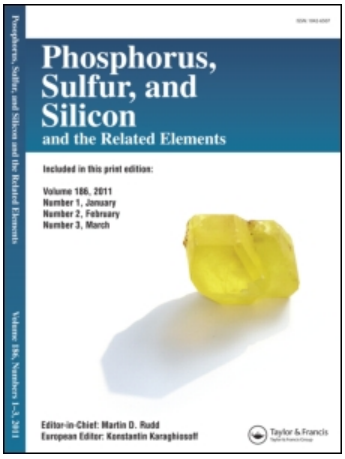

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Structure-Function Studies on Creatine Kinase

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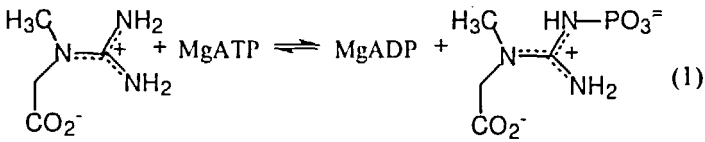
The accumulated evidence concerning the catalytic role of rabbit muscle creatine kinase will be discussed in light of the recently reported x-ray structures of an arginine kinase and a creatine kinase from chicken mitochondrion.

Keywords: Creatine kinase

INTRODUCTION

Creatine kinase (CK: EC 2.7.3.2) catalyzes the reversible transfer of a phosphoryl group (PO_3^-) from adenosine-5'-triphosphate (ATP) to creatine (Cr) to form phosphocreatine (PCr) and adenosine-5'-diphosphate (ADP), as shown in eq.1 [1].



(1)

The reaction has an absolute requirement for a divalent metal ion (e.g., Mg^{2+} or Mn^{2+}) which complexes with the nucleotide substrates but not directly with the enzyme [2]. CK plays a major role in the bioenergetics of muscle and brain activities of vertebrates [3]. In resting skeletal muscle, for example, high levels of phosphocreatine are

normally seen. CK serves a key role in bioenergetics allowing the rapid production of ATP, the principal energy source for muscle and brain action, upon demand, by catalyzing the back reaction shown in eq.1. CK levels in the blood are widely monitored clinically as an *ex post facto* indicator of the severity of myocardial infarctions [4]. CK also serves as a prototype of kinases which are a widespread class of enzymes that use MgATP as a phosphoryl group donor, including the protein kinases that are so important in signal transduction [5].

In a host of papers that have emerged from my laboratory and those of others, a mechanistic picture of rabbit muscle CK that contains considerable details about the core catalytic process has emerged [5,6]. For example, we know from electron paramagnetic resonance studies using creatine analogue complexes in the presence of Mn^{2+} and oxygen-17-labeled nucleotides at the α- and β-phosphorus positions that the required metal ion is bound to the pro S oxygen in each of these two positions [6]. Since MnATP is an α,β,√-tridentate complex, we know that of the 17 possible stereoisomers (among all of the possible mono-, di- and tridentate complexes), only one of these is bound to the enzyme (Figure 1) [6].

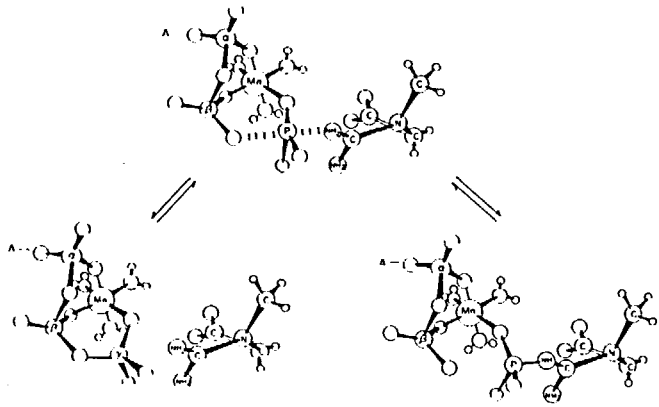


Figure 1. Schematic representation of the structures for the central complexes of CK and for a plausible transition state. (Reproduced with permission from Ref. 6. © 1985 American Chemical Society.)

We know that CK binds very tightly to the so-called transition-state analogue complex consisting of MgADP, NO_3^- and creatine [7]. From work done in the Knowles laboratory we know that the overall phosphoryl transfer process occurs with complete inversion of configuration indicating an S_N2 "in line" displacement reaction [8]. We know that the nitrogen on creatine that becomes phosphorylated is the one that is further removed from the negatively charged carboxymethyl substituent [9,10]. And we know from NMR studies using NOE measurements that the bound MgATP has an *anti* glycosidic torsional angle ($X=78\pm10°$) [11]. What has clearly been lacking until recently is information about precisely what amino acid side chain residues are intimately involved in the catalytic process.

This situation has been largely remedied beginning with sequence alignments which were used to discover which residues were conserved among various guanidino kinases (e.g., creatine kinases and arginine kinases). Most of the key catalytic residues that had been implicated earlier using pH [12], spectroscopic [11,13] and chemical modification [15,16] studies were then examined using site-directed mutagenesis (e.g., cysteine [17], histidine [18] and arginine [19]). And two relevant crystallographic studies have shed considerable light on the precise roles of these catalytic residues. Fritz-Wolf et al. [20] revealed for the first time the three-dimensional structure of a creatine kinase, in this case the enzyme isolated from the chicken mitochondrion. It is 65 percent identical in sequence to the CK from rabbit skeletal muscle and provided valuable insights into the structural elements that are at or near to the ATP binding site in particular. Finally, Zhou et al. [21] have recently presented a structure of the transition state analogue (MgADP, NO_3^-, arginine) complex of arginine kinase. This work permits important inferences to be made concerning the roles of similar amino acid residues in the closely related creatine kinase reaction.

Acknowledgments
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