PHOSPHOFRUCTOKINASE FROM *E. COLI*: EVIDENCE FOR A TETRAMERIC STRUCTURE OF THE ENZYME

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Phosphofructokinase from *E. coli* is a key enzyme in the control of glycolysis. It is inhibited by phosphoenolpyruvate and activated by diphosphonucleosides. It shows cooperative interactions with respect to one of its substrates, fructose-6-phosphate, but not towards the second, ATP. The inhibitor decreases the affinity of the enzyme for fructose-6-phosphate whereas the activators increase it [1]. The kinetic data can be fitted quantitatively by the equations derived from the concerted transition theory [2] if it is assumed that the enzyme molecule possesses four identical binding sites for each class of stereospecific ligand. As a consequence of this assumption, it was likely to infer that the enzyme was made up of four identical subunits.

In the present paper, ultracentrifuge studies, in dilute buffer or in 6 M guanidine hydrochloride, and disc gel electrophoresis experiments in the presence of 0.1% sodium dodecylsulfate are reported, leading to the conclusion that phosphofructokinase from *E. coli* is indeed an oligomeric protein made up of four subunits of identical molecular weight.

Phosphofructokinase has been purified through a new procedure different from that recently proposed by Griffin, Houck and Brand [3]; it includes phase partition, heat treatment, ammonium sulfate fractionation and DEAE-Sephadex column (Blangy, unpublished results). The specific activity of the final enzyme preparation was 195 units/mg *. Ultracentrifugation and polyacrylamide electrophoresis revealed the preparation to be homogeneous.

As shown in fig. 1a, in Tris-HCl buffer 0.1 M, pH 8.2, the enzyme sediments as a symmetrical peak. When the concentration of protein was varied from 0.5 to 5 mg/ml, no significant variation of the $s_{20,w}$ value was observed, the mean value being equal to 7.8 (fig. 1b).

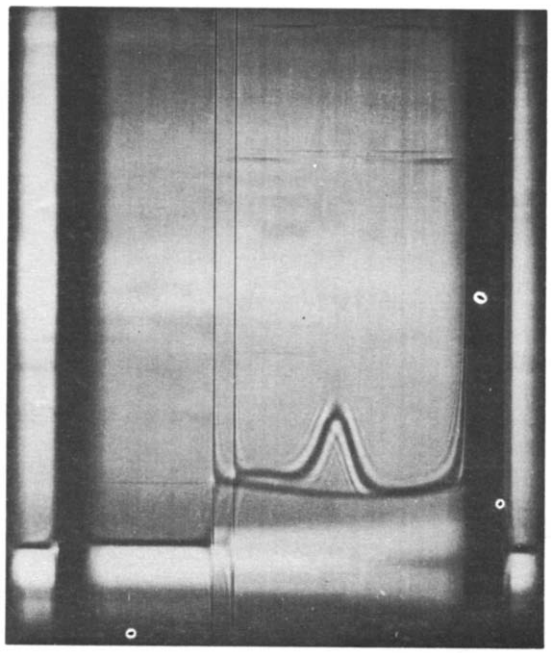

Fig. 1a. *Sedimentation pattern of phosphofructokinase from E. coli.* Same conditions as fig. 1b except: Protein concentration 5 mg/ml. Photograph was taken at a bar angle of 55°, 32 min after the rotor had reached the required velocity. This preparation was slightly contaminated (less than 5%).

* One unit of activity is defined as the amount of enzyme which catalyzes the formation of 1 μmole of fructose-1-6-diphosphate per minute under the conditions of the standard assay (see ref. [3]).

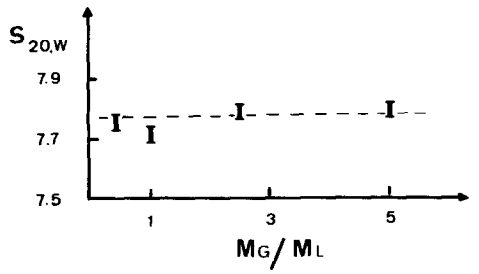

Fig. 1b. *Sedimentation velocity as a function of enzyme concentration.* Sedimentation velocity was determined in a Spinco ultracentrifuge Model E equipped with an automatic scanning attachment. A double sector cell was employed. Measurements were made every 4 min after the rotor had reached the required velocity. Buffer: Tris-HCl 0.1 M, pH 8.2, with NaCl 0.3 M, $MgCl_2$ 10^{-2} M and EDTA 10^{-3} M. Temperature 25°C. Velocity: 52,000 rpm.

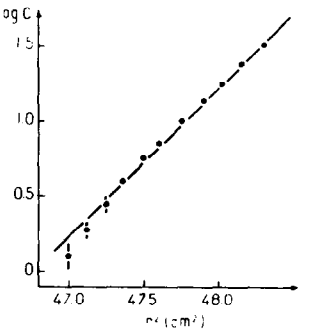

Fig. 2. *Sedimentation equilibrium.* Same conditions as fig. 1. A small amount of FC-43 was added to provide a clear interface at the bottom of the cell. The height of the column of protein was 3 mm. Protein concentration: 0.5 mg/ml. Velocity: 17,000 rpm. After 15 hr, equilibrium was attained and schlieren photographs were taken at a bar angle of 80°. Equilibrium sedimentation profiles were analyzed with a Precision Grinding Ltd. microcomparator.

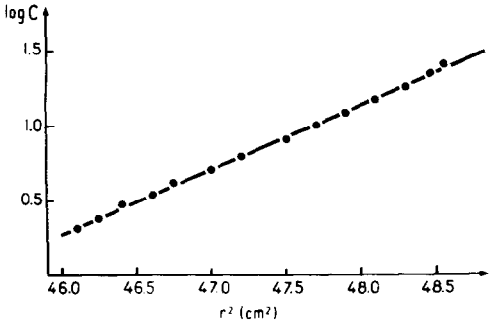

Fig. 3. *Sedimentation equilibrium in 6 M guanidine hydrochloride.* Phosphofructokinase (0.2 mg/ml) was dialyzed for 36 hr against 6 M guanidine hydrochloride in Tris buffer 0.1 M, pH 8.2, with NaCl 10^{-2} M, EDTA 10^{-2} M and β-mercaptoethanol 10^{-2} M. Sedimentation was performed in the same buffer, at 25°C. Velocity: 28,000 rpm. After 48 hr equilibrium was attained and equilibrium sedimentation profile was obtained by means of the scanner.

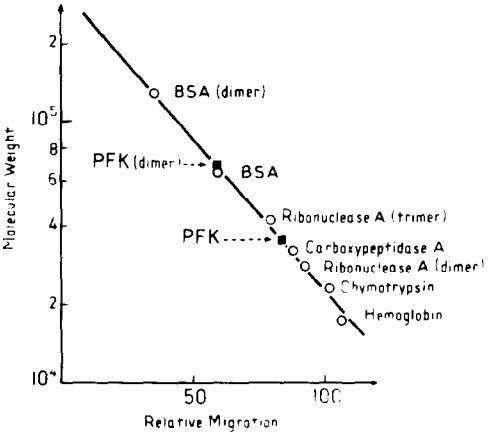

Fig. 4. *Molecular weight estimation of phosphofructokinase by electrophoresis in SDS-polyacrylamide gels.* Proteins (1 mg/ml) were dialyzed for 6 hr against 0.1 M phosphate-Na, pH 7.1, with sodium dodecylsulfate (SDS) 1% and β-mercaptoethanol (β-Me) 1% at 37°C, and for 16 hr against 0.01 M phosphate-Na, pH 7.1, with SDS 0.1% and β-Me 0.1%; 0.1 ml of protein solution was then mixed with 25 µl of glycerin and layered on the top of 5% polyacrylamide – 0.1% gel prepared according to Maizel [7]. Electrophoresis was performed at room temperature for 4 hr at 7 V/cm. The migration of bromophenol blue was used as the reference. The gels were fixed in 20% sulfosalicylic acid for 16 hr, stained with 0.25% Coomasie Blue for 5 hr and destained with 7% acetic acid.

Using high speed sedimentation equilibrium [4] under the same conditions, and assuming a value of 0.75 for the partial specific volume, we found for the molecular weight a value of 142,000 ± 7,100 (fig. 2).

In 6 M guanidine hydrochloride, phosphofructokinase sediments as a single symmetrical peak, indicating the homogeneity of the subunits.

Assuming that the partial specific volume of the protein is the same in 6 M guanidine hydrochloride as in dilute buffer [5], the molecular weight of the subunit calculated from sedimentation equilibrium profile was found equal to 36,500 ± 1,800 (fig. 3).

Disc gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate [6] reveals two protein bands, the molecular weight of which are respectively 35,000 ± 2,000 and 68,000 ± 4,000 (fig. 4). In view of the agreement between these data and the results of ultracentrifugation in 6 M guanidine, we identified the lower molecular weight species with the fully dissociated protein; the higher molecular weight component could be interpreted as a dimeric form of the enzyme stabilized by disulfide bonds resulting from the treatment of the protein which had not been previously carboxymethylated.

The results reported here show that phosphofructokinase from *E. coli* is an oligomeric protein made up of four subunits of identical molecular weight. As indicated at the beginning, the kinetic data could be quantitatively accounted for by the concerted transition theory [2] assuming the enzyme to be a tetramer and the transition to be entirely concerted. However, it had been shown that almost all the results could also be fitted assuming a hexameric molecule in which the transition would be partially concerted (i.e. a molecule which could exist to some extent in a hybrid state). The present data, which are in perfect agreement with the structure postulated on the basis of kinetic data only, permit to confirm the hypothesis of a fully concerted transition occurring in a tetrameric enzyme.

Further studies including a direct determination of the number of binding sites should allow a more complete description of the relations between the molecular structure of the protein and its regulatory interactions.

References

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