Crystal structure of isocitrate dehydrogenase from *Bacillus subtilis*

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Introduction

Isocitrate dehydrogenase from *B. subtilis* (BsIDH) is a homodimeric Krebs cycle enzyme that catalyzes the conversion of isocitrate to α-ketoglutarate with concomitant reduction of $NADP^+$ to NADPH. The *E. coli* homologue (EcIDH) is regulated by reversible phosphorylation on a sequestered active site serine (S113) [1]. Phosphorylation essentially inactivates the enzyme by electrostatic repulsion and steric hindrance with the polyanionic substrate, isocitrate [1, 2]. Transfer of the phosphate moiety from ATP to the target serine is catalyzed by a large 136 kDa bifunctional homodimer, IDH kinase/phosphatase (IDH-K/P) [3].

Although the mechanism of inactivation in EcIDH has been delineated, very little is known about the precise protein-protein interface between EcIDH and IDH-K/P. We have addressed this issue by more closely examining the substrate specificity of IDH-K/P. Specifically, we assessed the ability of BsIDH to serve as an alternate substrate since it is 69% identical to its *E. coli* counterpart and 100% identical around the phosphorylation site. Unexpectedly, kinetic data revealed that BsIDH is actually an extremely poor substrate of IDH-K/P, with Michaelis constants approximately 60-fold higher for the kinase and 300-fold higher for the phosphatase. Therefore, solving the crystal structure of BsIDH and comparing it to that of the *E. coli* homologue became an immediate goal.

Methods and Materials

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Rod-shaped crystals of BsIDH (7.9 mg/ml) were grown via hanging drop vapor diffusion against a solution of 100 mM citrate (pH 4.9), 23% PEG4K, and 18% propylene glycol. The protein crystallized as a dimer in the asymmetric unit and a single crystal diffracted to 1.5 Å resolution. It belongs to the monoclinic space group $P2_1$ and has the following unit cell dimensions: a = 73.69 Å, b = 73.29 Å, c = 80.90 Å, and b = 109.48^O. Diffraction data were collected at the APS undulator beamline 19-ID of the Structural Biology Center (SBC-CAT) with an x-ray wavelength of 1.0332 Å. Intensities were integrated on site with DENZO and scaled with SCALEPACK [4].

The structure of BsIDH was solved by molecular replacement employing a monomer of EcIDH as the phase probe. A cross-rotational search followed by Patterson-correlation refinement and translational searches as implemented in X-PLOR [5] revealed two unambiguous peaks that were henceforth treated as the two monomers of BsIDH. Because of large discrepancies between BsIDH and the search model, particularly around a 13-amino-acid insert in BsIDH, extensive rebuilding in the program O [6] was required. This was followed by runs of simulated annealing, positional, and individual B-factor refinement against bulk-solvent corrected data, as implemented in X-PLOR [5]. Alternate cycles of model-building and reciprocal-space refinement, along with the later addition of approximately 600 solvent molecules brought the R-factor and R-free [7] down to their current values of 19.1% and 24.6%, respectively. The present model of BsIDH has 844/846 amino acids, 620 waters, five molecules of propylene glycol, and alternate conformations for 16 residues. The model exhibits good stereochemistry with 90.2% of the amino acids in the most favored region of the Ramachandran diagram [8].

Results and Discussion

Analysis of the BsIDH structure demonstrates that it is extremely similar to the EcIDH, as was predicted from the extensive sequence homology. Like EcIDH, it functions as a homodimer physiologically and consists of large, small, and clasp domains. The active site amino acids are virtually superimposable, with only minor perturbations in the positions of a few residues. The primary deviation between the two proteins is the marked secondary structure difference spanning a ~35 residue region in the small domain. In EcIDH, this area is composed of nonpolar residues in the form of two antiparallel β-strands; in BsIDH, on the other hand, this area is comprised of primarily polar residues adopting a α-helical configuration. Furthermore, the presence of a 13-amino-acid insert in BsIDH extends this region further across the active site cleft of the other monomer.

A second, more subtle difference between the two enzymes centers on a three-residue loop (98–100 in BsIDH; 107–109 in EcIDH) that is bent inward in BsIDH, narrowing the opening to the active site cleft from 19.1 Å to 14.6 Å. A third difference focuses on an apparent heterogeneity between the two monomers of BsIDH. The two monomers of EcIDH are related by a crystallographic twofold axis, and they are exactly the same. However, the two monomers of BsIDH are related by a noncrystallographic dyad axis and have an RMSD > 0.6 A.

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