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Homodimeric and expanded behaviour of trimethylamine dehydrogenase in solution at different temperatures

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Abstract. Earlier studies using x-ray crystallography have shown that trimethylamine dehydrogenase (TMADH) from methylotropic bacteria exists as homodimers in the crystalline state. In this present hydrodynamic study we show that this is true also in dilute solution conditions and investigate the degree of swelling or relaxation of the protein in solution. Analytical ultracentrifugation was used to determine the molar mass and to investigate whether the homodimeric nature of this molecule in crystal form – as visualized by x-ray crystallography – is reproduced in dilute solution at temperatures between 4 and 40 °C. The globular solution structure determined at 4 and 40 °C is in good agreement with crystallographic data although trimethylamine dehydrogenase was found to be either more asymmetric in solution – or highly hydrated –, a phenomenon found to increase with temperature. In agreement with the crystallographic structure, the enzyme sediments as a homodimer with a molar mass of (163,000±5,000) g/mol. The concentration dependence of the sedimentation coefficient in the range of 0–1 mg/ml, indicates that no association or dissociation occurs. These findings are additionally supported by sedimentation equilibrium data in the concentration range of 0 to 1.8 mg/ml. Finally, from the sedimentation coefficient distribution at various temperatures, it was concluded that the enzyme is conformationally flexible and assumes an even more expanded structure at higher temperatures which is in good agreement with the hydrodynamic calculations performed.

Key words: Trimethylamine dehydrogenase – Analytical ultracentrifugation – Hydrodynamics – Homodimers

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Introduction

Trimethylamine dehydrogenase (EC 1.5.99.7) is an iron-sulphur flavoprotein and a member of a growing family of enzyme molecules which contain flavin-binding β/α barrel domains (Scrutton 1994; Raine et al. 1994). The enzyme catalyses the oxidative demethylation of trimethylamine according to the equation

$$(CH_3)_3N + H_2O \rightarrow (CH_3)_2\,NH + HCHO + 2H^+ + 2e^-$$

The enzyme is responsible for the ability of some methylotrophic bacteria to subsist on trimethylamine as the sole source of carbon. The crystal structure of trimethylamine dehydrogenase from *Methylophilus methylotrophus* (bacterium W_3A_1) has been solved at 2.4 Å resolution (Lim et al. 1986). The enzyme from crystallography appears as a homodimer and comprises three domains, a larger amino terminal eight-fold β/α barrel domain (about 380 residues) and two smaller domains at the C-terminus of the enzyme. The covalently attached flavin, the iron-sulphur centre and the substrate binding residues are located in the β/α barrel domain. The other two domains contain 5-stranded parallel β-sheets flanked by helices and other structural elements, and are similar in fold to the dinucleotide-binding domains of glutathione reductase (Scrutton 1994). ADP is bound to this part of the enzyme and occupies a position equivalent to the ADP moiety of FAD in the FAD-binding domain of glutathione reductase. This observation has led to the proposal that the C-terminal domains of trimethylamine dehydrogenase are the vestigal remains of dinucleotide-binding domains present in an ancestral protein which were modified by a process of divergent evolution (Scrutton 1994; Lim et al. 1988).

The *tmd* gene encoding trimethylamine dehydrogenase has been cloned (Boyd et al. 1992) which, in turn, has allowed the enzyme to be expressed in the heterologous host *Escherichia coli* (Scrutton et al. 1994). The apoenzyme subunit molar mass calculated from the inferred amino acid sequence is 81,623 g/mol (including the N-terminal methionine which is absent when the protein is expressed in *Methylophilus methylotrophus*). In this study we show

that the dilute solution data are consistent with the crystallographic data for TMADH in that the enzyme is a homodimer of approximate molar mass 163,000 g/mol but relaxes to a considerably more expanded form in dilute solution through molecular hydration and an increase in asymmetry. Detailed investigations of sedimentation velocity and equilibrium data at increased temperatures suggested that these effects increased with temperature although TMADH remained as a homodimer.

Experimental

Trimethylamine dehydrogenase was purified from *Methylophilus methylotrophus* (bacterium W_3A_1) as previously described by Steenkamp and Mallinson (1976) except that the last gel filtration step was replaced by a hydrophobic interaction chromatographic separation using phenyl sepharose (Scrutton et al. 1994). For analytical ultracentrifugation experiments, TMADH was exhaustively dialysed against potassium phosphate-chloride buffer, pH 7.5 (I=0.1). Enzyme concentrations were determined from the 443 nm absorbance of oxidised enzyme using an extinction coefficient of 27.3 mM^{-1} cm^{-1} in potassium phosphate-chloride buffer, pH 7.5 (I=0.1) (Kasprzak et al. 1983).

The partial specific volume of the polymer was calculated from the amino acid composition of TMADH using a formula given by Perkins (1986) giving $\bar{v}=0.731$ ml/g. The molar mass of TMADH was calculated to be 163,000 g/mol, consistent with the homodimeric molar mass calculated from the amino acid sequence of TMADH by Boyd et al. (1992).

To investigate the concentration dependence of the sedimentation coefficient at 4 °C, TMADH solutions were prepared using phosphate-chloride buffer, pH 7.5 (I=0.1) as solvent containing TMADH at concentrations ranging from 0.10–1.00 mg/ml. The temperature dependence of the sedimentation coefficient was studied using a 0.5 mg/ml solution at 4, 15, 20 and 30 °C. All sedimentation equilibrium experiments were performed in phosphate-chloride buffer at 4 & 40 °C, with the TMADH loading concentration ranging from 0.05–1.8 mg/ml.

The Beckman Optima XL-A (Beckman, Spinco division) analytical ultracentrifuge equipped with modern scanning absorption optics was used in all investigations. The sedimentation velocity experiments were carried out at various speeds between 25,000 rev/min and 50,000 rev/min, depending on the temperature of the experiment, whereas the sedimentation equilibrium experiments were performed at 10,000, 13,000 and 20,000 rev/min. The sedimentation coefficients as well as the sedimentation equilibrium data were obtained using the absorption optics of the Optima XL-A, scanning at 240, 280 and 443 nm. For the evaluation of the sedimentation equilibrium data, the "MSTARA" program was used which is described elsewhere (Harding et al. 1992). For the sedimentation velocity experiments, KELF double sector centerpieces and for the sedimentation equilibrium experiments 6-channel KELF "Yphantis cells" (Yphantis 1964; Teller 1973) have been used.

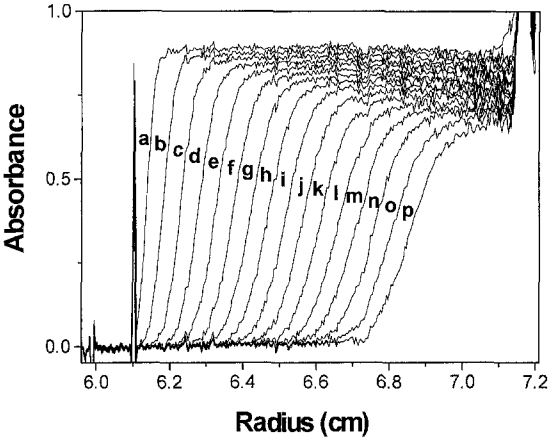

Fig. 1. Spectrophotometric sedimentation velocity patterns for TMADH in phosphate-chloride buffer at 4 °C, 45,000 rev./min and 4 °C in a Beckman Optima XL-A analytical ultracentrifuge. Experimental scans (A_{280}) at *a*)=8, *b*)=18, *c*)=28, *d*)=38, *e*)=48, *f*)=58, *g*)=68, *h*) =78, *i*)=88, *j*)=98, *k*)=108, *l*)=118, *m*)=128, *n*)=138, *o*)=148, *p*)=158 min of ultracentrifugation

Results

Sedimentation velocity

At 4 °C, the sedimenting TMADH boundary is quite well defined as seen in Fig. 1. The well defined boundary suggests a narrow molar mass distribution of the sample. Furthermore, it can be seen that there is no evidence for heterogeneity in the TMADH preparation as only a single sedimenting species was observed. All sedimentation coefficients wer evaluated at least three times to minimize the errors resulting from the graphical evaluation. The derived sedimentation coefficients in the buffer $s_{T,b}$ have been corrected to that at 20 °C in water $s_{20,w}$ using the standard formula (Tanford 1961):

$$s_{20,w} = \left[\frac{(1-\bar{v}\rho)_{20,w}}{(1-\bar{v}\rho)_{T,b}}\right]\left(\frac{\eta_{T,b}}{\eta_{20,w}}\right)s_{T,b} \qquad (1)$$

with $\bar{v}$=partial specific volume of the polymer, ρ=density, η=viscosity and s=sedimentation coefficient with the indices w=water, b=buffer and T=temperature.

The concentration dependence of $s_{20,w}$

The first point of interest in this study was to determine the concentration dependence of the sedimentation coefficient $s_{20,w}$ according to $s_{20,w}=s^0_{20,w}(1-k_sc)$ which provides information about a possible concentration dependent association/aggregation of the molecules. In this equation $s^0_{20,w}$ is the corrected sedimentation coefficient at 20 °C at infinite dilution, c the concentration and k_s the concentration dependent regression coefficient. The concentration dependence of $s_{20,w}$ at 4 °C is shown in Fig. 2. After the correction of the solution concentrations for radial dilution, the following values are obtained:

$s^0_{20,w}=(8.49\pm0.10)$ S $k_s=(61.37\pm24.77)$ ml · g^{-1}

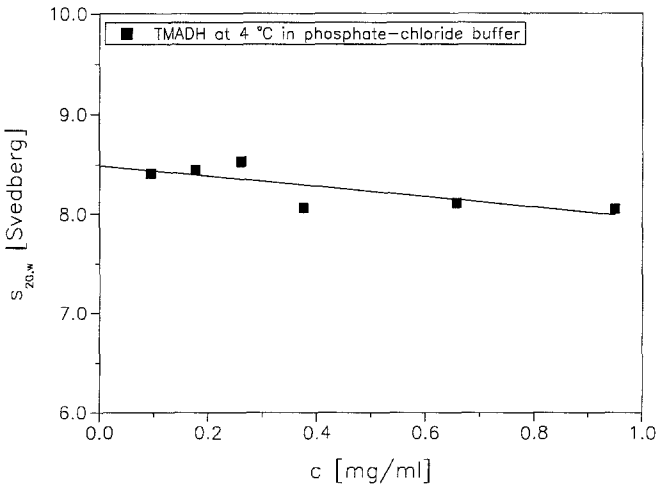

Fig. 2. Concentration dependence of $s_{20,w}$ for TMADH in phosphate-chloride buffer at 4 °C

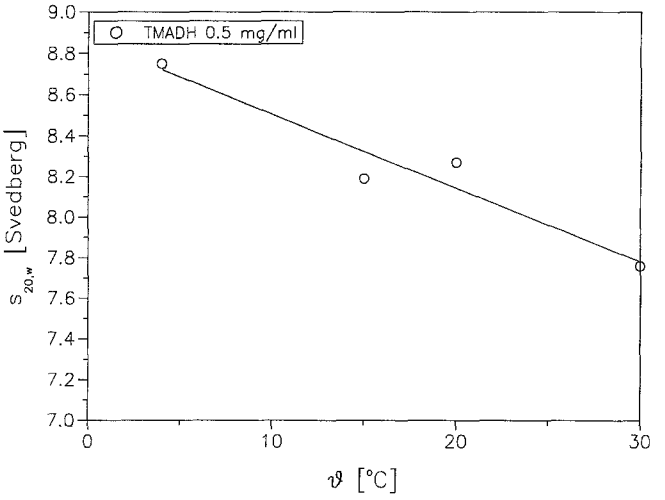

Fig. 3. Temperature dependence of the corrected sedimentation coefficient $s_{20,w}$ for TMADH in a 0.5 mg/ml solution in phosphate-chloride buffer (pH 6.8, I = 0.10). The line is included to indicate only the trend in the data

The positive k_s value indicates the absence of either association or dissociation behaviour in the concentration range investigated. The above given values for $s^0_{20,w}$ and k_s can also be used to derive more information about a possible association of the molecules (Harding and Rowe 1982). These simulation calculations clearly confirmed that no interaction between the TMADH molecules occurs in phosphate-chloride buffer at 4 °C. The TMADH in solution is therefore homodimeric at all concentrations studied in the sedimentation velocity experiments and this is in agreement with studies on the enzyme in the crystallized state (Lim et al. 1986). To obtain information on the question of a possible interaction between the TMADH molecules at different temperatures, the temperature dependence of the sedimentation coefficient was investigated for TMADH in a 0.5 mg/ml solution. Furthermore, this investigation might also reveal any conformational changes of TMADH at higher temperatures. The experiments were carried out in the temperature interval 4 – 30 °C and the results are presented in Fig. 3. The data clearly indicate that at elevated temperatures TMADH sediments more slowly which is suggestive of some conformational flexibility in the enzyme dimer.

Sedimentation equilibrium

The apparent weight average molar masses $M_{w,app.}$ have been determined for different concentrations at 10,000, 13,000 and 20,000 rev/min at 4 and 40 °C and 3 different wavelengths (Fig. 6). The $M_{w,app.}$ obtained for the different wavelengths agreed well. The results for 20,000 rev/min generally had larger error ranges of up to ±15,000 g/mol due to the relatively small amount of data points suitable for the evaluation resulting from the steep concentration gradient in the high speed equilibrium experiment performed at 20,000 rev/min. Nevertheless, the $M_{w,app.}$ agree for different rotational speeds. The equilibrium results clearly indicate the absence of any associative or dissociative behaviour in the temperature range

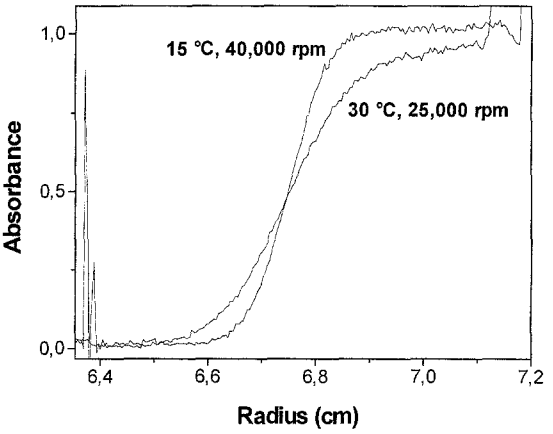

Fig. 4. Sedimenting boundaries of a 0.5 mg/ml solution of TMADH in phosphate-chloride buffer at different temperatures and different rotational speeds scanned at 240 nm. The zoom function of the Microcal Origin software has been used to show more detail

of 4 to 40 °C. The predicted homodimeric value of 163,000 g/mol (dashed line on Fig. 6) from the amino acid composition is clearly confirmed (Fig. 6) within the experimental error at all temperatures and rotational speeds selected: only 8 of the 34 values determined lie outside ±5,000 g/mol (±3%) of this value. Furthermore, the data point for the lowest concentration (i. e.<0.1 mg/ml) indicates no tendency for dissociation at low concentrations, indicating the subunits are quite strongly bound.

Discussion

Sedimentation velocity

From the $s_{20,w}$ vs. c plot (Fig. 2) no evidence of any dissociative or associative behaviour is seen (i. e. increase of $s_{20,w}$ with c): The observed small decrease of $s_{20,w}$ with

c indicates a slight non-ideality of TMADH, but the concentration range covered is too small to accurately describe non-ideal behaviour. This is reflected in the large error in the concentration dependence coefficient k_s (±40%), an unavoidable feature of the absorption optical system in this concentration range.

It can clearly be seen in Fig. 3 that the temperature has a significant effect on the corrected sedimentation coefficient $s_{20,w}$. From 4–30 °C, the $s_{20,w}$ value is decreased by nearly 1 S. This cannot be explained by temperature-dependent partial degradation of TMADH because TMADH is stable and retains activity in this temperature range (see also the constant $M_{w,app.}$ for 4 and 40 °C, Fig. 6). Also, as shown above, partial dissociation of TMADH does not occur and, therefore, dissocation of the dimer cannot account for the observed decrease in the sedimentation coefficient with temperature.

As sedimentation coefficients depend on the solution structure, any change in the sedimentation coefficient as a function of temperature must be attributed to some structural reorganisation of the TMADH dimer. The decrease in s for TMADH with increasing temperature is likely to be caused by a change from a compact globular structure to a somewhat more extended or swollen (through hydration) structure. With increasing temperature, single symmetric boundaries were obtained (Fig. 4) showing no evidence of associative or dissociative phenomena.

If one bears in mind the very well defined sedimenting boundaries at 4 °C (Fig. 1), the sedimentation coefficient distribution of the sample must have considerably increased at 30 °C. The observed boundary spreading is unlikely to be explained by increased diffusion at higher temperatures alone (the experiment was designed to suppress diffusion as much as possible by using high rotational speeds), as the velocity of the sedimenting boundary was comparable at both temperatures.

Further insight into the conformational flexibility of TMADH at higher temperatures can in principle be obtained, if the distribution of the sedimentation coefficients g(s*) is calculated for different temperatures according to the time derivative analysis described by Stafford (1992). This analysis does not take the diffusion of the molecules into account as it only determines sedimentation coefficients. The results for the temperatures 15, 20 and 30 °C are given in Fig. 5. Clearly, the sedimentation coefficient distribution of TMADH is broadened with increasing temperatures. In Fig. 5 the weight average sedimentation coefficient corrected for water, $s_{20,w}$ (as indicated by the lines in Fig. 5) is first shifted to higher s-values with increasing temperature and then to lower ones again in contrast to the results for $s_{20,w}$ in Fig. 3. As in this case the $s_{20,w}$ values from the g(s*) distribution are too high, only the qualitative finding that the sedimentation coefficient distribution gets broader with increasing temperature can be treated as reliable.

Sedimentation equilibrium

The weight average molar mass M_w = (163,000±5,000 g/mol) (Fig. 6) is consistent with the crystallographic and

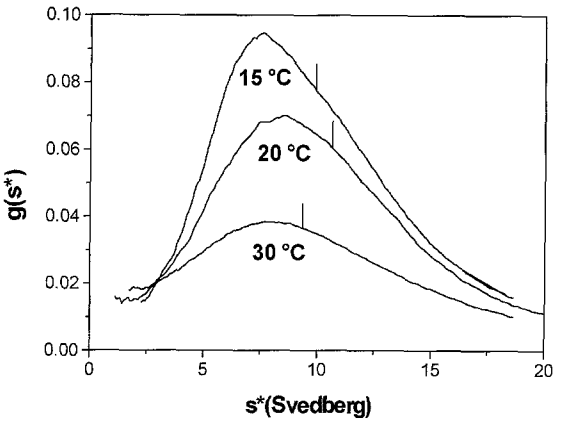

Fig. 5. Sedimentation coefficient distribution g(s*) for TMADH (corrected using the correction factors given in equation 1) in phosphate-chloride buffer at different temperatures

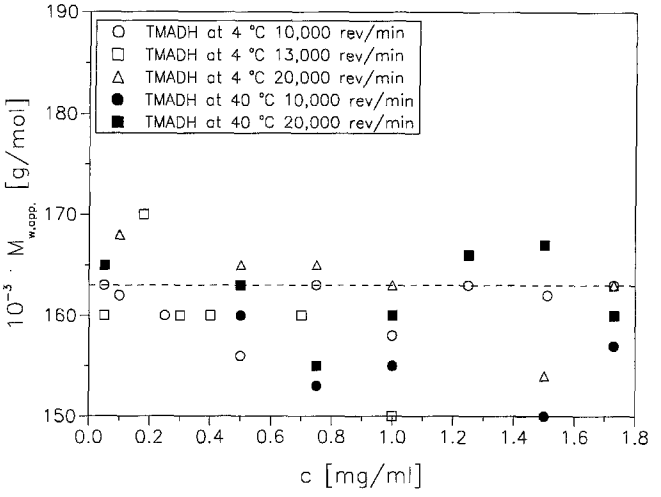

Fig. 6. Concentration dependence of the apparent molar mass $M_{w,app.}$ for TMADH in phosphate-chloride buffer at 4 & 40 °C from sedimenation equilibrium data derived at different rotational speeds. The dashed line indicates the TMADH homodimer $M_{w,app.}$ of 163,000 g/mol based on the amino acid sequence. The values have been derived at 240, 280 and 443 nm. Concentrations given are the ultracentrifuge cell loading concentrations

protein sequence data (Lim et al. 1986; Boyd et al. 1992). No clear concentration dependence was observed in the range studied (0–1.8 mg/ml) and this is probably a result of the fact that non-ideality effects are likely to be negligible in this concentration range (Teller 1973; Laue 1992). This is clearly confirmed by sedimentation equilibrium results (see Fig. 7). No evidence for non-ideality can be found as the in A vs ξ plots are linear in most cases (from the results given above, it can be assumed, that TMADH is not self-associating and not too polydisperse which might cancel out the effect of non-ideality) though some of the plots slighty bend upwards indicating either polydispersity or self-association. As these plots are the minority and contradict all other results presented here, they seem to result from some experimental artefacts for these particular samples.

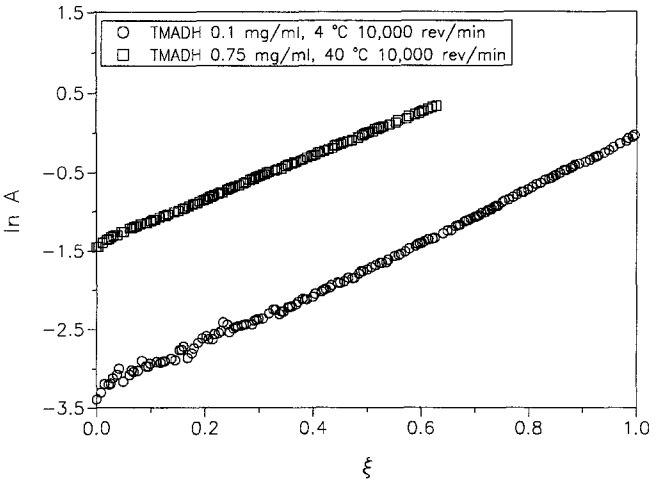

Fig. 7. Plot of in A (A = absorbance at 240 nm) vs $\xi = (r^2 - \text{Meniscus}^2)/(\text{Bottom}^2 - \text{Meniscus}^2)$ for TMADH at two different concentrations and temperatures in phosphate-chloride buffer at 4 °C

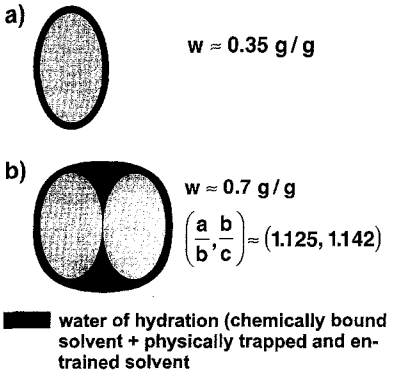

Fig. 8. Possible model for the TMADH homodimer in dilute solution. **a** monomer, **b** homodimer

Shape and hydration calculations for TMADH at 4 °C

It is possible, on the basis of the measured M and $s^0_{20,w}$ data together with the known crystallographic dimensions of TMADH (Lim et al. 1986) to make some observations on the overall shape and/or hydration (i. e. amount of "expansion" or "associated water") of the TMADH homodimer in solution. For these, we need to calculate the frictional ratio f/f_0 and approximate the homodimer shape as either a prolate ellipsoid of revolution or a general tri-axial ellipsoid. For this, we need to use the following two equations relating f/f_0 with M and $s^0_{20,w}$, and the shape parameter P (also known as the "frictional ratio due to shape") with f/f_0 and the hydration w (i. e., mass of water associated or "bound" per mass of protein) of the homodimer:

$$\left(\frac{f}{f_0}\right) = \left[\frac{M(1-\bar{v}\rho_0)}{N_A\left(6\pi\eta_0 s^0_{20,w}\right)}\right]\left(\frac{4\pi N_A}{3\bar{v}M}\right)^{1/3} \qquad (2)$$

{where N_A is Avogadros number, and ρ_0 and η_0 the density and viscosity of water at 20 °C} and

$$P = \left(\frac{f}{f_0}\right)\left[\frac{w}{\bar{v}\rho_0} + 1\right]^{-1/3} \qquad (3)$$

P, named after F. Perrin who first evaluated these (Perrin 1936), has been worked out for ellipsoids of revolution in terms of the axial ratio a/b, and for general triaxial ellipsoids in terms of the 2 axial ratios a/b, b/c. Computer programs are available for this type of hydrodynamic analysis uch as "ELLIPS2" (Harding 1995) – see also (Harding 1982). Using values of M = (163,000±5,000) g/mol, $s^0_{20,w} = (8.49\pm0.10).10^{-13}$ s, $\bar{v} = 0.7312$ ml/g, $\rho_0 = 0.9982$ g/ml and $\eta_0 = 0.01$ poise, we estimate $f/f_0 = 1.26\pm0.06$.

1. Axial ratio a/b calculation of the TMADH homodimer, based on $f/f_0 = 1.26$ and assuming a value w for the hydration of 0.35 g/g. From Eq. (3), and using a "typical" protein value of 0.35 g H_2O/g protein for the hydration, w, a value of P = 1.1 is predicted. For a prolate ellipsoid of revolution this corresponds to an a/b between 2 and 3. This is a little more asymmetric as could be inferred from the crystallographic dimensions of 80 Å×70 Å×60 Å (Lim et al. 1986). So the TMADH homodimer might relax to a more asymmetric structure in dilute solution compared to the crystal state. An alternative and perhaps more plausible explanation is that the hydration of the homodimer is higher than for a "typical" monomeric protein.

2. Hydration w of the homodimer, based on $f/f_0 = 1.26$ and the known crystallographic dimensions of 80 Å × 70 Å × 60 Å. (Lim et al. 1984). It is possible to predict the P and hence w values based on approximating the homodimer to a general triaxial ellipsoid (of semi axes $a \geq b \geq c$ and axial ratios a/b, b/c). Taking (a/b, b/c) = (1.125, 1.142) from the crystallographic dimensions, a value for P of 1.004 is predicted. From Eq. (3) this gives an estimate for w of ≈0.7 g H_2O/g protein. This is quite high for a protein (corresponding to almost a doubling of the effective volume of the homodimer in dilute solution compared to the crystalline state), but could be explained by a high degree of trapped or physically entrained solvent for the homodimer (see Fig. 8 b).

Cases 1 and 2 represent the two extremes, and the most probable explanation of the M and $s^0_{20,w}$ data lies somewhere between these extremes. Unfortunately, with just one shape parameter (P) it is impossible to pin both the hydration and the shape unequivocally. To do this additional hydrodynamic measurements are required (so that for example the hydration independent shape functions $k_s/[\eta]$ or Π can be used), but such analyses require much more sample than is currently available.

Shape and hydration of TMADH at higher temperatures

It is clear from Fig. 3 that there is a small but nonetheless significant decrease in the corrected (apparent) sedimentation coefficient, $s_{20,w}$, as the temperature is increased from 4 °C to 30 °C. Since the molar mass appears, within

the experimental error, invariant in this range (Fig. 6) this must mean there is either a further increase in asymmetry or swelling of the homodimer due to increased hydration.

Although the $s_{20,w}$ values in Fig. 3 are apparent ones (i.e. at a finite concentration of ≈0.5 mg/ml), and not $s^0_{20,w}$ values, it is still possible to make an approximate estimate to the change in the asymmetry/hydration. Figure 3 suggests an ≈10% steady decrease in $s_{20,w}$ in the range 4–30 °C, and hence, by Eq. (2) a ≈10% increase in f/f_0. This corresponds to a further relaxing of the molecule from its "tight" crystalline state, either through a further increase of its axial ratio, or an increase in its hydration w (to ≈0.8 g/g) or (more likely) a combination of both effects.

In conclusion, we think it is reasonable to say that the TMADH system is another example of how a crystallographic picture of a macromolecule doesn't always tell the complete story, and whereas although we have confirmed the homodimeric nature of this molecule in dilute solution, it appears to swell considerably (a swelling which, not surprisingly, increases with temperature) from its "tight" crystalline state.

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References

Boyd G, Mathews FS, Packman LC, Scrutton NS (1992) Trimethylamine dehydrogenase of bacterium –286 W3A. Molecular cloning, sequence determination and over-expression of the gene. FEBS Lett 308: 271

Harding SE (1982) A computer program for evaluating the hydrodynamic parameters of a macromolecule in solution for any given value of its axial dimensions. Comput Biol Med 12: 75–80

Harding SE (1995) On the hydrodynamic analysis of macromolecular conformation. Biophys Chem 55: 69–93

Harding SE, Rowe AJ (1982) Modelling biological macromolecules in solution III. The lambda-R intersection method for triaxial ellipsoids. Int J Biol Macromol 4: 357–361

Harding SE, Horton JC, Morgan PJ (1992) MSTAR: A FORTRAN program for the model independent molecular weight analysis of macromolecules using low speed or high speed sedimentation equilibrium. In: Harding SE, Rowe AJ, Horton JC (eds) Analytical Ultracentrifugation in Biochemistry and Polymer Science. Royal Society of Chemistry, Cambridge UK, pp 275–294

Kasprzak AA, Papas EJ, Steenkamp DJ (1983) Identity of the subunits and the stoichiometry of prosthetic groups in trimethylamine dehydrogenase and dimethylamine dehydrogenase. Biochem J 211: 535

Laue TM (1992) Short column sedimentation equilibrium analysis for rapid characterization of macromolecules in solution. Beckman Technical Information DS-835, Spinco Business Unit, Palo Alto, California

Lim LW, Shamala N, Mathews FS, Steenkamp DJ (1984) Molecular structure of trimethylamine dehydrogenase from the bacterium V3A at 6.0 angstroms resolution. J Biol Chem 259: 14458–14462

Lim LW, Shamala N, Mathews FS, Steenkamp DJ, Hamlin R, Xuong N (1986) Three dimensional structure of the iron-sulfur flavoprotein trimethylamine dehydrogenase at 2.4 angstroms resolution. J Biol Chem 261: 15140–15146

Lim LW, Mathews FS, Steenkamp DJ (1988) Identification of ADP in the iron-sulfur flavoprotein trimethylamine dehydrogenase. J Biol Chem 263: 3075–3078

Perkins SJ (1986) Protein volumes and hydration effects. The calculations of partial specific volumes, neutron scattering matchpoints and 280-nm absorption coefficients for proteins and glycoproteins from amino acid sequences. Eur J Biochem 157: 169–180

Perrin F (1936) Mouvement Brownian d'un ellipsoide II. Rotation libre et depolarisation des fluorescences. Translation et diffusion de molecules ellipsoidales. J Physique Radium 7: 1–11

Raine ARC, Scrutton NS, Mathews FS (1994) On the evolution of alternate core packing in 8-fold beta/alpha barrels. Protein Sci 3: 1889–1892

Scrutton NS (1994) Alpha/beta barrel evolution and the molecular assembly of enzymes: emerging trends in the flavin oxidase/dehydrogenase family. Bioessays 16: 115–122

Scrutton NS, Packman LC, Mathews FS, Rohlfs RJ, Hille R (1994) Assembly of redox centres in the trimethylamine dehydrogenase of bacterium W3A1. J Biol Chem 269: 13942–13950

Stafford WF (1992) Boundary analysis in sedimentation transport experiments: A procedure for obtaining sedimentation coefficient distributions using the time derivative of the concentration profile. Anal Biochem 203: 295–301

Steenkamp DJ, Mallinson J (1976) Trimethylamine dehydrogenase from methyloptropic bacterium. Isolation and steady-state kinetics. Biochim Biophys Acta 429: 705–719

Tanford C (1961) Physical Chemistry of Macromolecules. John Wiley and Sons, New York, p 381

Teller DC (1973) Characterization of proteins by sedimentation equilibrium in the analytical ultracentrifuge. Methods Enzymol 27D: 346–441

Yphantis DA (1964) Equilibrium ultracentrifugation of dilute solutions. Biochemistry 3: 297–317