Physiol. Res. 51: 35-41, 2002

Myofibrillar Creatine Kinase Activity Inferred from a 3D Model

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Received January 16, 2001
Accepted June 6, 2001

Summary

Myofibrillar creatine kinase (CK) that buffers ATP during fluctuating muscle energy metabolism has been selected for studies of conformational changes underlying the cellular control of enzyme activity. The force field was computed for three energetic states, namely for the substrate-free CK molecule, for the molecule conjugated with the MgATP complex, and for the molecule conjugated with the pair of reactants MgATP-creatine. Without its substrates, the enzyme molecule assumes an inactive "open" form. Upon binding of the MgATP complex, the CK molecule takes up a reactive "closed" conformation. Subsequent binding of creatine yields a nonreactive "intermediary" conformation. Acid-base catalysis is considered to be the basic principle for the reversible transfer of the phosphoryl group between the substrates. The results indicate that the substrate-induced energy minimizing conformational changes do not represent a sufficient condition for CK activity and that some other essential component of physiological control at the cellular level is involved in the transition from the intermediary to the closed structure of the molecule.

Key words
Muscle • Energetics • Myofibrillar creatine kinase

Introduction

Creatine kinase (CK)(EC 2.7.3.2.) plays a key role in muscle energy metabolism by buffering the cellular ATP concentration during different rates of ATP turnover in a resting or a contracting muscle. The turnover ranges from 10 μmol ATP g^{-1} s^{-1} in the muscles of humming birds hovering above a flower, and muscles of a running man which turn over 1 μmol ATP g^{-1} s^{-1}, to resting values in mammalian muscles including man of about 20-fold lesser turnover rate, i.e. 0.05 μmol ATP g^{-1} s^{-1}. It is evident that such an activity range, particularly during transient states of fluctuating energy demands in a certain muscle of a given species, requires appropriate control of CK activity.

The specific activity of CK measured in skinned fibers of the rat psoas muscle reached 6.97 μmol ATP min^{-1} mg^{-1} protein (Gregor *et al*. 1999). The enzyme is found at three locations in the muscle cell: on the myofibril, in the membrane of mitochondria, and in the cytoplasm. Activity evaluation of the myofibrillar CK itself, after elimination of the effect of substrate channelling by myosin ATPase, results in 7.6 nmol PCr min^{-1} mg^{-1} protein (Gregor *et al*. 1999) which represents the basal activity value for myofibrillar control.

The control of CK activity is realized by conformational changes of the CK molecule, in addition to slight changes in the concentration of the substrates in the Lohmann's reaction, which is thought to be at or near equilibrium in a muscle cell (Kushmerick 1983). For

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further quantitative measurements of the conformational changes, a 3D model of myofibrillar CK is constructed here, with the derived consequences for its activity.

Methods

The model has been created by homology modeling, on the basis of the reported structure of the isoenzyme monomer from the octameric mitochondrial CK isoform (Fritz-Wolf *et al.*1996), using a Swiss-Prot Viewer, Swiss-Prot Model (Guex and Peitsch 1997) and SYBYL 6.6 software (Tripos GmbH, Germany). Data on the primary sequence alignment for mitochondrial CK (Fritz-Wolf *et al.* 1996) and on the primary sequence for myofibrillar CK (Rao *et al.* 1998) were obtained from The Protein Data Bank, maintained as a web site by the Research Collaboratory for Structural Bioinformatics, Germany.

The force field energy minimizations and docking of the reaction substrates were performed using the SYBYL 6.6 package, running on MIPS 3 - IRIX 6.5. The geometry of creatine was obtained from CSD (Cambridge Small Molecule Database) and the geometry of the MgATP complex from the PDB database, Brookhaven, U.S.A. The loop consisting of a sequence alignment specific for myofibrillar CK has been built using the Swiss-Prot viewer loop function and SYBYL 6.6 software.

Three conformations were computed: the substrate-free CK molecule, the molecule conjugated with the MgATP complex and the molecule conjugated with the pair of reactants MgATP - creatine.

The initial procedure started with the primary sequence alignment for myofibrillar CK. Subsequently, the structurally conserved regions and the structurally variable region were built from corresponding locations in the homologous mitochondrial protein. The resulting structure was subjected to a simulated annealing procedure, in which the dynamics ran 2 ns at 700 ^{o}K and then the structure annealed for 10 ns to 300 ^{o}K in order to toughen it. This annealing procedure was run 50 times for each of the three conformations.

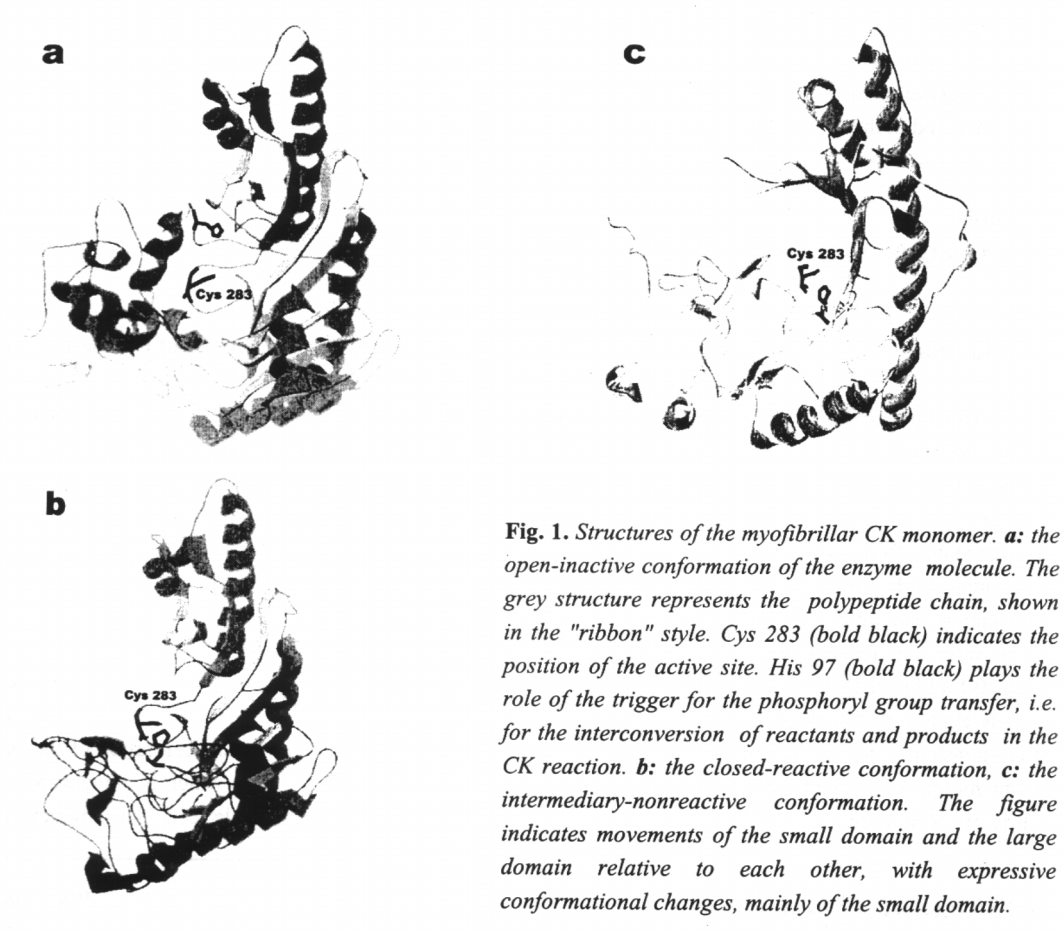

Fig. 1. *Structures of the myofibrillar CK monomer.* ***a:*** *the open-inactive conformation of the enzyme molecule. The grey structure represents the polypeptide chain, shown in the "ribbon" style. Cys 283 (bold black) indicates the position of the active site. His 97 (bold black) plays the role of the trigger for the phosphoryl group transfer, i.e. for the interconversion of reactants and products in the CK reaction.* ***b:*** *the closed-reactive conformation,* ***c:*** *the intermediary-nonreactive conformation. The figure indicates movements of the small domain and the large domain relative to each other, with expressive conformational changes, mainly of the small domain.*

Results

The monomer consists of a small (residues 1 - 117) and a large domain (residues 118 - 381). In comparison with the published structure of the mitochondrial isoform, the large domain comprises the most structurally different region, the loop of twelve residues between Thr 322 and Asp 335. Six amino-acid residues of the loop are identical with the mitochondrial isoform; six residues of the loop, Ser 323, Val 324, Phe 325, Gly 331, Ser 332 and Phe 334, are different (or Val 324 at a different place). Among the very six different residues, three are hydrophilic (Ser 323, Ser 332, Gly 331) and three are hydrophobic (Val 324, Phe 325, Phe 334). Due to the difference, the loop of myofibrillar CK could thus be involved in an isoform - specific function (binding to the myofibrillar surface). The homology between our model and the solved X-ray structure of the mitochondrial isoform has been found to be identical at the level 343/374 (343 amino-acid residues from 374 residues of the isoform), and using the amino-acid similarity, the positivity score equaled 347/374.

Without its substrates, the enzyme molecule takes an inactive "open" form. In this open conformation, when the two domains appear to be at the greatest distance apart, the interatomic distance between the proximate N^1 of His 97 and S of Cys 283 equals 0.766 nm (Fig.1a).

There are ten energetically favorable sites for MgATP alignment, nine sites for binding of creatine (Cr) and ten sites for binding of phosphocreatine (PCr) over the surface of the CK molecule. However, a selection of the "essential" cysteine Cys 283 (for "essential" see Discussion) determines one position only for the combination MgATP - Cr, as well as for the combination MgADP - PCr. Two other inconvenient combinations, i.e. MgATP - PCr and MgADP - Cr, are spatially excluded. The former combination has no space for docking, the latter has both substrates too far apart, as for the mechanism of the reversible transfer of a phosphoryl group between ATP and Cr, as presented below. The essential Cys 283 locates the substrate - binding sites in the cleft between the domains.

Fig. 2 shows the decrease of the radius of CK molecule gyration upon binding of the MgATP complex, which indicates movements of the domains relative to each other. The coordination number of Mg is six. One bond forms a chelate complex with an oxygen atom in a carboxyl group of Glu 231. Two bonds form chelate complexes with two oxygen atoms in a carboxyl group of Glu 232. The fourth bond binds an oxygen atom in a chelate within a carboxyl group of Asp 233, clamping the MgATP complex to the side of the CK molecule. The remaining two bonds form a chelate complex with oxygen atoms in the alpha phosphoryl group of ATP. In such a way, the beta and the terminal gamma phosphoryl groups remain untied for the reversible transfer of the terminal phosphate. The correct dock is also achieved by the furan ring of ATP ribose which perfectly follows the pyrrole ring of Trp 228, where two polar OH groups of the former ring and the π-electron cloud of the latter reflect the considerable resonance stabilization energy. The enzyme molecule takes a reactive "closed" conformation. In this closed conformation, the interatomic distance between the proximate N^1 of His 97 and S of Cys 283 equals 0.277 nm (Fig. 1b), thus suggesting a shift of this part of the small domain toward the active site by 0.489 nm.

Subsequent bonding of Cr triggers considerable changes in the shape and size of the molecule, yielding the "intermediary" conformation - with respect to the above mentioned intramolecular distance - somewhere between the open and the closed conformations. Contrary to the chelate complex dock for ATP, Cr is posited by weak electrostatic interactions. The negatively charged oxygen of the carboxyl group in the entering free Cr forms an intramolecular hydrogen bond to a proximate nitrogen of the positively charged guanidinium group. When Cr is docked by CK, the intramolecular hydrogen bond in Cr is split and the negative charge of the carboxylic oxygen is attracted by the nitrogens of both NH_2 groups of Arg 320 (at interatomic distances of 0.298 and 0.313 nm, respectively). In such a way, the positive charge of a quanidinium group is restored, introducing a necessity for the proton to be withdrawn from the guanidinium group during the phosphorylation of Cr. The additional electrostatic interaction is formed with the deprotonized amide group of Asn 286. Since the oxygen in the resonance-stabilized amide anion is remote from Cr, there is a negative charge on the amide nitrogen which forms an interaction with the positively charged nitrogen of Cr, docking the positively charged NH_2 group toward the SH group of Cys 283. The correct position is also supported by weak interactions between the tertiary N in the guanidinium group of Cr and the NH_2 group of Gly 73 and between hydrophobic CH_3 groups of Cr and Val 72. The bonding energy of the two substrates (MgATP + Cr) with protein in its intermediary

Fig. 2. *The radius of CK molecule gyration in the closed (blue) and the open (red) conformation.*

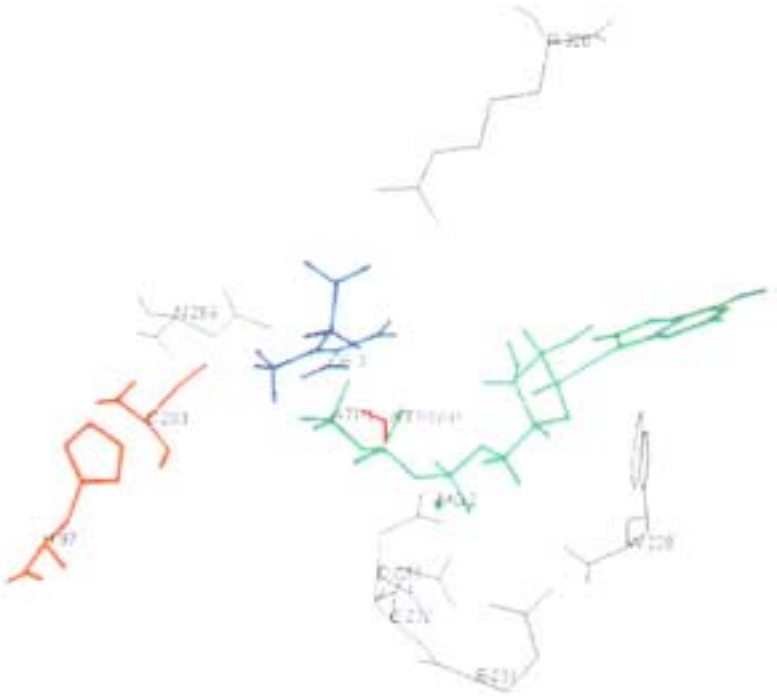

Fig. 3. *The computed active site structure (simplified). The residues of the peptide chain involved in substrate binding (for ATP: Glu 231, Glu 232, Asp 233 and Trp 228; for Cr: Arg 320 and Asn 286) are shown in black, the MgATP complex and Cr as the substrates in green and blue, respectively. His 97 as a "trigger" and "essential" Cys 283 are in orange, the hydrolyzing water molecule in red.*

conformation equals -120.1 kJ/2 mols. In this intermediary conformation, the interatomic distance between the proximate N^1 His 97 and S of Cys 283 equals 0.620 nm (Fig. 1c).

The conformational changes decisively affect the hydrophilic interaction coat of the CK molecule and prevent water molecules from entering the active site during catalysis. We have used 8221 water molecules in order to simulate in situ conditions of the protein. Movement towards the closed protein molecule excludes water molecules from the cleft, leaving just one molecule (No.5041) in the active site at half the distance between N^4 of His 97 and γP of ATP (N^4-γP = 0.344 nm).

Acid-base catalysis is considered to be the basic principle underlying the reversible transfer of a phosphoryl group between the substrates. In spite of the fact that acid-base catalysis has become a textbook mechanism, there is a particular point of interest concerning the active site structure of our model. It must catalyze both the rephosphorylation of Cr in the resting state of a muscle and the phosphorylation of MgADP during muscle work.

The computed active site for the intermediary conformation is presented in Fig. 3. Under the closed conformation, the six-membered ring system of central atoms is established, among which six pairs of electrons (marked with arrows in Fig. 4) are exchanged. The corresponding central atoms include: two nitrogens N^1 and N^4 of His 97 (reversibly unprotonated-protonated), S of Cys 283, N of Cr (the reactive guanidinyl atom in the guanidinium group), γP of ATP and O of the distance (N^4-γP) vaulting water molecule 5041. Using this structure, the acid-base catalysis is realized, according to the scheme presented in Fig. 4.

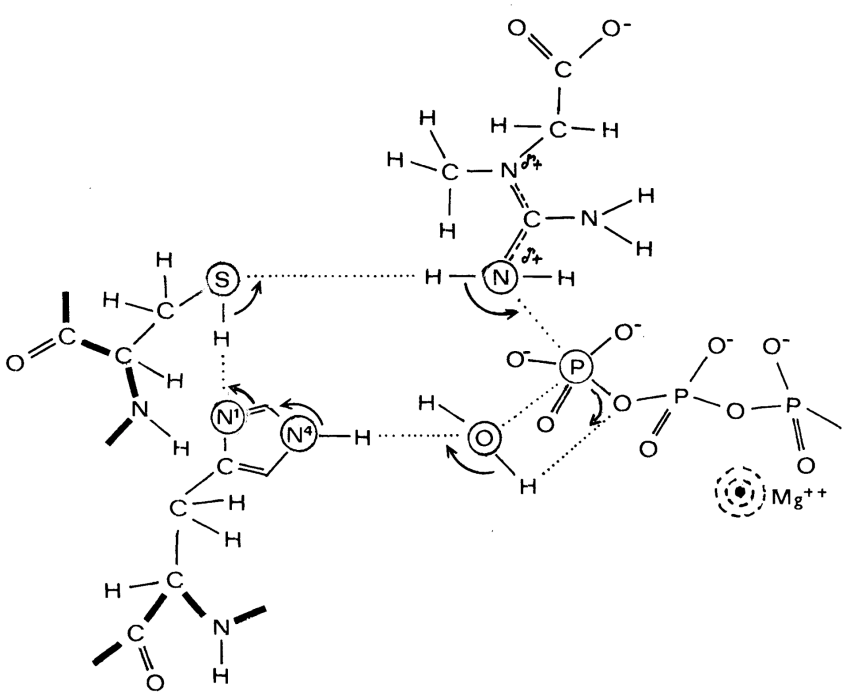

Fig. 4. *The catalytic mechanism of CK. In the schematic representation, only three phosphoryl groups of ATP, Cr, His 97, Cys 283 and a hydrolyzing water molecule are shown. Six atoms of the six -membered ring system are indicated by circles, among which six pairs of electrons (marked with arrows) are exchanged. Dotted lines represent weak interactions where new covalent bonds are formed by the donation of a pair of electrons from the respective neighboring bases. Bold black sticks of two residues (His 97 and Cys 283) form the primary structure of the polypeptide chain. In principle, the orientation is maintained as in Fig. 3.*

The simultaneous transfer of six electron pairs is triggered by insertion of His 97 into the ring system. As the initial step of the sequence, His 97 in its unprotonated form orients two structures: the water molecule No.5041 hydrolyzing ATP and the nonpolar SH-group on a side chain of Cys 283, inducing two respective weak interactions. One nitrogen of His 97 (N^4), by pairing with a hydrogen of the water molecule, causes electrons to move towards the water oxygen, making it an attacking nucleophilic group for the phosphoryl γP, when the γP atom is unshielded by the positively charged nitrogen in the guanidinium group of Cr. The nucleophilic attack towards γP starts when the proton of the positively charged guanidinium group in Cr is withdrawn by Cys

283, which leaves Cr as the conjugate base for the attack at the site of γP. As a result of the transition state, the intermediate structure presented in Fig.4 is ready for the covalent bond P-O hydrolysis of the terminal phosphoryl group of ATP. However, after the hydrolysis, the proton from the N^4 atom site of His 97 should be attracted by the water oxygen forming again a complete water molecule leaving His 97 still unprotonated and open for bonding of the thiol proton from Cys 283 by the N^1 atom. When N^1 nitrogen of His 97, proximate to S of Cys 283, is protonated, the proton of the positively charged guanidinium group can be withdrawn and accepted by S of Cys 283. Then the ring-membered sequence is terminated by the rephosphorylation of Cr. Unprotonated His 97 is withdrawn from the active site by movement of the CK molecule towards its open conformation, and the cycle is terminated.

In other words, concerning the acid-base catalysis, His 97 in its unprotonated form can be considered as a conjugate base and as a nucleophile it attracts the water hydrogen at the onset of hydrolysis, yielding the classical hydrogen bond. The shift of electrons toward the water oxygen makes it a stronger nucleophile attacking group for hydrolysis of the phosphoryl group from ATP. Unshielded γP is attached by unprotonated Cr as the conjugate base and tied up by a new covalent bond.

The cycle goes in a reverse direction for the phosphorylation of MgADP. In order to trigger it, a proton should be donated from outside into His 97. Protonated His 97 (under the newly formed closed conformation) triggers a shift of the proton into the guanidinium group of Cr.

Discussion

The structure of myofibrillar CK and the enzyme activity interactions presented in this communication are fully consistent with some earlier proposals and results. Cys 283 was selected as "essential" for this study on the basis of the observation that covalent modifications resulted in an inactive enzyme (Wu *et al.* 1989). Such inactivation was also observed when iodoacetamidofluorescein, as a thiol-reactive fluorophore, was covalently bound during the fluorescence resonance energy transfer measurement of the CK molecule (Gregor *et al.* 2001).

Binding of ATP or ADP in the absence of Mg did not lead to a significant change in the size or shape of the CK molecule (Forstner *et al*. 1998). On the other hand, binding of the MgATP complex as the first substrate induces formation of the reactive closed conformation and prevents water molecules from entering the active site and from wasteful ATP hydrolysis. This finding is in accordance with the initial velocity studies at pH=7, which indicate a rapid equilibrium ordered mechanism for the phosphorylation of Cr with MgATP added to the enzyme *prior* to Cr (Schimerlik and Cleland, 1973).

His 97, as the trigger for the phosphoryl group transfer in this study, represents a single group with a pK near 7 that acts as an acid-base catalyst and must be unprotonated in the direction of Cr rephosphorylation and protonated for MgADP phosphorylation (Cook *et al*. 1981). The docking role of Trp 228 is in accordance with its recently proposed role (Hagemann *et al.* 2000).

The basic question, whether a substrate-induced conformational change is required for CK activity, or alternatively, whether CK acts as a "coenzyme" bringing the substrates into oriented steering proximity, may be answered in the following way. The three structures found in this study (open-inactive, closed-reactive and intermediary-nonreactive) suggest that the substrate-induced conformational change would be required for CK activity. This conclusion based upon the model behavior does not support the view on coenzyme catalysis (Stroud 1996). However, the intermediary conformation brings both domains of the flexible CK molecule too far apart from each other for spontaneous catalysis. Taken together, our results would indicate that the substrate-induced energy minimizing conformational changes do not represent a sufficient condition for CK activity and that some other necessary component of physiological control at a cellular level is involved (e.g. Ca^{2+}, pH, structural myofibrillar changes), in the transition from the intermediary to the closed structure of the molecule.

Acknowledgements

This work was supported by the CR Ministry of Education Grant VZ 113100003 and by the CR Ministry of Education Grant VS 96141.

Preliminary results presented at the Congress of Czech and Slovak Physiological Societies, Hradec Králové, February 2 – 4, 2000 (Mejsnar et al. 2000).

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Reprint requests

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