VISCOMETRIC COMPARISON OF THE ASYMMETRY PROPERTIES OF PHOSPHOFRUCTOKINASES FROM PIG KIDNEY AND RABBIT MUSCLE*

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SUMMARY: A direct comparison of the asymmetry properties of phosphofructokinases (PFK) from two functionally different mammalian tissues has been made by determining the intrinsic viscosities of rabbit muscle PFK and pig kidney PFK at 3.5°C. The intrinsic viscosity of the muscle PFK is 6.9 cc/g, which is significantly higher than for typical globular proteins (3 to 4 cc/g). Furthermore, the intrinsic viscosity of the kidney PFK, 34.0 cc/g, is dramatically higher, indicating a highly asymmetric enzyme. Hence, both phosphofructokinases are asymmetric, but they differ markedly in degree of asymmetry and, therefore, in structure. These studies open up an important new area of investigation of this key group of asymmetric, regulatory enzymes.

Recent investigations (1-3) in this laboratory have indicated that pig liver phosphofructokinase is structurally asymmetric, in contrast to most enzymes, which are fairly globular. The present study was undertaken to determine whether this characteristic is exhibited by other phosphofructokinases. Since previous studies (1-3) have shown differences in physical properties (including both size and solubility) between phosphofructokinases from glycolytic tissues, such as muscle, and gluconeogenic tissues, such as liver and kidney, we chose a tissue source from each category. This paper describes viscometric studies on phosphofructokinases from pig kidney and rabbit muscle.

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MATERIALS AND METHODS

Rabbit muscle PFK (fructose 6-phosphate kinase, type III) was obtained from Sigma Chemical Company. Purification of pig kidney PFK was as described previously (1,2) with the same modifications as for pig liver PFK (3). The solvent for viscosity measurements contained 50 mM imidazole (pH 7.4), 110 mM ammonium sulfate, 5 mM $MgCl_2$, 100 mM dithiothreitol, 0.1 mM ATP, and 0.1 mM FDP. It was filtered through a 0.45 μ Metricel filter. Just prior to each viscosity experiment, the PFK, kept at 4°C, was solubilized in the solvent and centrifuged in a Sorvall SS-34 rotor at 18,000 rpm (39,000 x g) for 15 minutes to remove any foreign particulate matter. The protein concentration was then measured (2). Viscosity measurements were made with a Cannon (Model 50) Ubbelohde semi-micro dilution viscometer in a 15-gal insulated water bath regulated at 3.50°C ± 0.02°C. Solvent and protein solution flow times were measured with an electric timer to the nearest 0.01 sec. Successively more dilute solutions of protein (from approximately 8 to 0.5 mg/ml) were obtained by carefully adding with a syringe solvent at 3.5°C directly to the initial protein solution in the viscometer. The average of 3 or 4 flow times was obtained and the reduced viscosity calculated at each concentration. Intrinsic viscosities were obtained from the y-axis intercept of a linear least squares fit of the reduced-viscosity-versus-protein-concentration data.

Reduced viscosities were calculated from the flow times using the following equation:

$$\eta_{Red} = \left(\frac{t\,\rho}{t_o\,\rho_o} - 1\right)/C$$

where t and ρ are the flow time and density, respectively, of the protein solution at concentration C, and t_o and ρ_o are the solvent flow time and density, respectively. The approximate solvent flow time was about 430 seconds at 3.5° for the viscometer used. The standard deviation in measurement of flow times for both enzymes was less than ± 0.5 sec. This resulted in standard deviations for the calculated values of reduced viscosity of less than ± 0.2 cc/g at concentrations above 2 mg/ml and less than ± 1.0 cc/g below 2 mg/ml.

RESULTS AND DISCUSSION

A plot of reduced viscosity versus protein concentration is shown in Figure 1 for both the rabbit muscle PFK and the pig kidney PFK. A linear least-squares analysis of the points for rabbit muscle PFK gives an intercept corresponding to an intrinsic viscosity, [η], of 6.93 ± 0.14 cc/g and a slope of -0.023; the latter value indicates that the reduced viscosity is essentially concentration-independent in this range. A similar analysis of the data points for pig kidney PFK, however, shows a markedly higher intrinsic viscosity value, 34.00 ± 0.59 cc/gm, and a steep slope of ± 3.67; the latter value indicates a very pronounced concentration dependence for the reduced viscosity. On the basis of these intrinsic viscosity values, and the general observation (4,5) that most globular proteins have intrinsic

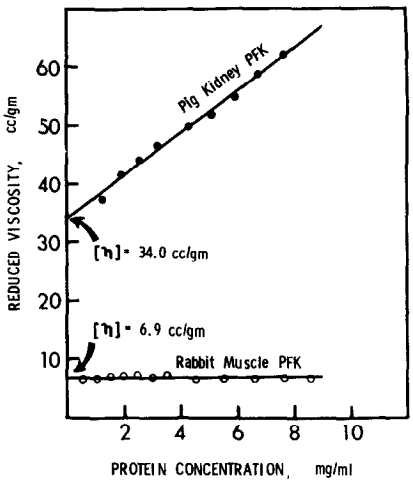

Figure 1. Comparison of the concentration dependence of the reduced viscosity at 3.5°C of the phosphofructokinases from rabbit muscle and pig kidney, see text for details.

viscosity values in the range of 3-4 cc/g, we conclude that phosphofructokinase from the gluconeogenic tissue, pig kidney, is markedly asymmetric while that from the glycolytic tissue, rabbit muscle, is only moderately asymmetric. The strong concentration-dependence exhibited by pig kidney PFK is further evidence for asymmetric shape since one cause of concentration dependence is interactions among molecules and these are greater for particles with greater asymmetry. Other types of studies with rabbit muscle phosphofructokinase have led to the conclusion that the enzyme is somewhat asymmetric (6-8).

The intrinsic viscosity values reported here are minimum values since orientation of the asymmetric molecules in solution would lessen their resistance to movement in the viscometer and result in lowered measured viscosity values. Thus, the true intrinsic viscosity values may be even greater, with corresponding implications for the asymmetry of the enzyme.

It is interesting that there is such a great difference in the physical shape of the enzymes from these two tissues, which have greatly different metabolic roles. The main flow of carbohydrate metabolism in muscle is in the direction of glycolysis, while in kidney the flow generally may be

either in the direction of glycolysis or in the direction of gluconeogenesis. The regulatory enzymes from these two sources apparently have different physical properties, to provide the necessary differences in regulatory properties.

Due to the susceptibility of the kidney PFK to oxidation even in the presence of unusually high concentrations of sulfhydryl reducing agents (100 mM DTT), this first study was carried out at low temperature (3.5°C) where the enzyme is most stable. Preliminary association-dissociation studies on pig kidney PFK and detailed studies on pig liver PFK (3), whose properties are similar to those of pig kidney PFK (1,2), indicate that the enzyme shifts with temperature between two different forms with different asymmetry properties. Further information about the shape of these forms may be derived from measurement of reduced viscosity over a wide temperature range; experiments along these lines are in progress in our laboratory.

It would be of great interest to know the intrinsic viscosity values for erythrocyte and heart muscle PFK. The human erythrocyte PFK (9-11), like pig kidney PFK and pig liver PFK (1-3), appears large yet shows unique patterns of association-dissociation. These patterns are different from those for pig kidney, pig liver (1-3) rabbit muscle (6,12-16) or sheep heart (17-19) phosphofructokinases.

Viscosity is a very powerful tool for analysis of asymmetry or changes in conformation of particles which are asymmetric, as phosphofructokinases appear to be on the basis of these studies. This opens up a new area of study on these important regulatory enzymes. For example in addition to the comparative studies suggested above of enzymes from different sources, it will be of interest to anlyze the allosteric transitions of various phosphofructokinases by viscometry to see whether structural changes can be detected, and if so, to determine their basis.

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