CONFORMATIONAL CHANGES OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE OBSERVED USING LASER LIGHT-SCATTERING SPECTROSCOPY

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has an important role in glycolysis, and in recent years it has been studied as a possible glycolytic control point. Both the yeast and rabbit muscle enzyme undergo temperature-dependent dissociation in the presence of ATP (1–3). This study further defines the physical behavior of this enzyme through measurements of its translational diffusion coefficient, D_t, and gives evidence for some conformational changes in the enzyme.

By measuring the half-width of the spectrum of the light scattered from a solution of enzyme, it is possible to determine D_t of the molecule (4–6). D_t can then be related, through hydrodynamic parameters, to molecular size and shape. By concurrently measuring the intensity of scattered light, molecular weight changes may also be followed. Thus in one experiment it is possible to obtain independent parameters related to conformation and molecular weight changes. Our results show that this enzyme has a distinct tendency to form aggregates (to which the scattered intensity is very sensitive), that it shows a temperature-dependent dissociation, and that, in the presence of ATP, there is a conformational change with temperature, distinct from the aforementioned changes in quarternary structure.

Rabbit muscle enzyme was obtained from the Sigma Chemical Co., St. Louis, Mo. (lot number 63C-9530). Crystals of the enzyme were collected by centrifugation and dissolved in buffer I (0.1 M imidazole, 1 mM dithiothreitol (DTT), 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.15). This solution was then dialyzed overnight against 1 liter of buffer I and run through a 1 × 18-in Sephadex G200 column (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). The enzyme was then reconcentrated using an Amicon filtration unit (Amicon Corp., Lexington, Mass.) and UM10 filters. Solutions not purified by column chromatography contained aggregates which completely dominated the scattering spectrum. A different lot of enzyme showed the same behavior. All samples were prepared within one day of the experiment, as it was found that a sample aggregated enough after one day's storage to significantly affect measured values of D_t. All purification steps were carried out at 4° C. Concentrations

were determined spectrophotometrically using the extinction coefficient ($A^{1\%}_{280}$ = 10) given by Fox and Dandliker (7). ATP (Sigma grade) was obtained from Sigma Chemical Co.

Purified samples were filtered through a Millipore filter having an average pore size of 0.05 μm directly into a clean scattering cuvette. Details of the general procedure for cleaning cuvettes, etc., have been described elsewhere (8), as have the details of the experimental apparatus (5). Using a 0.3% enzyme solution, D_t could typically be measured in 1 min with an accuracy of 1–2%. All scattering measurements were made at 90° to the incident light path.

Fig. 1 shows the variation with temperature of the translational diffusion coefficient corrected to standard conditions, $D_{20,w}$, and the relative scattered intensity for GAPDH in buffer I with no ATP. It is seen that $D_{20,w}$ is relatively constant from 0.5° C to about 26° C. At 0.5° C, our value of D_t is some 10% lower than that obtained by Fox and Dandliker (9) who used classical techniques. At temperatures slightly higher than 26° C, $D_{20,w}$ decreases rapidly indicating large particle formation, or aggregation. The relative scattered intensity decreases steadily from its value at 0.5° C reaching a minimum in the vicinity of 22–26° C where assays of the enzyme are typically performed. Since the scattered intensity at constant mass concentration is proportional to the molecular weight of the species in solution, the intensity results indicate that the protein is dissociating, with maximum dissociation between 22° and 26° C.

Assuming the tetrameric enzyme is dissociated only to dimers, we calculate from the

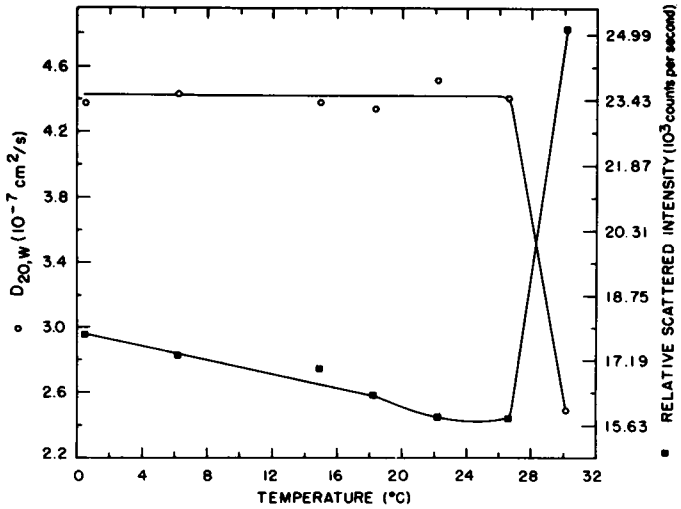

FIGURE 1 $D_{20,w}$ and relative scattered intensity vs. temperature for GAPDH. Each point shown is the average of seven or eight measurements. The values of 30°C are unstable with time. Solutions were held at each temperature for 25–30 min and temperature increases were about 1°/min. Circles indicate values of D, and squares indicate values of intensity. Standard deviations for the scattering values are typically less than 1%. Enzyme concentration is 0.33% in buffer I. No ATP is present.

decrease in scattered intensity that approximately 23% of the original enzyme has dissociated by 22° C; assuming dissociation to monomers only gives 15% dissociation. The reason there is no apparent change in the measurement of D_t corresponding to the decrease in scattered intensity lies in the nature of the experimental technique. The whole enzyme will have a greater scattering power than the subunits due to its larger size. Hence, it will contribute more to the scattered signal, and weight the measured value of D_t in favor of the whole enzyme. It was shown earlier (8) for the dissociation of ribosomes that the percentage change in D_t as a function of percent dissociation was substantially less than the percentage change of the relative scattered intensity.

If the temperature of the enzyme solution is subsequently raised to 30.2° C, the relative scattered intensity increases rapidly which implies the formation of large particles or aggregates as does the sharp decrease in D_t at the same temperature. At 26.6° C, or lower, the scattered intensity was stable over the 7–9 min period required for measurements. These data were readily reproduced from one experiment to another.

Fig. 2 shows the results of an experiment testing the reversibility of the dissociation. Upon raising the temperature from 0.2° C to 24.5° C, the relative scattered intensity

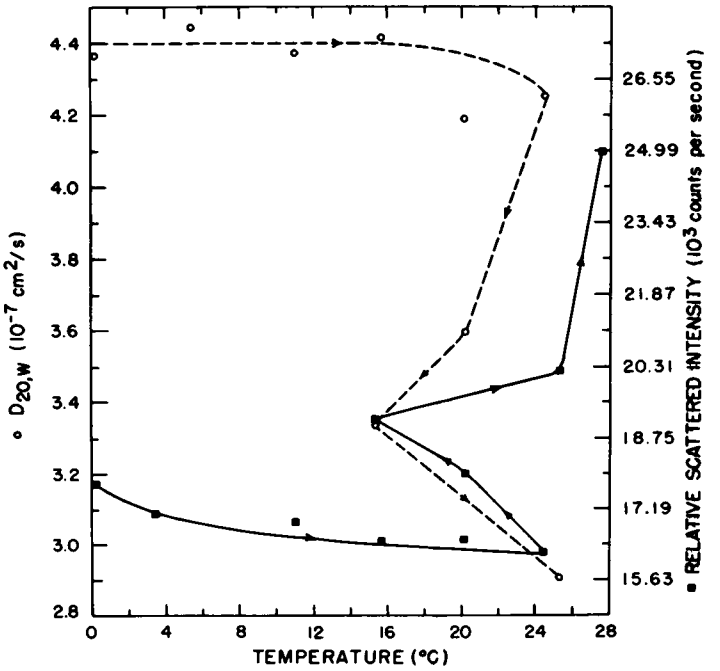

FIGURE 2 $D_{20,w}$ and relative scattered intensity vs. temperature. Each point shown is the average of five or six measurements. The values at 25.3° and 27.6° are unstable with time. Solutions were held at each temperature for 15–20 min. Temperature increases were about 1°/min and decreases around 0.15°/min. Arrows indicate the direction of temperature change. Enzyme concentration is 0.33% in buffer I.

decreases approximately 10%, but upon subsequent cooling to 20.2° C, and then 15.3° C, the intensity increases sharply indicating aggregation. Subsequently raising the temperature to 25.3° C and 27.6° C causes the intensity to increase still further. This time, however, the intensity is not stable, but increases with time (not shown). At 24.5° C and lower temperatures, the intensity was stable over the 6–7 min necessary for measurements. The variation in $D_{20,w}$ also indicates the formation of larger particles on cooling in much the same way the scattered intensity does. The spontaneous formation of aggregates we have observed over 24 h, even after gel filtration and storage at 4° C, further indicates the instability of the tetramers. This observation may be of considerable importance to those trying to interpret the complex reaction kinetics of the enzyme.

It has been shown that at 0° C, both rabbit muscle and yeast glyceraldehyde-3-phosphate dehydrogenase dissociate into subunits under the influence of ATP, and this has been interpreted as being related to a possible control mechanism in glycolysis (1, 2). This interpretation has been criticized because the dissociation takes place very slowly, and only at low temperatures. It was further suggested (3) that ATP causes a change more subtle than dissociation which makes the enzyme more susceptible to proteolysis and would allow enzymatic digestion to function as a control mechanism. Fig. 3 is a plot of $D_{20,w}$ and relative scattered intensity against temperature for a solution of enzyme where the solution has been made 0.003 M in ATP 25 min before the first measurement. As the temperature is raised from 0.1° C to 21.9° C, the relative intensity is seen to stay constant whereas $D_{20,w}$ starts to decrease in the 10–14° C region. At 22° C, $D_{20,w}$ is 7% smaller than at lower temperatures, but the relative intensity is unchanged. The fact that $D_{20,w}$ decreases with no accompanying change in molecular weight indicates that a conformational change has taken place. Since $D_{20,w}$ is inversely proportional to the radius of an equivalent hydrodynamic sphere (Stokes-Einstein equation) this 7% decrease in $D_{20,w}$ indicates a substantial change in the

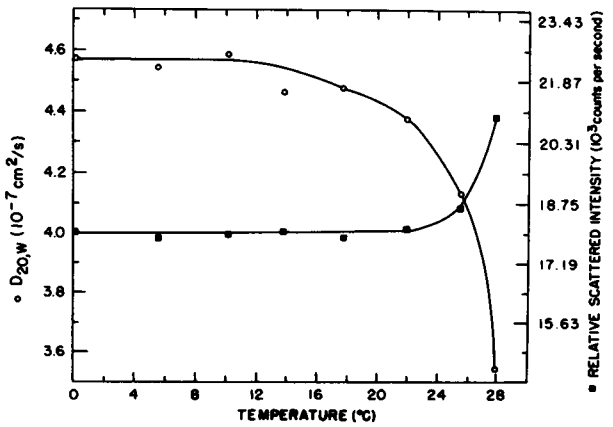

FIGURE 3 $D_{20,w}$ and relative scattered intensity vs. temperature for a solution of enzyme that is also 0.003 M in ATP. Enzyme concentration is 0.4% in buffer I.

hydrated shape of the enzyme, and provides direct evidence for the conformational change previously deduced (3). When the temperature is raised from 22° to 25.5° C aggregation commences, some 3–4° C earlier than for the same situation without ATP. Both effects here are markedly different from the behavior of the enzyme without ATP (Fig. 1).

Finally it should be remarked that the enzyme concentration used here is 5–10 times greater than that used by Constantinides and Deal (2) to obtain a maximum amount of dissociation in the presence of ATP. Also, the time scale of our measurements is shorter. The substantial agreement between results obtained by such different means indicates the general importance of the ATP effect on this enzyme.

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