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Purification of rabbit liver phosphofructokinase and its properties under simulating *in vivo* conditions

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Abstract. Phosphofructokinase (EC 2.7.1.11) from rabbit liver was purified to homogeneity. Preincubation of enzyme results in nonlinearity of enzyme activity with enzyme concentration. Therefore $K_{0\cdot5}$ of enzyme for fructose 6 phosphate in the absence or presence of fructose 2,6 bisphosphate or polyethylene glycol or in the presence of both was determined at physiological concentrations of its various effectors by taking the initial rate obtained by adding the enzyme last. They decrease the $K_{0\cdot5}$ value from 4·1 mM to about 0·2mM. The $K_{0\cdot5}$ of enzyme for fructose 2,6 bisphosphate was also determined under the above conditions. It is about 4·3 μM. Transient kinetics of phosphofructokinase at varying concentrations of enzyme in the presence of fructose 2,6 bisphosphate or polyethylene glycol or in the presence of both were studied. It was found that although they decrease $t_{1/2}$ *i.e.* the time to reach half the maximal steady rate by about 5–8 fold, it was about constant at varying concentrations of the enzyme. These results indicate that fructose 2,6 bisphosphate and polyethylene glycol decrease $K_{0\cdot5}$ of the enzyme for fructose 6 phosphate not by associating the enzyme to higher aggregates, but by a different mechanism.

Keywords. Phosphofructokinase; glycolysis; fructose 2, 6 bisphosphate.

Introduction

Many reports have appeared in the last 3 years on the mechanism of regulation of liver phosphofructokinase [adenosine 5'–triphosphate (ATP): D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11] (Hers and Van Schaftingen 1982, Uyeda *et al*., 1982; Pilkis *et al.,* 1982a). Van Schaftingen *et al.* (1981) and Uyeda *et al.* (1981) suggested that fructose 2,6 bisphosphate ($F2,6P_2$) in liver which is most effective metabolite in activating phosphofructokinase is sufficient to keep phosphofructokinase active under *in vivo* conditions, while in its absence, Reinhart and Lardy (1980a) had shown that phosphofructokinase was hardly active at concentrations of ATP, fructose 6 phosphate (F6P) and other effectors corresponding to their *in vivo* levels in the liver at pH 7·0, and 37°C.

All the above studies on this enzyme were done by preincubating the enzyme at μg

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Abbreviations used: $F2,6P_2$, Fructose 2,6 bisphosphate; F6P, fructose 6 phosphate; SDS, sodium dodecyl sulphate; EDTA, ethylenediaminetetra acetic acid; $F1,6P_2$, fructose 1,6 bisphosphate; BSA, bovine serum albumin; AMP, adenosine 5'–monophosphate; P_i, inorganic phosphate; NADH, nicotinamide adenine dinucleotide adenine; ADP, adenosine 5'–diphosphate.

protein range for 2 min or 4 min (Pilkis *et al.,* 1982b) in the assay mixture, and taking the initial rate after addition of F6P, or taking the rate after the first 5 min after starting the reaction (Van Schaftingen *et al*., 1981). No information was available whether under these conditions the enzyme activity was proportional to its concentration without which the information thus collected cannot be extrapolated to *in vivo* conditions, where the enzyme is present at many times the concentration of the enzyme in the cuvette. This information acquires importance since it was shown earlier by Tejwani (1973), that the rate of liver phosphofructokinase was not proportional to enzyme concentration at nonsaturating F6P concentration in the absence of its positive effectors, and the $K_{0.5}$ of the enzyme for F6P through alteration of which the enzyme is basically regulated (Ramaiah, 1974) is markedly altered by preincubation of enzyme (Ramaiah and Tejwani, 1973).

Therefore, in the present paper, the enzyme from rabbit liver was purified to homogeneity, and the conditions under which the activity is proportional to enzyme concentration were determined. The enzyme activity was proportional to enzyme concentration when it is estimated in the presence of various effectors of the enzyme at their physiological concentrations. The $K_{0\cdot5}$ of the enzyme for F6P in the absence or presence of $F2,6P_2$, or polyethylene glycol or in the presence of both was determined at physiological concentrations of its effectors and temperature. Reinhart (1983, 1980) suggested that $F2,6P_2$ and polyethylene glycol associate phosphofructokinase beyond tetramers, and thus decreases $K_{0\cdot5}$ of the enzyme for F6P. This could be verified by studying the effect of $F2,6P_2$ and polyethylene glycol on transient kinetics of phosphofructokinase. Therefore the effects of $F2,6P_2$ and polyethylene glycol on the transient kinetics of the enzyme were studied. Based on these studies, it is concluded that the phosphofructokinase activity under simulating *in vivo* concentrations of many of its effectors corresponds closely to the glycolytic rate in the normal liver, and that polyethylene glycol and $F2,6P_2$ decrease $K_{0\cdot5}$ of the enzyme for F6P perhaps by a common mechanism of stabilising the active form of the enzyme, rather than by associating the enzyme beyond tetramers.

Materials and methods

Materials

All chemicals used were of analytical grade. The biochemicals, polyethylene glycol 8000 and sodium dodecyl sulphate (SDS) were obtained from Sigma Chemical Company, St. Louis, Missouri, USA.

Rabbit livers

Rabbits were fed with laboratory diet *ad lib.* and were sacrificed by injecting air into the ear veins. Livers were quickly removed, blotted and given a quick rinse with homogenisation buffer, and then used for purification, or stored at –70°C till further use.

Composition of various buffers

Homogenization buffer: Contained 50mM Tris-HCl, 50mM β-mercaptoethanol and 5 mM Ethylenediaminetetra acetic acid (EDTA) at pH 8·0.

Suspension buffer: Contained the same as homogenization buffer, except EDTA and, in addition, 5mM $MgCl_2$,0·1 mM fructose 1,6 bisphosphate ($F1,6P_2$) and 0·35 mM ATP.

Dilution buffer: Contained 50mM Tris-HCl, 1M $(NH_4)_2SO_4$, 2mM ATP and 140mM β-mercaptoethanol at pH 8·0

Storage buffer: Contained 50mM Tris HCl, 0·7 M $(NH_4)_2SO_4$, 10 mM K_2HPO_4, 10 mM ATP, and 0·33 mM EDTA at pH 7·5.

Methods

Phosphofructokinase activity was determined as described earlier (Ramaiah and Tejwani, 1973). The auxiliary enzymes (200 μl aldolase+150 μl triosephosphate isomerase and α-glycerophosphate dehydrogenase were of Sigma Chemical Company products, No. A.6523 and G-1881 respectively) were dialyzed overnight at 0–4°C against 800 ml of 10mM Tris-HCl buffer pH 8·0 during which time the buffer was changed once to free them of $(NH_4)_2SO_4$. The amount of the auxiliary enzymes that were used to assay phosphofructokinase were sufficient to ensure that the lag observed at high concentration of F6P were not due to any limitation of these enzymes. Reaction was started by addition of the enzyme. The enzyme was applied on a perspex spatula and then stirred into the assay mixture in the cuvette. The time taken for stirring and closing the cuvette chamber was about 3 to 5 sec. The specific activity of phosphofructokinase is expressed as units per mg of protein, where 1 unit is defined as the formation of 1 μmol of $F1,6P_2$ per min, which was computed using the molar extinction coefficient of NADH as 6270.

Lyopholized $F2,6P_2$ kindly supplied by Van Schaftingen and Hers was estimated by hydrolyzing it to F6P which was estimated using excess commercial muscle phosphofructokinase as described by Van Schaftingen and Hers (1981).

Protein estimation: Protein was estimated by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin as standard.

SDS- polyacrylamide gel electrophoresis

Phosphofructokinase and marker proteins (thyroglobulin, bovine serum albumin and ovalbumin) at final concentrations of 0·5 mg/ml were incubated for 2h at 37°C in 50 mM sodium phosphate buffer, pH 7·1, containing 1% SDS and 1% β-mercaptoethanol. 25μg protein samples were applied on the gels along with Bromophenol Blue and a drop of glycerol. Electrophoresis in 5 % gel was performed at 5 m amperes/gel (Weber and Osborn, 1969).

Staining and destaining procedure: At the end of the run, the gels were immersed in 5% trichloroacetic acid for 12 h and later stained with Coomassie Brilliant Blue. Destaining was done with 20 % methanol and 7 % acetic acid solution.

Results

All operations, unless otherwise mentioned, were carried out at 0–4°C.

Purification scheme

Homogenization: About 200g of fresh or frozen livers were thawed, minced with scissors, and homogenized in two volumes of homogenization buffer in a Waring Blendor for 2×30 sec. The homogenate was centrifuged in an SS-34 rotor at 20,000 *g* for 45 min.

Heat-alcohol step: 1 ml of *β*-mercaptoethanol (14·29M) was added per 100ml of homogenate supernatant. After mixing, 20 ml of 95 % alcohol per 100 ml of the above solution was added slowly, and the solution was kept in a water bath at 41–42°C for 45 min with occasional stirring. At the end of the incubation, it was centrifuged in an SS-34 rotor at 20,000 *g* for 45 min and the clear, reddish supernatant was filtered through Whatman No. 1 filter paper. The enzyme can be stored at –70°C at this step without loss of much of its activity for at least 12 h.

Magnesium chloride precipitation: The filtrate from the previous step was cooled to – 3°C, and 1 M $MgCl_2$ was added slowly to a final concentration of 50 mM while the solution was constantly stirred. It was kept at –3°C for 30 min and then centrifuged in an SS-34 rotor at – 3°C at 20,000 *g* for 45 min. The brown pellets obtained were pooled and suspended in 2 ml of suspension buffer per 100 g of wet weight of tissue.

Differential centrifugation: The suspension obtained in the preceding Step 3 was spun for 1 h in rotor Ti 55·2 at 34,000 *g*. The precipitate thus obtained had negligible activity and was discarded. The supernatant was further centrifuged at 105,000 *g* for 1 h.

Extraction of phosphofructokinase by F6P: The pellet obtained in step 4 was gently swirled with a solution of 2 mM F6P (0·2 ml per 100 g of original weight wet tissue) for about 20min and the supernatant was gently pipetted out. This procedure was repeated once more and the supernatants were pooled. Most of the phosphofructo-kinase in the pellet was thus removed. The enzyme can be stored at this step at –70°C overnight without any loss of activity.

Precipitation of phosphofructokinase: The supernatant thus obtained in Step 5 was diluted four times with dilution buffer and centrifuged in rotor Ti 55·2 at 105,000 *g* for 45 min. The pellet so obtained was gently swirled with 2mM F6P solution as described in Step 5. Steps 5 and 6 were thus repeated four times which results in the further purification of phosphofructokinase.

$(NH_4)_2SO_4$ *Precipitation*: The pellet obtained in Step 6 was suspended in storage buffer (0·2 ml per 100 g original wet weight.tissue). This was diluted 6 times with a buffer containing 1·8 M $(NH_4)_2SO_4$, 50 mM Tris HCl, 140 mM *β*-mercaptoethanol at pH 8·0, so that the final $(NH_4)_2SO_4$ concentration was 1·5 M. This was kept at 0°C for 30 min with occasional stirring, and then centrifuged in rotor Ti 55·2 at 20,000 *g* for 20 min. The precipitate obtained was suspended in storage buffer (0·2 ml per 100 g of wet tissue) and distributed into small tubes and stored at –70°C until used. A typical

purification data are presented in table 1. The specific activity is about 100 units/mg protein.

Table 1. Purification of phosphofructokinase from rabbit liver.

Fraction	Volume (ml)	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification fold	Recovery (%)
Homogenate supernatant	412	19809	657	0·033	1	100
Heat alcohol filtrate	335	3350	534	0·16	5	81
Magnesium chloride precipitate suspension	3·4	57·46	342	6·00	182	52
First 2 mM F6P extract	2·4	13·84	322	23·00	697	49
$(NH_4)_2SO_4$ precipitate suspended in storage buffer	0·4	1·60	157	98·00	2970	24

Attempts were first made to purify rabbit liver phosphofructokinase according to the method of Massey and Deal (1973). However, it could not be purified beyond a specific activity of 6–11 by this method. Steps 1 to 3 were essentially the same as described by Massey and Deal (1973), except that ATP concentration in suspension buffer has to be 0·35 mM instead of 0·1 mM ATP for the stability of the enzyme and the 3rd step was done at –3°C since otherwise the enzyme was inactivated.

Stability of the purified enzyme

The enzyme stored as described above, loses about 20 % of its original activity within a week. However, there was no further loss of activity for at least one month of storage.

Homogeneity and the size of the subunit of phosphofructokinase

SDS gel electrophoresis of the enzyme showed a single band. The subunit molecular weight of rabbit liver phosphofructokinase determined as described by Weber and Osborn (1969) using ovalbumin, thyroglobulin, and bovine serum albumin (BSA) as standards, was found to be 80,000 (data not shown).

Physiological conditions for measuring phosphofructokinase activity

In view of the enzyme concentration dependent changes on $K_{0.5}$ value of the enzyme for F6P (Ramaiah and Tejwani, 1973), one should study the properties of the enzyme at 50 μg protein enzyme per ml, which is the estimated concentration of the enzyme in the cell (Reinhart and Lardy, 1980b), so as to apply the information directly to *in vivo* conditions. However, the rate will be too fast and cannot be measured accurately. But if the enzyme at physiological concentration and in the presence of its various effectors at physiological concentrations was added to an enzyme assay mixture and initial rates were taken, the properties of the enzyme at that enzyme concentration can be assumed to be true under *in vivo* conditions as well. The assay mixture described in table 2 was

Table 2. Physiological concentrations of various affectors of phosphofructokinase in the liver which were used in the assay mixture.

ATP	3 mM	Bergmeyer (1974)
Citrate	0·2 mM	
F6P	0·1 mM	
AMP	1·0 μM	Uyeda *et al.* (1981)
P_i	5·0 mM	Veech *et al.* (1970)
$MgCl_2$	5·0 mM	Reinhart and Lardy (1980a)
KCl	120·0 mM	
Phosphofructokinase	50·0 μg/ml	Reinhart and Lardy (1980b)

taken as close to the physiological concentrations of various effectors of phosphofructokinase except for ADP, $F1,6P_2$ and $F2,6P_2$, which were not included in the routine assay of enzyme.

Enzyme activity was proportional to enzyme concentration

The enzyme from the storage buffer was first diluted to 50μg protein/ml of physiological assay mixture (table 2) at 0°C and to this was added 3·8 μM $F2,6P_2$. Various aliquots of this enzyme solution were added last to the assay mixture containing 3·8 μM $F2,6P_2$ and the initial rate was plotted against enzyme concentration as shown in figure 1. The activity is proportional to the enzyme concentration under

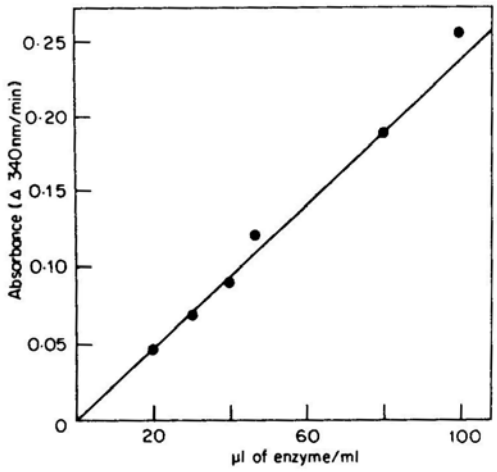

Figure 1. Enzyme activity was assayed at 37°C with different concentrations of the enzyme in the presence of 3·8 μM $F2,6P_2$ in physiological assay mixture which contained at final concentrations 50mM Tris-HCl pH 7·0,5mM $MgCl_2$,5mM P_i, 120mM KCl,0·2mM citrate, 1 μM AMP,0·1 mM F6P, 3 mM ATP, 0·2 % BSA, 0·2 mM NADH and dialyzed aldolase, α-glycerophosphate dehydrogenase and triose phosphate isomerase as described in methods. The enzyme was diluted to 50 μg protein/ml in the above physiological assay mixture excluding F6P at 0°C and was used as an enzyme source. Rate in the 1st one min after addition of enzyme was plotted against enzyme concentration.

these conditions. In the absence of $F2,6P_2$ the enzyme activity could be measured only at 5 μg protein or above/ml of physiological assay mixture. The rate was found to be proportional to the enzyme concentration.

$K_{0.5}$ of enzyme for F6P

The activity of enzyme at fixed concentration of enzyme was estimated at varying concentrations of F6P under conditions described in figure 1, with or without a fixed concentration of 3·8 μM $F2,6P_2$ or 25 μM $F2,6P_2$, or 20% polyethylene glycol, and plotted as shown in figure 2. It can be seen that 20 % polyethylene glycol or 3·8 μM

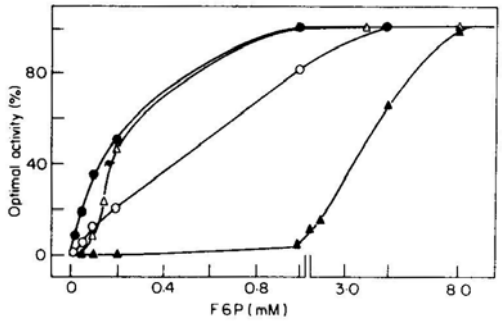

Figure 2. $K_{0.5}$ value of the enzyme for F6P in physiological assay mixture as described in figure 1 (rate in the 1st one min of enzyme activity was plotted).
(▲), No $F2,6P_2$ or polyethylene glycol; (O), 3·8μM $F2,6P_2$;(●),25μM $F2,6P_2$;(Δ),20% polyethylene glycol (BSA was not added whenever polyethylene glycol was used in physiological assay mixture).

The enzyme which was diluted as described in figure 1 was used as the enzyme source in the graph (▲), In other cases (O), (●), (Δ), the enzyme contained in addition 3·8 μM $F2,6P_2$ or 25 μM $F2,6P_2$ or 20% polyethylene glycol respectively.

$F2,6P_2$ or 25 μM $F2,6P_2$ lower the $K_{0.5}$ values of enzyme for F6P by about 7–18 fold. The $K_{0.5}$ value for F6P is 4·1mM in the absence of $F2,6P_2$ or polyethylene glycol. It is reduced to 0·58 and 0·2mM in the presence of 3·8 μM or 25 μM $F2,6P_2$ respectively. While it is 0·22 mM in the presence of 20 % polyethylene glycol or in the presence of both polyethylene glycol and 25 μM F2,6P2 (the data in the presence of both not shown). In the physiological range of F6P *i.e.* 0·1 mM F6P (Bergmeyer, 1974) and other conditions as described in figure 2 the enzyme activity is about 30 % of its maximal activity showing thereby that the glycolytic rate will be quite considerable under physiological conditions in contrast to zero values expected based on the observations in figure 2 and of Reinhart and Lardy (1980a) before the discovery of $F2,6P_2$ by Van Schaftingen *et al.* (1980) and Uyeda *et al.* (1981).

$K_{0.5}$ value of enzyme for $F2,6P_2$

The activity of the enzyme was measured at varying concentrations of $F2,6P_2$ under conditions described in figure 1 with a fixed concentration of F6P at 0·1 mM and the

data were plotted as described in figure 3. It can be seen that the curve is sigmoidal and the $K_{0.5}$ is about 4·3 μM.

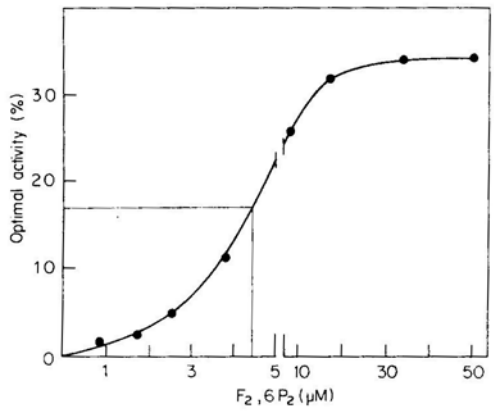

Figure 3. $K_{0.5}$ value of enzyme for F2,6P_2 in physiological assay mixture. The conditions of assay were the same as in figure 1 except that F6P was fixed at 0·1 mM and F2,6P_2 was varied (rate in the 1st one min of enzyme activity was taken).

Acceleration of rate of reaction at high concentration of F6P

The results described so far were done after prior dilution of enzyme to 50 μg protein/ml and following the initial reaction after the addition of the enzyme last. The initial activity is maximum at low F6P concentrations, while at high F6P, there was acceleration of activity with time. Similar results were obtained earlier for the partially purified enzyme (Ramaiah and Tejwani, 1973) and for the pure enzyme from rat liver (Reinhart and Lardy, 1980a). In the literature many such results were explained as due to dissociation of the enzyme as a result of dilution, and that substrate induced acceleration is related to the association of the enzyme (Frieden, 1970). Recently, Reinhart (1983) suggested that F2,6P_2 may activate phosphofructokinase through its effect on promoting aggregation of the enzyme. But this was studied on enzyme labeled with pyrene butyrate, and assuming that polarisation values of fluorescence reflect the overall aggregation state of the enzyme (Reinhart, 1983). A study of whether such association of the enzyme was responsible for the acceleration of the enzyme activity, was done with pure enzyme preparation by measuring its activity at varying short time intervals after addition of the enzyme. The rate at any instant of time was obtained by drawing a tangent at that point on a recorder tracing of the enzyme activity curve. The data were plotted as described in figure 4. It is clear from the graph that it is a straight line indicating a first order process. Addition of F2,6P_2 or polyethylene glycol individually, or together decreases the $t_{1/2}$, *i.e.* the time required to reach half of the maximum steady state rate, by about 5–8 fold, and remains essentially unaltered over a five fold range of enzyme concentration as described in table 3.

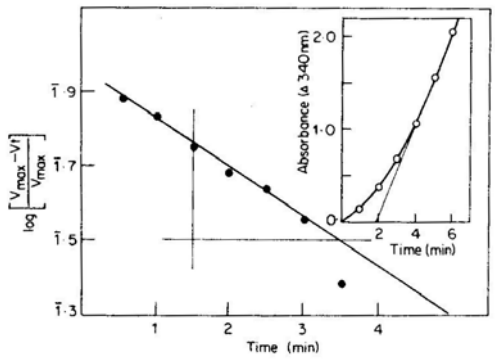

Figure 4. The enzyme was diluted as described in figure 1 and was added last to physiological assay mixture as described in figure 1 except that F6P concentration was at 5 mM. The V_t was taken from the slope of the tangent drawn from the reaction progress curve (*inset*) at any time t, the rate being ΔA340nm/min. V_{max} is the maximal steady rate under these conditions. Log $\frac{(V_{max} - V_t)}{V_{max}}$ was plotted against time t.

Table 3. Enzyme assays were carried out in physiological assay mixture as described in figure 1 except that F6P was present at 5 mM and with or without 25 μM $F2,6P_2$ or with 20 % polyethylene glycol, or with 25 μM $F2,6P_2$ and 20 % polyethylene glycol together, and various amounts of the enzyme diluted as described in figure 1 were added, $t_{1/2}$ *i.e.* the time required to reach half of the maximum steady state rate was calculated from reaction progress curve.

	$t_{1/2}$ values in minutes			
Concentration of Enzyme μg protein/ml	Physiological assay mixture	Physiological assay mixture with 25 μM $F2,6P_2$	Physiological assay mixture with 20% polyethylene glycol	Physiological assay mixture with 20% polyethylene glycol and 25 μM $F2,6P_2$
0·50	2·0	0·2	0·35	—
0·75	1·9	0·25	0·45	—
1·00	1·9	0·32	—	0·35
1·25	—	—	—	0·33
1·50	1·9	0·32	0·45	0·33
2·50	2·2	0·32	0·30	0·25

Discussion

Reinhart and Lardy (1980a) were the first to study kinetic properties of phosphofructokinase of liver under conditions close to physiological situations, and observed that at 3 mM ATP, physiological concentrations of positive effectors [$F1,6P_2$, AMP and inorganic phosphate (P_i)] and F6P, its activity was zero and suggested additional mechanisms to increase the enzyme affinity for F6P (1980b,c). However, this conclusion was reached before the discovery of $F2,6P_2$ by Van Schaftingen *et al.* (1980) and Uyeda *et al.* (1981) which is the most

effective activator of phosphofructokinase. Later, Van Schaftingen *et al.* (1981) and Uyeda *et al.* (1981) suggested that $F2,6P_2$ present in the liver would keep the phosphofructokinase sufficiently active, but their data cannot be extrapolated to *in vivo* situation in view of the lack of information on enzyme proportionality and due to enzyme incubation induced changes in $K_{0.5}$ of the enzyme for F6P (Ramaiah and Tejwani, 1973). In addition estimation of $F2,6P_2$ by more sensitive method of Van Schaftingen *et al.* (1982), indicated its value to be 6 μM in the liver of fed rat (Hue *et al.* 1983) rather than 27 μM as estimated by Kuwajima and Uyeda (1982), or 15 μM as mentioned by Van Schaftingen *et al.* (1981). In the present studies, it was shown that the enzyme activity was proportional to enzyme concentration under conditions described here. The $K_{0.5}$ of the enzyme for F6P and $F2,6P_2$ determined at 37°C, and at physiological concentrations of various effectors of the enzyme, and at 3·8 μM or 25 μM $F2,6P_2$ were shown to be in the range of 0·58 to 0·2 mM and 4·3 μM respectively. The glycolytic rate in the liver of fed animal is about 26% of the maximal activity of phosphofructokinase present in the liver (Reinhart and Lardy, 1980a). The activity of phosphofructokinase at 6 μM F2, $6P_2$ is about 23% of its maximal activity (figure 3), suggesting that $F2,6P_2$, if present at 6μM in the liver of fed rat, will be sufficient to explain the glycolytic rate in the liver of fed rat.

The role of phosphorylation-dephosphorylation of phosphofructokinase in its regulation is conflicting. The experiment of Sakakibara and Uyeda (1983) indicate that phosphorylated phosphofructokinase has higher $K_{0.5}$ value for F6P and for $F2,6P_2$, while these are contradicted by Pilkis *et al.* (1982b).

Reinhart (1980,1983) suggested that polyethylene glycol and $F2,6P_2$ may decrease $K_{0.5}$ of the enzyme for F6P by aggregating the enzyme to more than tetramers. But the constant $t_{1/2}$, *i.e.* the time to reach half the maximal steady rate, when rate was measured by the addition of saturating concentration of F6P in the presence of polyethylene glycol or $F2,6P_2$ or in the presence of both at varying concentrations of the enzyme indicate that perhaps no association of the enzyme was responsible for the 5–8 fold decrease in $t_{1/2}$ in their presence as compared to the $t_{1/2}$ in their absence (table 3). The studies on enzyme crosslinked with glutaraldehyde in the presence of its activators or at inhibitory concentration of ATP indicate also that association of enzyme may not be responsible for the acceleration of activity when the reaction was started by the addition of enzyme at high concentration of ATP and F6P (G. Roman Reddy and A. Ramaiah, unpublished results). Polyethylene glycol may not bind to the enzyme unlike that of $F2,6P_2$, but yet may favour the active conformor of enzyme with low $K_{0.5}$ for F6P. Polyethylene glycol may do so by preventing the enzyme to a great extent to exist in the form with high $K_{0.5}$ for F6P, and thus favour the other form to exist to a larger extent. The observations of Lee *et al.* (1981) on lysozymes led them to suggest that polyethylene glycol may help to maintain certain conformations of proteins under conditions in which proteins might otherwise assume other structures.

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Hers and his colleagues, Laboratoire de Chimie Physiologique, Universite de Louvain and International Institute of Cellular and Molecular Pathology, VCL-75·39, avenue Hippocrate 75, B-1200 Bruxelles, Belgium. Preliminary experiment on the purification of phosphofructokinase of rabbit liver was carried out by Jasminder Kaur.

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