DISSOCIATION OF MAMMALIAN D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE INTO MONOMERS

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1. Introduction

The molecular weight of mammalian D-glyceraldehyde-3-phosphate dehydrogenase is 145,000 [1–3]. The enzyme is composed of 4 identical subunits [4] and can be dissociated into subunits at extreme pH [3], or by guanidine hydrochloride [2], detergents [5], etc.

The purpose of our investigations was to study the dissociation of tetrameric D-glyceraldehyde-3-phosphate dehydrogenase under conditions compatible with enzyme action. In the present work the concentration dependence of the number-, weight- and *Z*-average molecular weights of D-glyceraldehyde-3-phosphate dehydrogenase was investigated over the 50–500 μg per ml concentration range at 5°.

Reversible dissociation into dimers at neutral pH in 0.1 M phosphate buffer at 5° was recently observed below 500 μg per ml protein concentration [6]. We have succeeded in detecting the dissociation of the enzyme both into dimers and monomers on dilution.

2. Materials and methods

Four times recrystallized enzyme obtained from porcine skeletal muscle [7] was dissolved in 0.1 M phosphate buffer, pH 7.5. To remove ammonium sulfate the enzyme solution was gel filtered on a Sephadex G-50 column equilibrated with the same buffer. All operations with the enzyme were carried out below 5°.

The concentration dependence of the number-, weight- and *Z*-average molecular weights of the enzyme was measured by the high speed equilibrium method of Yphantis [8]. The enzymic activity did not change during the experiments as controlled by Warburg's optical test before and after ultracentrifugation.

The sedimentation equilibrium runs were carried out with a MOM G-120 analytical ultracentrifuge equipped with Rayleigh interferometer optics. The initial protein concentration was about 200 μg per ml, the solution column height in the ultracentrifuge cell varied between 0.15–0.25 cm. The appropriate rotor speed and the time required to attain the equilibrium were calculated as suggested by Van Holde and Baldwin [9]. Accordingly, 7 to 10 hr runs were made at 5° with a rotor speed of 25,000 rpm. The protein concentration at different positions of the cell was calculated from the interference fringe displacements [10]. The partial specific volume was 0.733 ml per g, obtained by the pycnometric method.

The second virial coefficient, B, was measured by light scattering in a Brice-Phoenix S-2000 photometer at 436 and 546 nm wavelengths.

3. Results and discussion

High speed sedimentation equilibrium experiments were performed and the apparent number-($M_{n,app}$), weight-($M_{w,app}$) and *Z*-average ($M_{z,app}$) molecular weights were calculated over the whole concentration range, according to the following equations [8, 11]:

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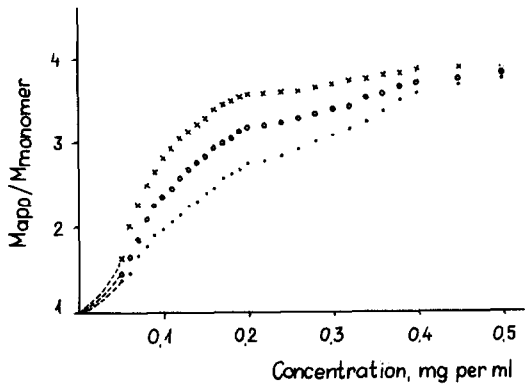

Fig. 1. Concentration dependence of the ratios of apparent average molecular weights to monomer. (.–.–.): $M_{n,app}/M_1$. (o–o–o): $M_{w,app}/M_1$. (x–x–x): $M_{z,app}/M_1$.

$$M_{w,app}(c) = \frac{2RT}{(1-\bar{V}\rho)\,\omega^2}\,\frac{d\ln c}{dr^2} \qquad (1)$$

$$M_{z,app}(c) = \frac{2RT}{(1-\bar{V}\rho)\,\omega^2}\,\frac{d^2c/(dr^2)^2}{dc/dr^2} \qquad (2)$$

$$M_{n,app}(c) = M_1 c \Big/ \int_0^c (M_1/M_{w,app}(c))\,dc \qquad (3)$$

where R is the gas constant, T is the temperature, $\bar{V}$ is the partial specific volume of the solute, ρ is the density of the solvent, ω is the angular speed of the rotor, r is the distance measured from the axis of revolution, c is the concentration at position r in the ultracentrifuge cell, measured in g per 100 ml, M_1 is the molecular weight of the monomer.

The values of eq. 1 and 2 were calculated point by point, by fitting exponential functions to every 3 adjoining experimental points, since we did not succeed in the analytical formulation of the $c(r)$ function obtained experimentally. The apparent number average molecular weight as a function of concentration ($M_{n,app}(c)$) can be calculated according to eq.3, by using the value 36,300 for the monomer weight, as determined from the amino acid sequence [4]. The integral in eq. 3 was evaluated with the aid of the trapezoidal rule. The concentration dependence of the different average molecular weight values is presented in fig. 1. These values exhibit a definite concentration dependence over the whole concentration range examined. The different average molecular weights converge at both ends of the concentration range approaching at the lower end the value of the monomer (36,300) and at the upper end that of the tetramer (145,000).

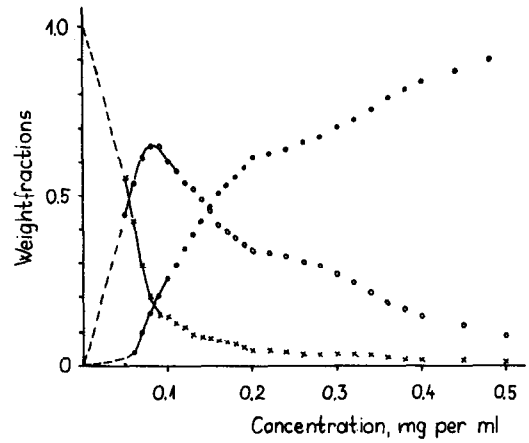

Fig. 2. Concentration dependence of the weight fractions of different species. (x–x–x): weight fraction of the monomer. (o–o–o): weight fraction of the dimer. (·–·–·): weight fraction of the tetramer.

For describing the subunit interactions in the dissociating system the following assumptions were made:

1. Dissociation proceeds in two steps, i.e. only monomers, dimers and tetramers exist. This assumption is supported by the results of Hoagland and Teller [6].

2. Both partial specific volume and refractive index increments of all species are equal.

3. The concentration dependence of the activity coefficient can be neglected. To support this assumption we measured the second virial coefficient, which is characteristic of the solvent–solute interaction. The correction caused by the second virial coefficient proved to be less than the inaccuracy of the molecular weight determination in the ultracentrifuge.

Taking these assumptions into account we calculated the relative concentration of the monomer, dimer and tetramer as a function of protein concentration (fig. 2).

As seen in fig. 2 at 40 μg per ml concentration, the amounts of monomer and dimer are equal, whereas at 160 μg per ml those of the dimer and tetramer are equal. From the concentration values at the intersection of different molecular species in fig. 2 the dissociation constants can be estimated: 2.2×10^{-6} M and 1.1×10^{-6} M were obtained for the tetramer–dimer and dimer–monomer systems, respectively. If we calculate the theoretical concentration distribution function for each molecular species using these figures, the curves obtained do not fit the experimental data in fig. 2.

Thus it appears that this dissociating protein system cannot be simply characterized by 2 dissociation constants since the phenomenon is more complex: the partial release of the bound coenzyme [12] for example, may also influence subunit interactions.

Summarizing, we can say that glyceraldehyde-3-phosphate-dehydrogenase dissociates at low temperature on dilution and dissociation proceeds to the monomer. The conditions under which dissociation occurs are compatible with the functioning of the enzyme.

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