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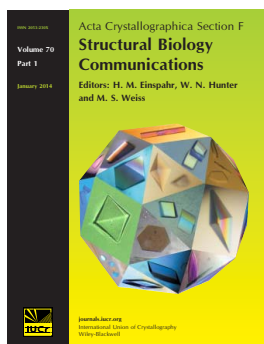
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Crystallization and preliminary crystallographic analysis of histamine dehydrogenase from *Natrinema gari* BCC 24369

Histamine dehydrogenase (HADH) catalyzes the oxidative deamination of histamine, resulting in the production of imidazole acetaldehyde and an ammonium ion. The enzyme isolated from the newly identified halophilic archaeon *Natrinema gari* BCC 24369 is significantly different from the previously described protein from *Nocardioides simplex*. This newly identified HADH comprises three subunits with molecular weights of 49.0, 24.7 and 23.9 kDa, respectively, and is optimally active under high-salt conditions (3.5–5 M NaCl). As a step in the exploration of the unique properties of the protein, the HADH heterotrimer was purified and crystallized. Crystals were obtained using the sitting-drop vapor-diffusion method from a solution composed of 0.2 M calcium chloride dihydrate, 0.1 M HEPES pH 7.5, 28% PEG 400. Diffraction data were collected at -173°C to a resolution limit of 2.4 Å on the Southeast Regional Collaborative Access Team (SER-CAT) beamline 22-ID at the Advanced Photon Source, Argonne National Laboratory. The crystals belonged to the monoclinic space group $C2$, with unit-cell parameters $a = 211.9$, $b = 58.6$, $c = 135.4$ Å, $\beta = 103.0^{\circ}$. The estimated Matthews coefficient is 3.21 Å³ Da⁻¹, corresponding to 61.7% solvent content.

1. Introduction

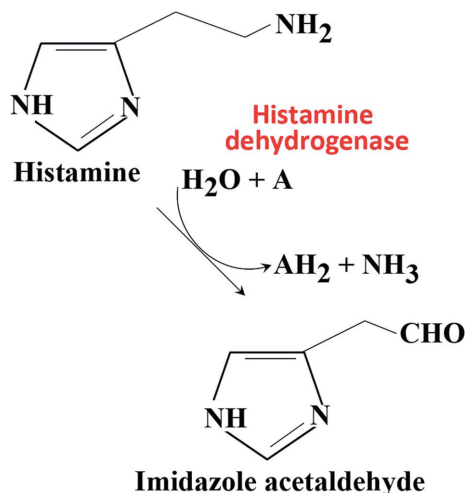
Histamine is an essential biogenic amine that is broadly present in prokaryotes and in tissues of animals and plants (Reed *et al.*, 2010), and was first identified as a mediator of biological functions in the early 1900s. Histamine exerts various effects on diverse physiological and pathological processes such as inflammation or gastric acid secretion and may also act as a neurotransmitter. Previous studies suggested that histamine is involved in other diseases such as allergic asthma (Broide *et al.*, 1991; Casale *et al.*, 1987; Jarjour *et al.*, 1991; Liu *et al.*, 1990; Wardlaw *et al.*, 1988; Wenzel *et al.*, 1988), atopic dermatitis (Johnson *et al.*, 1960; Juhlin, 1967), chronic urticaria (Greaves & Sondergaard, 1970; Kaplan *et al.*, 1978), multiple sclerosis (Tuomisto *et al.*, 1983) and psoriasis (Petersen *et al.*, 1998), as well as rheumatoid and psoriatic arthritis (Frewin *et al.*, 1986; Crisp, 1984).

Histamine dehydrogenase (HADH) catalyzes the oxidative deamination of histamine to produce imidazole acetaldehyde and an ammonium ion (Fig. 1; Bakke *et al.*, 2005; Fujieda *et al.*, 2005). Focusing on histamine oxidation, it is important to understand the physiological, biochemical and functional properties of HADH which are responsible for its oxidative deamination activity for future applications (Sato *et al.*, 2005; Reed *et al.*, 2010). To date, there have been only a few reports on the molecular and catalytic properties of HADH, including the enzymes from *Nocardioides simplex* (Limburg *et al.*, 2005), *N. simplex* IFO 12069 (Siddiqui *et al.*, 2000) and *Rhizobium* sp. 4-9 (Sato *et al.*, 2005). Both HADH from *N. simplex* IFO 12069 and *Rhizobium* sp. 4-9 were shown to have a molecular mass of about 150 kDa and consisted of two identical subunits (Bakke *et al.*, 2005; Siddiqui *et al.*, 2000).

The histamine-degrading activity of *Natrinema gari* BCC 24369, a halophilic archaeon first identified in anchovy fish sauce, is mediated through the presence of intracellular HADH (Tapingkae *et al.*, 2008,



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**Figure 1**

Schematic diagram showing the degradation of histamine by histamine dehydrogenase.

2010). This enzyme is a heterotrimer and its activity requires high levels of salt (unpublished data). Reed *et al.* (2010) reported that HADH from *N. simplex* is a homodimer consisting of 76 kDa subunits. The crystal structure of HADH from *N. simplex* was determined at 2.7 Å resolution, showing a high similarity to the structure of trimethylamine dehydrogenase (TMADH) from *Methylophilus methylphilus* (bacterium W3A1; Dietmann & Holm, 2001). Each identical subunit contains 6-S-cysteinyI-FMN and [4Fe-4S] as redox-active cofactors and also a single ADP molecule.

In this study preliminary to the future determination of its three-dimensional structure, we report the crystallization and preliminary X-ray characterization of HADH from *N. gari* BCC 24369, an enzyme which, unlike the protein from *N. simplex*, is a heterotrimer rather than a homodimer. A comparison of the structures of these enzymes with shared function but different subunit organization should extend our knowledge of the family of histamine dehydrogenases.

2. Materials and methods

2.1. Culture and growth condition for *N. gari* BCC 24369

N. gari BCC 24369 was grown on agar plates with a halophilic medium (pH 7.2). The inoculum was prepared by inoculating a loopful of cultures into 5 ml halophilic medium with an initial pH of 7.2 containing 5 g l⁻¹ yeast extract, 5 g l⁻¹ casamino acids, 1 g l⁻¹ sodium glutamate, 3 g l⁻¹ trisodium citrate, 20 g l⁻¹ MgSO₄·7H₂O, 2 g l⁻¹ KCl, 250 g l⁻¹ NaCl, 36 mg l⁻¹ FeCl₃ and 0.36 g l⁻¹ MnCl₂·4H₂O under sterile conditions, autoclaving at 121°C for 15 min and incubating at 37°C in a shaker incubator (Certomat BS-1, Sartorius, Göttingen, Germany) at 200 rev min⁻¹ for 7 d. Cells were cultivated by inoculating 5% (v/v) of seed cultures into 200 ml halophilic medium containing 500 p.p.m. of histamine (free-base) in a 500 ml Erlenmeyer flask, and incubating at 37°C in a shaker incubator at 200 rev min⁻¹ for 7 d.

2.2. Enzyme preparation

Cells were harvested by centrifugation of cultured broth at 10 000g at 4°C for 15 min. The pellet was washed twice with 4.5 M NaCl and suspended in 50 mM Tris-HCl pH 7.0, referred to as standard buffer (SB) containing 4.5 M NaCl at a ratio of 1:1 (weight of the cell paste/volume of Tris-HCl buffer) and sonicated for a total of 2 min by a

**Figure 2**

Crystals of histamine dehydrogenase from *N. gari* BCC 24369.

Vibra Cell VCX60 (Sonics and Materials Inc., USA). The supernatant was collected by centrifugation at 15 000g for 30 min (4°C) and was referred to as crude enzyme extract.

2.3. Purification of histamine dehydrogenase

The crude enzyme extract was heated rapidly to 60°C; it was maintained at this temperature for exactly 15 min and then immediately cooled in iced water. The resulting precipitate was discarded after centrifugation at 15 000g at 4°C for 30 min. The supernatant was collected and dialyzed against SB for 12 h at 4°C. In the current study, HADH was purified using a multi-step procedure that included anion-exchange chromatography on a HiTrap Q XL column (0.7 × 2.5 cm, GE Healthcare), Vivaspin ultrafiltration with 50 000 MWCO and gel-filtration chromatography on a Superose 12 10/300 GL column (1.0 × 30 cm, GE Healthcare). All of the purification steps were performed in a cold chamber using an ÄKTAexplorer purification system (GE Healthcare). The purity of the purified HADH was confirmed by native gel electrophoresis. Tricine SDS-PAGE was performed by the method of Schagger (2006) with slight modification. Purified histamine dehydrogenase (HADH, 15 µg) was mixed in a 1:1 (v:v) ratio with the SDS-PAGE sample buffer (0.125 M Tris-HCl pH 6.8 containing 4% SDS, 20% glycerol, 2% β-mercaptoethanol, 0.002% bromophenol blue) and boiled for 15 min. Electrophoresis was carried out in an ATTO AE-6530 Dual mini-slab system. Tricine as the trailing ion in the cathode buffer was used at a concentration of 0.1 M. The separation gel had a total concentration (*T*) of 12.5% with a cross-linking (*C*) of 3% and the stacking gel had 4% *T* and 3% *C*. Separation was carried out at a constant current of about 15 mA per gel for 6 h. After electrophoresis, the gels were stained with 0.125% Coomassie Brilliant Blue R-250 in 45% methanol and 10% acetic acid and destained with 30% methanol and 7% acetic acid.

2.4. Crystallization

The protein was concentrated to a concentration of about 30 mg ml⁻¹ and immediately used for crystallization. Initial crystallization trials were performed using a Phoenix robot (Art Robbins Instruments, Mountain View, California, USA) with several crystallization screens. Several hits were observed in Crystal Screen HT (Hampton Research, Aliso Viejo, California, USA) after a few days. Crystals from condition B11 (0.2 M magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 30% polyethylene glycol 400) had anomalous shapes and were not suitable for data collection. Better crystals were

Table 1

Statistics of X-ray data collection.

Values in parentheses are for the highest resolution shell.

Space group	<i>C2</i>
Unit-cell parameters (Å, °)	$a = 211.9, b = 58.6, c = 135.4,$ $\beta = 103.0$
Resolution (Å)	20–2.4 (2.6–2.4)
No. of measurements	121230
No. of unique reflections	58422
$\langle I/\sigma(I) \rangle$	5.0 (1.35)
Completeness (%)	91.3 (85.6)
Multiplicity	2.08 (1.71)
$CC_{1/2}$	99.2 (16.8)
$R_{\text{merge}}^{\dagger}$ (%)	10.4 (44.4)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity of the i th measurement of reflection hkl and $\langle I(hkl) \rangle$ is the average intensity of that reflection obtained from multiple observations.

obtained from condition B2 (0.2 M calcium chloride dihydrate, 0.1 M HEPES sodium pH 7.5, 28% PEG 400). These crystals were red, thin plates (Fig. 2); they grew to a final size of around $0.1 \times 0.05 \times 0.005$ mm in about two weeks at 20°C.

2.5. X-ray data collection and processing

Diffraction data were collected on the Southeast Regional Collaborative Access Team (SER-CAT) beamline 22-ID at the Advanced Photon Source, Argonne National Laboratory. Single crystals were transferred into a cryoprotectant solution (mother liquor with extra 20% glycerol) for approximately 2 min and were then flash-cooled at -173°C in a stream of liquid nitrogen. The crystals of HADH diffracted X-rays to a resolution of 2.4 Å. Diffraction data were indexed, integrated and scaled with *XDS* (Kabsch, 2010). The crystal used for data collection belonged to space group *C2*, with unit-cell

parameters $a = 211.9, b = 58.6, c = 135.4$ Å, $\beta = 103.0^\circ$. Based on its volume and the molecular mass of histamine dehydrogenase, the asymmetric unit is most likely to contain one heterotrimeric molecule of the enzyme. The estimated Matthews coefficient is $3.2 \text{ \AA}^3 \text{ Da}^{-1}$, corresponding to 61.7% solvent content (Matthews, 1968). Data-processing statistics for the best crystal are shown in Table 1.

3. Results and discussion

We isolated and purified histamine dehydrogenase from the newly identified, extremely halophilic archaeon *N. gari* BCC 24369, which was initially identified in Thai fish sauce. The purity of the purified histamine dehydrogenase was confirmed by its migration as a single band on native gel electrophoresis. SDS-PAGE indicated the presence of three bands with molecular weights of 49.0, 24.7 and 23.9 kDa (Fig. 3). The molecular weight of the purified enzyme was evaluated to be around 97.6 kDa, a value that differs from those found for other organisms. Unlike the HADHs from *N. simplex* and *Rhizobium* sp. 4-9, which are homodimers, HADH from *N. gari* BCC 24369 is a heterotrimer with a smaller molecular mass that is highly soluble and optimally active at pH 6.5–8.5 and 40–60°C in the presence of 3.5–5.0 M NaCl (unpublished data). The amino-acid sequence of HADH still remains to be determined and the necessary experiments are under way. In order to characterize the three-dimensional structure of this novel HADH, the enzyme was crystallized in a step preliminary to its future structure determination. This protein was easy to crystallize; however, the crystals obtained under most conditions were not well shaped and exhibited very weak X-ray diffraction. The crystals used for data collection were thin plates and could diffract X-rays to a resolution limit of 2.4 Å (Fig. 4), but they exhibited very high mosaicity, which initially prevented indexing and integration. Different strategies for reducing the mosaicity included optimization of cryoprotectant and annealing and were ultimately successful in improving the diffraction to the point that useful data could be obtained (Table 1). Attempts to solve the

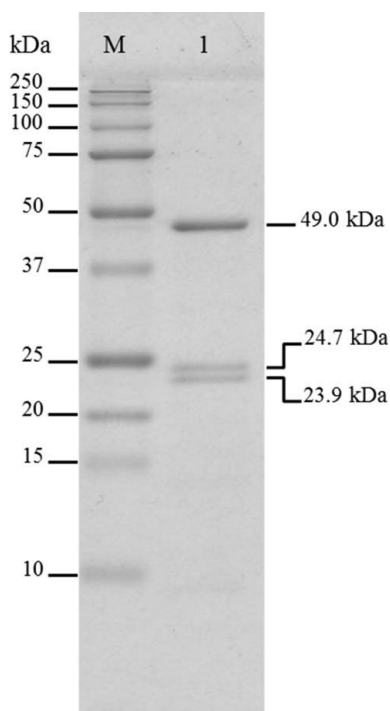


Figure 3

Tricine SDS-PAGE pattern of the purified HADH from *N. gari* BCC 24369 (lane 1). Lane *M*, molecular-weight standard (labelled in kDa).

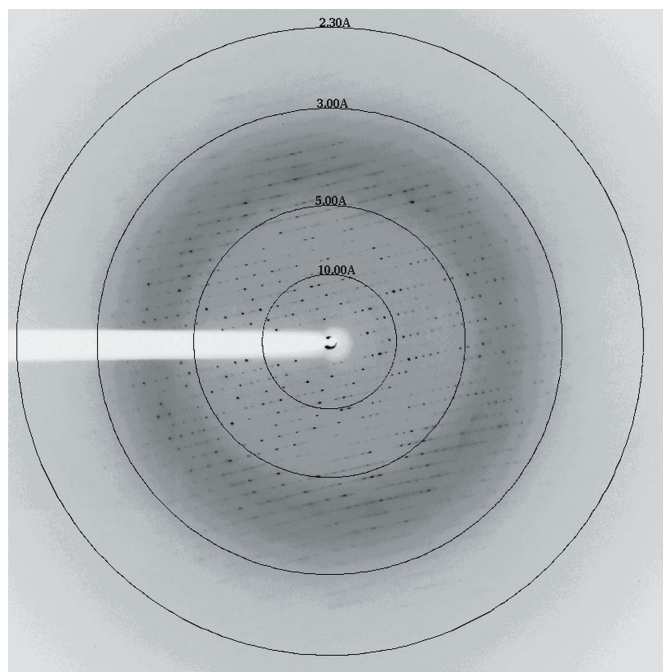


Figure 4

Diffraction pattern of HADH from *N. gari* BCC 24369 collected on the SER-CAT beamline 22-ID at the APS, Argonne National Laboratory.

structure by molecular replacement were hampered by the lack of the amino-acid sequence of HADH and, as expected, using the structure of HADH from *N. simplex* (PDB entry 3k30; Reed *et al.*, 2010) as a starting model did not succeed. We are currently trying to collect MAD data utilizing the anomalous signal of zinc (which is bound to the protein in a 1:4 molar ratio), as well as searching for standard heavy-atom derivatives. Several data sets have been collected and structure-determination trials are in progress.

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