a review, see Wallimann *et al.*, 1992). It plays a key role in the energy homeostasis of cells and tissues with high and fluctuating energy demands. The creatine kinase/phosphocreatine system serves as an energy-buffering system, an energy-transport system and a regulatory system of subcellular ATP/ADP ratio. A number of isoenzymes of creatine kinase have been found with different intracellular compartmentation and functions. The cytosolic isoform of creatine kinase is a dimeric protein composed of two different types of subunits with similar molecular weigtht of 43 kDa: M, the predominant form in muscle tissue and B, the primary form in brain tissue. Thus, three kinds of cytosolic creatine kinase (MM, MB and BB) are formed. The increased level of MB creatine kinase in serum is clinically used as the diagnostic marker for myocardial infarction. Mitochondrial creatine kinase, although encoded for by nuclear genes like cytosolic creatine kinase, is located at the inner mitochondrial membrane and exists in two oligomeric forms: a dimer and an octamer of four dimers. Some 20 members of the creatine kinase isoenzyme family have been sequenced and sequence comparisons show extensive homologies both at the DNA and amino-acid level (Babbit *et al.*, 1986; Mühlebach *et al.*, 1994). Biochemical and biophysical studies were carried out on the catalytic mechanism and conformation of various isoforms of creatine kinases (for recent references, see Raimbault *et al.*, 1997; Forstner *et al.*, 1997; Leydier *et al.*, 1997; Yang & Zhou, 1997; Yang *et al.*, 1997).

Preliminary crystallographic analysis has been reported for cytosolic creatine kinases from rabbit muscle (McPherson, 1973; Hershenson *et al.*, 1986) and bovine heart (Gilliland & Olsson, 1983) and for mitochondrial creatine kinase from chicken cardiac muscle (Schnyder *et al.*, 1990, 1991). However, considerable microheterogeneity was detected in purified MM creatine kinase (Hershenson *et al.*, 1986) and BB creatine kinase (Quest *et al.*, 1990), which causes difficulty in growth of creatine kinase crystals of high quality suitable for atomic level X-ray studies. The only crystal structure reported to date is that of mitochondrial creatine kinase from chicken cardiac

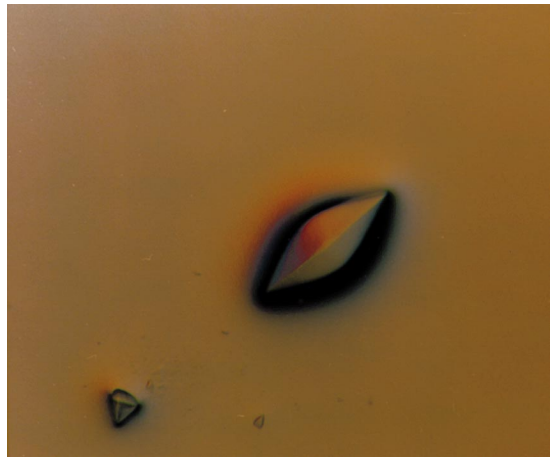

Figure 1
The crystal of human muscle creatine kinase. The longest dimension is approximately 0.6 mm.

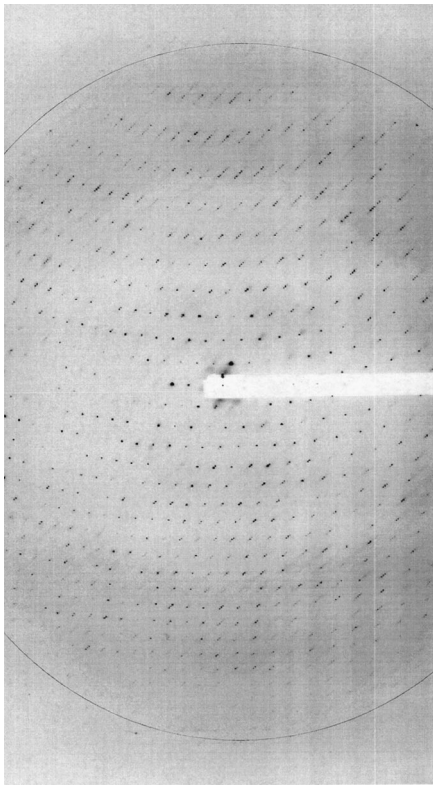

Figure 2
Oscillation photograph of human muscle creatine kinase using synchrotron radiation. Oscillation angle: 1°. The black circle corresponds to 3 Å resolution.

muscle in an octameric isoform at 3 Å resolution (Fritz-Wolf *et al.*, 1996). Here we report the preliminary crystallographic studies on MM creatine kinase from human muscle.

2. Experimental and results

The MM creatine kinase was purified from human skeletal muscle as previously described by Ritter *et al.* (1981) and showed a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and a single spot on two-dimensional gel electrophoresis (isoelectric focusing and gradient gel). The protein in 0.05 *M* Tris–HCl pH 8.0 buffer containing 8 m*M* mercaptoethanol was lyophylized after three dialyses against double-distilled water to remove low molecular weight solutes. Crystallization trials were performed by the hanging drop vapor diffusion method at 291 K. The lyophilized protein was dissolved in double-distilled water (pH 5.8) to a concentration of 10 mg ml^{-1}. The droplet was a mixture of 5 µl of protein solution and 5 µl of 40% 2-methyl-2,4-pentanediol (MPD) and was equilibrated over the reservoir of 0.7 ml 40% MPD. Eye-shaped crystals appear within one or two weeks with a size of 0.6 × 0.2 × 0.2 mm (Fig. 1).

Crystallographic characterization and data collection were performed on a 430 mm Weissenberg camera with 200 × 400 mm imaging plates using synchrotron radiation (wavelength 1.00 Å) at beamline BL6A, KEK, Japan. A typical oscillation photograph is shown in Fig. 2. Data at 3.3 Å resolution were processed with programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1999). The data set in the range of 40–4.0 Å has an $R_{\rm merge}$ of 12.2% and mean I/σ of 9.0, and is 89.0% complete. The crystal belongs to the enantiomorphous space group $P6_222$ or $P6_422$ with cell parameters of $a = b = 89.11$ and $c = 403.97$ Å. The asymmetric unit of the crystal contains two subunits which results in a V_m value of 2.7 Å^3 Da^{-1} corresponding to the solvent content of 54% unit-cell volume (Matthews, 1968). The values lie within the range observed for normal protein crystals. The further molecular-replacement studies using the structure of mitochondrial creatine kinase as an initial model are under way.[1]

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[1] Supplementary data, including data-processing statistics are available from the IUCr electronic archive.

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