A Shadow of the Past: Acetate Kinase, an Ancient Member of the ASKHA Phosphotransferase Superfamily

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Introduction

Acetate kinase, an enzyme widely distributed in the Bacteria and Archaea domains, catalyzes the phosphorylation of acetate. Acetyl phosphate is a precursor of important metabolic intermediates such as acetyl-CoA and is also a potential regulator of bacterial signal-transduction pathways. Many arguments over the enzyme mechanism and the existence of a phosphorylated intermediate have been shared in the past.

We have postulated the evolutionary relationship between acetate kinase and other phosphotransferases. The only enzymes that are identified as similar to the acetate kinases by sequence comparison programs are the propionate and butyrate kinases. Through secondary-structure prediction based upon comparative sequence analysis, it seems that acetate kinase would possess a topology in common with glycerol kinase, hexokinase, actin, and Hsc70. To help address all of these issues, we have solved the structure of acetate kinase from *Methanosarcina thermophila*.[1]

Methods and Materials

Expression and purification of homogeneous acetate kinase as well as conditions for the crystallization of the native enzyme bound to ATP have been described elsewhere. The multiple isomorphous replacement (MIR) data (native and derivatives) were collected at 277 K at a local data collection facility. Selenomethionyl crystals required incubation at 20°C for a minimum of three weeks, followed by transfer to 37°C. Selenomethionyl data were collected at the Advanced Photon Source BIOCARS beamline BM-14D at100 K. Data were processed (DENZO/ SCALEPACK) and the phases were calculated (CCP4). Patterson maps were used to locate heavy atoms in MIR. To locate selenium atoms in multiwavelength anomalous diffraction (MAD), a map was calculated with MIR phases and the anomalous difference from the peak MAD wavelength (0.9794 Å). Initial phases and positions of the MIR and MAD solutions were refined in the CCP4 program MLPHARE. Multicrystal density modification was performed using DMMULTI. The model was built using the program O. Refinement was carried out using XPLOR and CNS. Noncrystallographic symmetry information was used in phase and model refinement. The coordinates at 2.5 Å resolution (R_{work}=16.0%, R_{free}=19.2%) and structure factors are deposited in the Protein Data Bank (1G99). The resolution has been improved to 1.7 Å recently.

Results and Discussion

The structure of acetate kinase was solved through the combination of two crystallographic methods. Electron-density maps produced independently through either multiple isomorphous replacement (MIR) or multiwavelength anomalous diffraction (MAD) using selenomethionine-substituted protein were incomplete. Multicrystal density modification was performed using the initial phases from both techniques and treating each domain as an independent group. This treatment significantly improved the quality of the electron-density maps, allowing 96% of the structure to be built into the native-protein map during the first round of model building.

As we had predicted, acetate kinase is similar to hexokinase, glycerol kinase, actin, and hsp70. We have named this group the ASKHA (acetate and sugar kinases/Hsc70/actin) superfamily of phosphotransferases. Comparison of the structures suggests that acetate kinase is similar to the ancient ancestor of this family, for several reasons. First, by structural observation, acetate kinase contains almost all additions in the core structure present in other known family members. Second, by reason, acetate is a common compound and would have been present in the environment before life existed; it is trapped within a hydrophobic barrier by phosphorylation; and, in fact, it is a building block of a fatty acid in a membrane.

An interesting feature shared by this family, not previously described, is a peculiar structure used to bind ADP. A residue in the unusual epsilon conformation is followed by a glycine (glycine-331 in acetate kinase); the amide of that glycine binds the α-phosphate of ADP. It is striking that this formation has been conserved over the course of the evolution of this superfamily.

Our structural work has suggested that acetate kinase is one of the first primeval enzymes, as it activates the common molecule acetate to a charged form that may be used to construct a cell membrane. In the future, we hope to extend our studies to related enzymes, in order to understand the factors that have been important in evolution of this enzyme family, especially in terms of substrate specificity and catalytic mechanism.

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Reference

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