Crystallization, Preliminary X-ray Diffraction and Structure Analysis of *Thermotoga maritima* Mannitol Dehydrogenase

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43

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Introduction

Industrial polyols are sugar alcohols that are used in the pharmaceutical, chemical and food-ingredient industries. Mannitol is used as a low-calorie and low-cariogenic sweetener (particularly in diabetic foodstuffs), as a pharmaceutical formulating agent (e.g. as a diureticin in the manufacture of intravenous fluids and tablets, in dental hygiene products and as a low-reactivity drug filler; and as a specialty chemical in other types of industries. SpecChemOnline estimates the global market for mannitol to be about 22 million Euros [1]. Currently, 50 000 tons per year of mannitol are produced by the hydrogenation of 50% fructose/50% glucose syrup at high pressures and temperatures using a Raney nickel catalyst. The fructose/glucose syrup is converted to a 30% mannitol/70% sorbitol mixture, from which mannitol is purified by low-temperature crystallization. Developing new simplified biological processes for mannitol production could lower production costs, increase the chemical yield and lower the downstream processing costs. Biological synthesis routes are now being developed [2] to lower production costs.

Mannitol is produced enzymatically from fructose by mannitol dehydrogenase (MtDH) using NAD(P)H as the cofactor. Many MtDH-encoding genes have been cloned and sequenced and their enzymes have been purified and characterized. These enzymes belong to three different dehydrogenase/reductase families. Firstly, some fungal MtDHs belong to the short-chain dehydrogenase/reductase family. These fungal MtDHs do not require metals for catalysis. Secondly, the medium-chain dehydrogenase/reductase family contains some bacterial MtDHs. These enzymes are often zinc-dependent enzymes. Thirdly, other bacterial MtDHs belong to a polyol-specific long-chain dehydrogenase group. These long-chain MtDHs are often monomeric; they are characterized by the catalytic consensus motif Lys-Xaa(4/5)-Asn-Xaa(2)-His and do not require metals for catalysis. None of the medium-chain MtDHs have known X-ray structures. However, all biological routes being currently developed for mannitol production use medium-chain MtDHs [2]. Thus, it is important to understand the structure–function relationships in this group of enzymes, particularly if enzyme engineering is required for process optimization. For this reason, we cloned and characterized the medium-chain MtDH from the hyperthermophilic bacterium *Thermotoga maritima*. Here, we describe the crystallization conditions, characterization and initial structural analysis of TmMtDH crystals.

Experimental

10 mg ml^{-1} TmMtDH protein in 50 mMTris pH 8.5 buffer solution was screened at room temperature against the MPD and Classics screening kits from Nextal Biotechnologies using the microbatch-under-oil method. Drops were formed by mixing an equal volume of TmMtDH protein solution and MPD screening kit solution and were covered with 100% mineral oil. The protein crystallized in two Nextal Classics conditions, but the largest and most defined crystals were found in the MPD screening kit solution G4 (30% MPD, 0.1 M Na HEPES pH 7.5). The MPD grown crystals were approximately 0.1 x 0.05 x 0.05 mm3 in size and were rectangular in shape. Crystals were flash-cooled without additional cryoprotectant because of the high concentration of MPD in the precipitant solution.

Diffraction data have been collected from a crystal of *Thermotoga maritima* mannitol dehydrogenase at the 08ID-1 beamline of the Canadian Light Source. The crystal diffracted to 3.3 Å resolution and belongs to space group $P2_12_12_1$, with unit-cell parameters a = 83.43, b = 120.61, c = 145.76 Å^3 [3].

A molecular-replacement solution for the diffraction data set from the TmMtDH crystal was found using the 3D-PSSM search engine. The sequence of ketose reductase (sorbitol dehydrogenase) from silver leaf whitefly (PDB code 1e3j) is 24% identical to that of TmMtDH. This identity level is low, so the structure of NADP(+)-dependent *Bacillus stearothermophilus* alcohol dehydrogenase (PDB code 1rjw), which has 23% sequence identity with TmMtDH, may also be used to aid in calculating phases using the program Phaser.

References

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Acknowledgements

LTJD is a Tier 1 Canada Research Chair in Structural Biochemistry. This research was funded by a CIHR Operating Grant and an NSERC Discovery Grant to LTJD and by a USDA grant (No. 2005- 35504-16239) to CV.