Crystal structure of nicotinamide mononucleotide adenylyltransferase from *Methanobacterium thermoautotrophicum* with bound NAD^+

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Introduction

NAD^+ plays a central role in cellular processes; it functions as a coenzyme in reduction-oxidation reactions and as a substrate in DNA ligation and protein ADP ribosylation reactions. Nicotinamide mononucleotide adenylyltransferase (NMNATase) catalyzes the synthesis of NAD^+ from nicotinamide mononucleotide and adenosine triphosphate (ATP). It is the final step in both the *de novo* and salvage NAD^+ biosynthetic pathways and thus is an essential protein in all organisms. It is therefore important to understand the reaction mechanism of NMNATase.

Results and Discussion

Recombinant NMNATase from *M. thermoautotrophicum* was expressed in *E.coli* and purified using Ni^{+2} chromatography. The protein formed crystals in $LiSO_4$ at pH 7.5 (Figure 1). The crystals were approximately 500 x 200 x 200 microns^3, belonged to space group $P6_322$, and had cell constants $\alpha = \beta = 89.084$ Å and $\gamma = 109.926$ Å. There was a single molecule of NMNATase in the asymmetric unit with a Matthews coefficient of 3.23 Å^3/Dalton and a solvent content of 62 %.

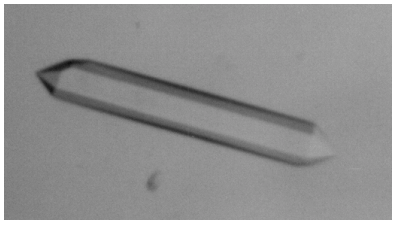

Figure 1: Crystal of NMNATase.

The structure of NMNATase was solved using the multiwavelength anomalous dispersion (MAD) method with selenium as the anomalous scatterer. Molecules of NAD^+ and SO_4^{-2} were bound in the active site of NMNATase and residues that are involved in product binding were identified (Figure 2). The structure has been refined to a final R_{work} of 21.3% and R_{free} of 24.4% to 1.9 Å resolution for data >2σ.

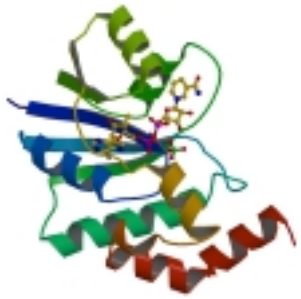

Figure 2: Ribbon structure of NMNATase with ball and stick NAD^+ and $SO4^{-2}$.

The structure of NMNATase adopts a Rossman fold and contains a conserved active site motif, HXGH. HXGH is also present in the active sites of glutaminyl tRNA synthetase and CTP:glycerol-3-phosphate cytidylyltransferase and has been implicated in binding the β- and γ-phosphates of ATP to stabilize the transition state. Ultimately, we would like to provide further information to delineate the biochemical mechanism via which NMNATase catalyzes the synthesis of NAD^+.

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