

Crystallization of an Archaeal Dihydroorotase

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Dihydroorotase (DHOase) catalyzes the reversible cyclization of N-carbamoyl-L-aspartate to L-dihydroorotate in the third step of the *de novo* biosynthesis of pyrimidines. It is a zinc-containing member of the amidohydrolase superfamily of metalloenzymes. The characterization of the enzyme from the hyperthermophilic and barophilic archaeon *Methanococcus jannaschii* has been carried out (Vitali et al, 2017). It is a monomer in solution with two Zn ions and exhibits hyperbolic kinetics. A homology model was also constructed using as template the *B. anthracis* protein. The recombinant enzyme was purified using ammonium sulfate precipitation, and cation exchange and hydrophobic interaction chromatographies. Initial crystallization conditions were obtained under oil by the High Throughput Crystallization Screening Center of the Hauptman Woodward Institute that samples 1536 different chemical conditions. The hits were modified for hanging drop and optimized using a wide range of precipitant and buffer pH. The crystals are trigonal P3₁2 or P3₂2 with cell constants $a = b = 111.3 \text{ \AA}$, $c = 101.2 \text{ \AA}$ and Matthews coefficient $V_m = 3.80 \text{ \AA}^3/\text{Da}$ for one molecule per asymmetric unit and $1.90 \text{ \AA}^3/\text{Da}$ for two molecules per asymmetric unit. The crystals diffract beyond 2.3 \AA resolution. The structural analysis of this protein will give insight into the variability within the dihydroorotase family of proteins and how its structure adapts to the high temperatures that are the normal environment of this organism.

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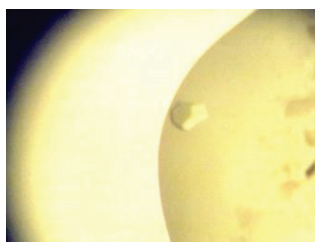


Fig. 1. Crystals of DHOase from *M. jannaschii*

References

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