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THE SUBUNIT SIZE OF ALCOHOL DEHYDROGENATES

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1. Introduction

Glyceraldehyde 3-phosphate dehydrogenase (GPDH, EC 1.2.1.12) from several sources and alcohol dehydrogenases (EC 1.1.1.1) from both yeast (YADH) and horse liver (the ethanol-active isozyme, $LADH_{EE}$) have all been shown to contain one reactive cysteine residue in a unique amino acid sequence corresponding to a minimum molecular weight of between 36,000 and 40,000 [1–4]. The molecular weights of the active enzymes have been shown to be 145,000 for GPDH [5], 150,000 for YADH [6], and about 80,000 for LADH [7]. On the basis of these results it has been proposed that GPDH and YADH are tetramers [3,8] and that LADH is a dimer [3,4], consisting in each case of similar and probably identical polypeptide chains with molecular weights of between 36,000 and 40,000.

These proposals are supported by the results obtained for the molecular weights of the three enzymes in the presence of dissociating solvents such as 5M-guanidine HCl or 8M-urea [5,9,10]; and confirmed in the case of GPDH by the elucidation of the complete amino acid sequence of its monomer chains [11,12].

2. Materials and methods

The ethanol active isozyme of horse liver alcohol dehydrogenase ($LADH_{EE}$) was prepared and kindly supplied by Dr. Å.Åkeson of the Biochemical Department, Nobel Medical Institute, Stockholm, Sweden [13], while the other enzymes were prepared as described in the literature [14,15].

Glyceraldehyde 3-phosphate dehydrogenase (8 mg/ml) was carboxymethylated by reaction with iodoacetic acid (3 mM) at pH 8.0 in 8 M-urea for 90 min at 30° [8]. The alcohol dehydrogenases were carboxymethylated fully by the following procedure. The protein was dissolved (about 10 mg/ml) in 0.1 M tris chloride, 1.0 mM EDTA, 5 M guanidine hydrochloride, apparent pH (glass electrode) 8.1, and incubated, under nitrogen, with dithiothreitol in a 1.25 fold excess of reagent sulphydryl groups over total protein sulphydryl groups for 3 hr at room temperature. Sufficient iodoacetic acid to react with all the dithiothreitol sulphydryl groups and to be in a two-fold excess over the protein sulphydryl groups was dissolved in the same buffer and then added to the protein solution. The mixture was then incubated under nitrogen at room temperature for 3 hr. In all cases the reaction was stopped by the addition of an excess of 2-mercaptoethanol and the protein solution then dialysed against 0.1 M sodium pyrophosphate, pH 8, containing 8 M urea (for the glyceraldehyde 3-phosphate dehydrogenase) or 6 M guanidine hydrochloride (for the alcohol dehydrogenases).

In order to avoid the inaccuracies inherent in the determination of the molecular weights of proteins in either 8 M urea or 6 M guanidine hydrochloride, the carboxymethylated proteins were then maleylated by reaction with maleic anhydride [16,17]. The solution of carboxymethylated protein in urea or guanidine hydrochloride was adjusted to pH 9 by the addition of 1.0 N sodium hydroxide and was then reacted with maleic anhydride in a 20 fold excess over the total

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amino groups, with the pH being maintained between 8.5 and 9.0 by the addition of 1.0 N sodium hydroxide. When the reaction was complete the protein was dialysed exhaustively against 0.5% (w/v) ammonium hydrogen carbonate and then finally against this buffer containing various concentrations of sodium chloride.

The maleyl proteins were examined in a Spinco Model E analytical ultracentrifuge equipped with both Schlieren and Rayleigh interference optics. Concentrations of about 5 mg/ml were used with the Schlieren optics for sedimentation velocity runs, and of from 0.5 mg/ml to 2.5 mg/ml for sedimentation equilibrium runs with column lengths of up to 5 mm and using the combined Schlieren/interference technique [18]. The equilibrium experiments were run until the interference fringe patterns remained constant over a period of 24 hr and the results were then calculated by plotting graphs of $1/r \cdot dc/dr$ against Δc, when any departure from ideal behaviour can be detected by its deviation from a straight line. The molecular weight may then be calculated from the slope of the line [19]. Partial specific volumes for the maleylated, carboxymethylated proteins were calculated from the amino acid compositions [20]. A value of 0.73 cm^3/g was obtained in each case; this and a solvent density of 1.00 at 20° were used for the calculations of the molecular weights.

3. Results and discussion

The *N*-maleyl derivative of S-carboxymethyl-YADH was submitted to ultracentrifugation in 0.5% (w/v) NH_3HCO_3 containing increasing concentrations of NaCl. The sedimentation constant was found to increase up to 0.2 N NaCl but did not show any further increase at 0.4 N NaCl. This concentration of NaCl was therefore used for all the subsequent experiments.

Sedimentation analyses of the maleylated, carboxymethylated proteins were performed at 57,100 rev/min in 0.5% NH_4HCO_3 – 0.4 N NaCl. A single symmetrical boundary was observed in each case and the results obtained are given in fig. 1. From these it was calculated that the three proteins possessed a similar sedimentation coefficient of about 1.7S. Their molecular weights were then measured by the method of sedimentation equilibrium and the graphs obtained by plotting $1/r \cdot dc/dr$ against Δc are shown in fig. 2a–c.

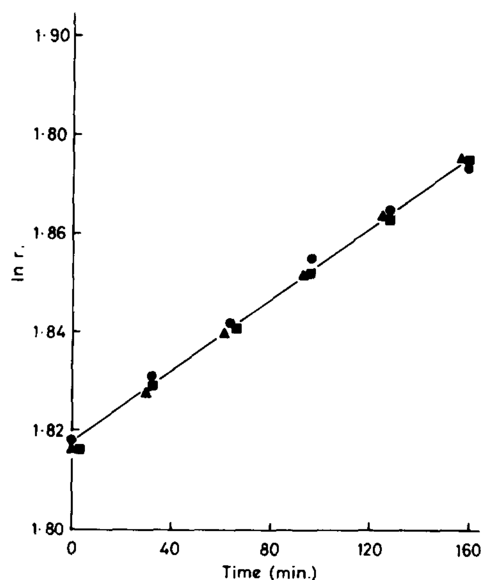

Fig. 1. Sedimentation velocity of maleylated, carboxymethylated enzymes at 57,000 rev/min in 0.5% (w/v) ammonium hydrogen carbonate, 0.4 N sodium chloride at 20°. ▲ yeast alcohol dehydrogenase; ■ liver alcohol dehydrogenase; ● pig glyceraldehyde 3-phosphate dehydrogenase.

Straight line graphs were obtained in each case, showing that the three proteins appear to be behaving in an ideal manner, and their approximate molecular weights were calculated to be: 33,000 for GPDH, 42,000 for YADH and 39,000 for LADH.

Although these values have not been measured with a high degree of precision, the similarity found between the sedimentation coefficients and the molecular weights of the three proteins is sufficient to establish that the subunits derived from GPDH, YADH and LADH are of similar size. Moreover since this value is in reasonable agreement with the size of the subunit in pig GPDH as calculated from its complete amino acid sequence [12] it seems unlikely that the method employed is subject to any inherent systematic error.

In the case of LADH, the strongly dissociating conditions of reduction with dithiothreitol in 6 M guanidine HCl containing 1.0 mM EDTA, followed by carboxymethylation of SH groups in the same solvent, would be expected to release both the "free" and "buried" zinc ions, and to cause complete dissociation of the enzyme [21]. Moreover maleylation of

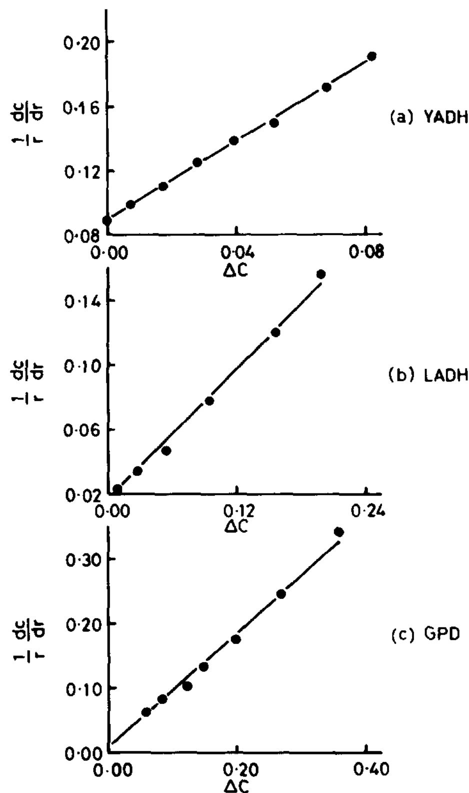

Fig. 2. Sedimentation equilibrium of maleylated, carboxymethylated enzymes in 0.5% (w/v) ammonium hydrogen carbonate, 0.4 N sodium chloride at 20°. Concentration (c) in arbitrary units. (a) yeast alcohol dehydrogenase at 15,220 rev/min; (b) liver alcohol dehydrogenase at 12,590 rev/min; (c) pig glyceraldehyde 3-phosphate dehydrogenase at 15,220 rev/min.

amino groups alone has been shown to cause the dissociation of polymeric proteins [22,23]. The fact that under these conditions a molecular weight of about 40,000 is found for both the alcohol dehydrogenases is in good agreement with the chemical evidence for the subunit structures of these enzymes [15,24,25,26] and thus provides additional evidence for the view that the yeast enzyme is a tetramer, while the liver enzyme is a dimer, and not a tetramer as previously claimed [21]. The lower values which have been reported [21, 27] for the molecular size of the monomer in LADH would appear to be due to the presence of artifacts which may [27] have been produced by non-specific cleavage of the protein chain cF [28].

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