Structures of Exopolyphosphatase and Butyrate, Members of the ASKHA Phosphotransferase Superfamily

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Introduction

The ASKHA phosphotransferase superfamily, named for acetate and sugar kinases, Hsc70, and actin, is a group of enzymes that share similar structures and transfer phosphoryl groups but play very different roles in the cell. We have recently solved the structure of acetate kinase, an enzyme widely distributed in the bacteria and archaea domains, by using data collected at Argonne National Laboratory [1]. In order to further our understanding of the wide range of enzymes in this family, we have solved the structures of two additional members of the family, a butyrate kinase from *Thermotoga maritima* and exopolyphosphatase of *Escherichia coli*.

The sequence of butyrate kinase is about 25% identical to the sequence of acetate kinase. Each enzyme catalyzes the phosphorylation of a similar substrate, acetate or butyrate, different only in the length of the carbon chain. The comparison of the two structures should provide an understanding of the specificity of the enzymes for such similar substrates.

Exopolyphosphatase (Ppx), in contrast, is a very distant relative of acetate kinase. Unlike the other known members of the ASKHA family, Ppx cannot use ATP as a source of phosphate; instead, its substrate is polyphosphate. The importance of the levels of polyphosphate in all cells, especially in response to stress, has begun to be elucidated by A. Kornberg at Stanford University. In order to further understand the different roles and structures of related enzymes, we have solved the structure of Ppx.

Methods and Materials

T. maritima butyrate kinase was cloned into a pET vector that encodes a C-terminal His-tag and was purified in several steps after growth in *E. coli*. Crystals of butyrate kinase belong to space group I422 with unit cell parameters of a = b = 97.45 Å and c = 58.18 Å. Phases were obtained on selenomethionine-containing butyrate kinase by using multiwavelength anomalous diffraction (MAD) data collected at the APS Bio Consortium for Advanced Radiation Sources (BioCARS) beamline 14-BM-D to a resolution of ~3.0 Å at wavelengths of 0.9799, 0.9796, and 0.9574 Å. A native data set of Se-Met butyrate kinase was collected to a resolution of 2.9 Å at a wavelength of 1.1 Å with a completeness of ~94%. Another native data set was collected to a resolution of 2.8 Å at a wavelength of 0.9 Å.

E. coli Ppx was overexpressed and purified to homogeneity. Diffraction-quality crystals were grown; they have an average size of $0.8 \times 0.4 \times 0.1$ mm. These crystals belong to space group $P4_12_12$ with unit cell parameters of a = b = 89 Å and c = 351 Å. Phases were obtained by MAD by using a mercury derivative, with data collected at BioCARS beamline 14-BM-D. An experimental electron density map was calculated to 3.25 Å by using the programs Solve and Resolve. A native data set was collected to 2.47 Å at BioCARS beamline 14-ID-B.

Results and Discussion

The structures of butyrate kinase and acetate kinase are similar, as expected. The unexpected differences between the two structures are quite interesting. While acetate kinase is a dimer, butyrate kinase is an octomer (i.e., a tetramer of dimers). Surprisingly, residues from one dimer in the octomer reach into the active site of a neighboring dimer.

The experimental electron density shows that Ppx does possess the conserved core structure of the ASKHA superfamily and that the C-terminal domain is made up of seven helices and one three-stranded beta-sheet. The structure of Ppx has been solved to 2.47 Å and is currently being refined. This structure will be the first polyP-utilizing enzyme structure to be solved.

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.

Acknowledgments

We thank the staff at the APS for their help. Use of the APS was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38. Use of BioCARS sector 14 was supported by the National Institutes of Health (NIH), National Center for Research Resources. This work was supported by a National Science Foundation (NSF) predoctoral fellowship and NIH biophysics training grant to J. Alvarado, an NSF CAREER award to D. A. Sanders, an NIH award and a March of Dimes and David and Lucille Packard Foundation fellowship to M. S. Hasson, and NIH Cancer Center support at Purdue University. The diffraction and computing facilities shared by the Structural Biology Group at Purdue have

been developed and supported by grants from NIH, NSF, the Lucille P. Markey Foundation, Keck Foundation, and Office of the University Executive Vice President for Academic Affairs.

Reference

[1] K. A. Buss, D. R. Cooper, C. Ingram-Smith, J. G. Ferry, D. A. Sanders, and M. S. Hasson, J. Bact. **183**, 680-686 (2001).