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The Catalytically Active Form of Histidinol Dehydrogenase from *Salmonella typhimurium*

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The active-enzyme-sedimentation procedure was used to identify the catalytically competent form of histidinol dehydrogenase (EC 1.1.1.23) isolated from *Salmonella typhimurium*. At pH 9.4 the active species has a sedimentation coefficient $s_{20,w}$ of 5.4 S, indicating that the dimer with a mol.wt. of approx. 83000 is the enzymically active form.

Histidinol dehydrogenase of *Salmonella typhimurium* has a mol.wt. of approx. 87000, as determined by sedimentation equilibrium at pH 6.5 (Loper, 1968). After dissociation with 6M-guanidine hydrochloride the molecular weight is decreased to 38000–42000 (Yourno, 1968; Loper, 1968). Polyacrylamide-gel electrophoresis in the presence of SDS reveals a single protein band (polypeptide chain) with a mol.wt. of 45000–53000 (Yourno *et al.*, 1970; Kohno & Yourno, 1971; Rechler & Bruni, 1971). The amino acid composition (Loper, 1968; Yourno, 1968) and chromatographic and genetic evidence (Loper, 1968; Greeb *et al.*, 1971) support the view that histidinol dehydrogenase is a dimer composed of identical polypeptide chains. However, Bitar *et al.* (1977) reported a mol.wt. of 47000 for histidinol dehydrogenase, which disagrees with the values of approx. 87000 reported previously.

The present study was undertaken to establish the catalytically competent form of histidinol dehydrogenase by using the active-enzyme-sedimentation procedure (Cohen & Mire, 1971).

Materials and Methods

Chemicals

The inorganic chemicals used were of analytical reagent grade. Glycine, succinic acid, Tris, bovine serum albumin and ribonuclease were obtained from Merck, Darmstadt, Germany, and DEAE-Sepharose CL-6B and Sephacryl S-200 (superfine) were from Pharmacia, Uppsala, Sweden. Glyceraldehyde 3-phosphate dehydrogenase and catalase were purchased from Boehringer, Mannheim, Germany, and ovalbumin, pepsin, myoglobin, L-histidinol and NAD^+ were from Sigma, München, Germany. α-Chymotrypsinogen was obtained from Calbiochem, Lahn, Germany, and L-leucine aminopeptidase, γ-globulins (bovine) and lysozyme were purchased from Serva, Heidelberg, Germany.

Glucose dehydrogenase from *Bacillus megaterium* (Pauly & Pfleiderer, 1975) was a gift from Dr. H. E. Pauly, Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität Stuttgart, Stuttgart, Germany; the ribosomes from *Micrococcus radiodurans* were a gift from Mrs. A. Müller of this institute.

Abbreviation used: SDS, sodium dodecyl sulphate.

Enzyme preparation

Histidinol dehydrogenase was isolated from *S. typhimurium* strain LT-2 hisO 1242 (Roth *et al.*, 1966) obtained through the courtesy of Dr. Roth, Johns Hopkins University, Baltimore, MD, U.S.A. The organism was grown in the minimal medium of Vogel & Bonner (1956) supplemented with 0.4% glucose and 0.2% nutrient broth. Purification of the enzyme followed closely the procedure described by Yourno & Ino (1968). Instead of DEAE-Sephadex A-50, however, DEAE-Sepharose CL-6B was used, and an additional chromatography on Sephacryl S-200 was performed. The purified enzyme had a specific activity of 12 units/mg and crystallized readily. The highest specific activity reported in the literature is 12.5 units/mg after several recrystallizations (Bitar *et al.*, 1977).

Enzyme assay

The enzyme activity was measured spectrophotometrically by following the formation of NADH at 340nm. One unit of enzyme is defined as that reducing 2μmol of NAD^+/min at 25°C. The substrate solution contained 0.05M-glycine/NaOH buffer, pH 9.4, 450μM-$MnCl_2$, 10mM-NAD^+ and 1.5mM-L-histidinol.

Centrifugation procedures

Sedimentation experiments were performed at

20°C in a Beckman–Spinco model E analytical ultracentrifuge equipped with a photoelectric scanner.

Active-enzyme-sedimentation experiments were performed as described by Cohen & Mire (1971) with a 12mm double-sector charcoal-filled band-forming type I Epon centrepiece. Enzyme solution (10μl corresponding to 40–50ng of protein) was layered on to 0.45ml of substrate solution. NADH production was recorded at 340nm, and the results were treated by the approximate method described by Cohen & Mire (1971).

Boundary-sedimentation and sedimentation-equilibrium studies by the conventional method were performed with 12mm double-sector charcoal-filled Epon centrepieces. Sedimentation equilibrium by the meniscus-depletion method was performed in an equilibrium six-channel charcoal-filled Epon centrepiece. Protein distribution was recorded at 280nm.

Evaluation of results

All molecular-weight calculations used a value for partial specific volume ($\bar{v}$) of 0.737 (Loper, 1968). Densities and viscosities are taken from data in the International Critical Tables.

Gel chromatography

The molecular weight of histidinol dehydrogenase was estimated by gel chromatography on Sephacryl S-200 (superfine) as described by Andrews (1964).

Polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis in the presence of SDS was performed by the method of Weber & Osborn (1969).

Results

Active-enzyme sedimentation

At very low enzyme concentrations and in the presence of its substrates (conditions that correspond to those used in steady-state kinetic experiments), histidinol dehydrogenase from *S. typhimurium* has a sedimentation coefficient $s_{20,w}$ of 5.4±0.1 S (mean± S.E.M., $n = 5$). The experiment is illustrated in Fig. 1. Mn^{2+}, which is known to stimulate the activity of histidinol dehydrogenase (Loper & Adams, 1965),

Table 1. *Sedimentation coefficient of histidinol dehydrogenase*

Results are means±S.E.M. for four to five determinations.

Conditions	$s_{20,w}$ (S)
Active-enzyme sedimentation	5.4 ±0.1
0.05M-Glycine/NaOH, pH9.4	5.4 ±0.1
0.02M-Tris/succinate, pH6.2,+0.75% NaCl	5.4 ±0.1
0.1M-Sodium phosphate, pH6.5	4.85±0.15

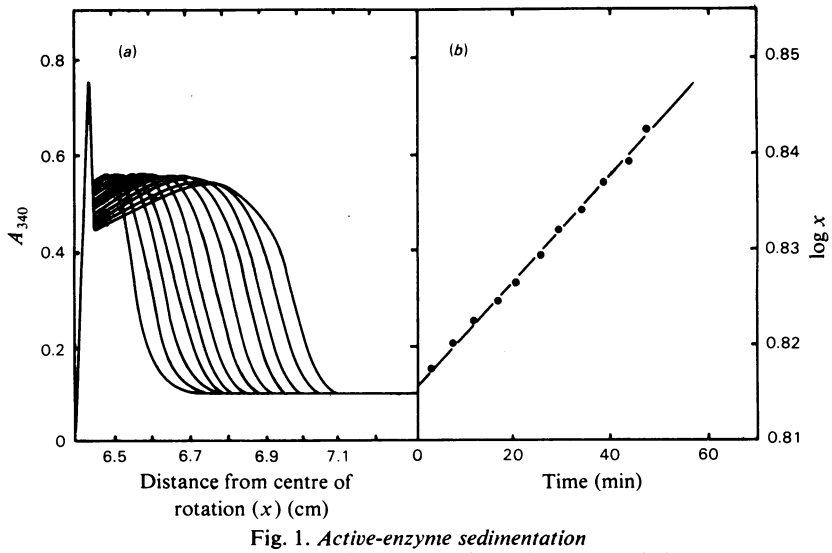

Fig. 1. *Active-enzyme sedimentation*

Sedimentation of histidinol dehydrogenase was performed at 20°C and 60000 rev./min. Enzyme (0.05μg) was layered on to the substrate solution. (*a*) The formation of NADH recorded at 340nm. A scan was performed every 4.5min. (*b*) Position of the enzyme band in the ultracentrifuge cell as a function of time, calculated by the approximate method of Cohen & Mire (1971).

Table 2. *Molecular weight of histidinol dehydrogenase*
Results are means ± S.E.M. for four to five determinations.

Conditions	Conventional method	Meniscus-depletion method
0.05 M-Glycine/NaOH, pH 9.4	—	80000 ± 6000
0.20 M-Tris/succinate, pH 6.2, + 0.75% NaCl	84500 ± 4000	83300 ± 3000
0.1 M-Sodium phosphate, pH 6.5	86000 ± 3000	83400 ± 4000
Gel chromatography	82500 ± 3000	
Polyacrylamide-gel electrophoresis + SDS	43000 ± 3000	

has no effect. In both the presence and absence of 450 μM-Mn^{2+} histidinol dehydrogenase sediments at $s_{20,w} = 5.4$S.

Velocity-sedimentation experiments

At concentrations of 1–1.5 mg of protein/ml, histidinol dehydrogenase sediments with a sharp single boundary and has a sedimentation coefficient $s_{20,w}$ of 5.4 ± 0.1 S. This is also true for different pH values, as shown in Table 1. The lower value of $s_{20,w}$ (4.85 S) in 0.1 M-sodium phosphate buffer, pH 6.5, is not due to dissociation of the enzyme, as is apparent from the molecular-weight determinations under the same conditions shown in Table 2.

Sedimentation-equilibrium measurements

The conventional and the meniscus-depletion sedimentation-equilibrium methods yield a mol.wt. of approx. 83000 for histidinol dehydrogenase. Table 2 shows the molecular weight of the enzyme determined under different conditions. At pH 9.4 it is slowly inactivated, and thus the mol.wt. of 80000 found under these conditions is the least reliable.

Gel chromatography

To confirm the molecular weight found by the sedimentation-equilibrium methods, histidinol dehydrogenase was also studied by gel chromatography. Its molecular weight is estimated to be 82000, which is in good agreement with the ultracentrifugation data (Table 2).

Polyacrylamide-gel-electrophoresis in the presence of SDS

The subunit molecular weight of histidinol dehydrogenase, estimated by this method, is 43000.

Discussion

The molecular weight of histidinol dehydrogenase from *S. typhimurium* has been reported to be 87000 (Loper, 1968). However, Bitar *et al.* (1977) claimed that it is 47000. In the present study we find, by gel chromatography, a mol.wt. of 82000 and, by sedimentation equilibrium, a mol.wt. of 83000. The enzyme has a sedimentation coefficient $s_{20,w}$ of 5.4S. These data are in substantial agreement with the mol. wt. of 87000 reported by Loper (1968). In addition, by the technique of active-enzyme sedimentation, we have demonstrated for the first time that the enzymically active form of histidinol dehydrogenase in the presence of its substrates and at the low concentrations used in steady-state kinetic experiments has a sedimentation coefficient of 5.4S. This corresponds to a mol.wt. of 83000. Recently, we repeated the active-enzyme-sedimentation experiments with histidinol dehydrogenase that had been purified by a modified procedure, in which the heat-denaturation step was omitted (H. Görisch, unpublished results). This enzyme preparation also yields a sedimentation coefficient $s_{20,w}$ of 5.4S. By polyacrylamide-gel electrophoresis in the presence of SDS, the molecular weight of the subunit was found to be approx. 43000. This finding is in fair agreement with earlier reports (Loper, 1968; Yourno, 1968; Yourno *et al.*, 1970; Kohno & Yourno, 1971; Rechler & Bruni, 1971). Thus the catalytically active species of histidinol dehydrogenase of *S. typhimurium* is a dimer with a mol.wt. of approx. 83000.

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