MS06-2-2 Structural and functional characterization of altronate oxidoreductase from Escherichia coli #MS06-2-2

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Abstract
Gram-negative bacteria metabolize various hexuronic acids, which is essential for the colonization of the intestine (1,2). Furthermore, enzymes that metabolize hexuronic acids are of high interest for the industrial utilization of pectin-rich biomass from, for instance, sugar beet pulp (3). In the metabolization of the hexuronic acid D-galacturonate, the enzyme altronate oxidoreductase (AOR, also known as tagaturonate reductase, EC1.1.1.58) catalyzes the reduction of D-tagaturonate to D-altronate. We have solved the crystal structure of AOR from Escherichia coli to a resolution of 2.2 Å in the absence of its substrate. The structure was solved by single anomalous dispersion utilizing the anomalous signal of tungsten present in the telluro-polyoxotungstate cluster anion [TeW6O24]6− included in the crystallization experiment. The polypeptide chain of AOR, comprising 483 amino acid residues, folds into two distinct domains, which are separated by a solvent-accessible cleft. Comparison with known crystal structures of other sugar and sugar acid metabolizing enzymes confirm that AOR belongs to the family of polyol-specific long-chain dehydrogenases (4) and that the cleft is the binding site for the substrates D-tagaturonate and NADH. Using molecular docking of D-tagaturonate and NADH, we identified amino acid residues that likely determine substrate specificity. To verify these findings, we established an absorption-based activity assay and showed that mutation of these residues abolishes the enzymatic activity.

References
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