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INTERACTION OF D-FRUCTOSE AND FRUCTOSE 1-PHOSPHATE WITH YEAST PHOSPHOFRUCTOKINASE AND ITS INFLUENCE ON GLYCOLYTIC OSCILLATIONS

KARLHEINZ KREUZBERG

University of Bonn, Institute of Botany, D-53 Bonn (G.F.R.)

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Summary

Fermentation of D-fructose- and D-glucose induced glycolytic oscillations of different period lengths in *Saccharomyces carlsbergensis*. Recent studies suggested, that D-fructose or one of its metabolites interacted with phosphofructokinase (ATP:D-fructo-6-phosphate 1-phosphofructokinase, EC 2.7.1.11), the core of the glycolytic 'oscillator'. In order to explore the kinetics of interaction, the influence of D-fructose and fructose 1-phosphate on purified yeast phosphofructokinase was studied. D-fructose concentrations up to 0.3 mM stimulated the enzyme, while a further increase led to competitive inhibition. The Hill coefficient for fructose 6-phosphate decreased from 2.8 to 1.0. Fructose 1-phosphate acted in a similar way, up to 1 mM activation and inhibtion competitive to fructose 6-phosphate at higher concentration (2.0—3.5 mM) with the same effect on the Hill coefficient. The inhibition patterns obtained with D-fructose or fructose 1-phosphate suggest a sequential random reaction mechanism of yeast phosphofructokinase with fructose 6-phosphate and $MgATP^{2-}$. The mode of interaction of phosphofructokinase with D-fructose and fructose 1-phosphate is discussed. The influence of both effectors resulted in altered enzyme kinetics, which may cause the different period lengths of glycolytic oscillations.

Introduction

The discovery of phosphofructokinase as the key regulatory enzyme in glycolysis (ref. 1, also see review, ref. 2) and the recognition of its central role for controlling metabolite levels in fermenting yeast [3] and beef-heart extracts [4] stimulated investigations of its regulation. There is evidence that sustained glycolytic oscillations are generated by a periodic change of phosphofructokinase activity [5,6] caused by the cooperative interactions of both substrates

and effectors with the enzyme. After feeding yeast cells with D-fructose instead of D-glucose, different period lengths of NADH oscillations are obtained [7—9]. This effect is not connected with different fermentation rates for both hexoses [10]. By estimation of the specific radioactivity of glycolytic intermediates in yeast cells supplied with ^{14}C-labelled hexoses we recently showed that D-fructose is almost quantitatively metabolized via phosphofructokinase in glycolysis [11]. Statistical analysis of NADH oscillations induced with a mixture of D-fructose and D-glucose excluded the existence of more than one oscillating system in anaerobic glycolysis [12]. The different period lengths after feeding with either D-fructose or D-glucose suggested a direct interaction of the ketose with phosphofructokinase, which seemed possible, since D-fructose reaches up to 10 times higher intracellular concentrations than D-glucose [9] and fructose 1-*P* is accumulated during metabolism of D-fructose [11]. In a previous study, a direct influence of D-fructose on phosphofructokinase from baker's yeast was shown [13]. All recent results point to a direct interaction of D-fructose with phosphofructokinase, which could alter the period lengths in NADH oscillations. In order to elucidate the mode of this interaction in detail, the following basic questions have been answered: (a) Can fructose 1-*P* act as substrate of phosphofructokinase from brewer's yeast? (b) In what manner do D-fructose and fructose 1-*P* influence enzyme affinity for fructose 6-*P*? (c) Do D-fructose and fructose 1-*P* alter the reaction mechanism of the enzyme?

Material and Methods

Phosphofructokinase was purified from *Saccharomyces carlsbergensis* ATCC 9080 using a combination of the methods described earlier [14—17]. Frozen yeast cells were suspended in phosphate buffer (50 mM potassium phosphate, 2 mM EDTA, 2 mM 2-mercaptoethanol pH 6.9) and homogenized by 2 passes through a French pressure cell. If not mentioned otherwise, all steps were carried out at 4°C. The pH of the homogenate was adjusted to 6.9 after the addition of $(NH_4)_2SO_4$ (1 M final concentration) and 100 mg/l phenylmethylsulfonylfluoride. After removal of cell debris by centrifugation, solid $(NH_4)_2SO_4$ was added to the supernatant solution to 30% saturation and allowed to stand for 30 min. The suspension was centrifuged for 30 min at 20 000 × *g*. 1 mg per 20 mg protein from a freshly prepared 1.5% protamine sulfate solution at pH 6.9 was then added to the supernatant solution. A pellet was obtained by centrifugation for 60 min at 27 000 × *g*. After the supernatant solution was brought to 55% saturation with solid $(NH_4)_2SO_4$, the suspension was stored in ice overnight. The protein precipitate, collected by centrifugation, was dissolved in 40 ml phosphate buffer and desalted by gel filtration on Sephadex G-50. The desalted protein was mixed with Cibacron Blue-Sephadex G-100, prepared according to the method of Böhme et al. [18], to a concentration of 20U/g. After stirring for 60 min, the suspension was transfered to a Büchner funnel and washed with phosphate buffer, until no more protein was detectable in the filtrate. Phosphofructokinase was eluted from the absorbent by addition of 500 ml phosphate buffer/1.33 M $(NH_4)_2SO_4$ and then precipitated by addition of solid $(NH_4)_2SO_4$ to 80% saturation. The protein was

dissolved in phosphate buffer/1.33 M $(NH_4)_2SO_4$/3 mM fructose 6-*P* (pH 7.2). The preparation was passed through Sepharose 6-B column (15 × 100 cm) equilibrated with the same buffer. The fractions with phosphofructokinase activity were collected and the protein precipitated by addition of solid $(NH_4)_2SO_4$ (80% saturation). After centrifugation this pellet was dissolved in 4 ml of the latter buffer and could be stored frozen in liquid N_2 for at least 3 months with only a slight loss of activity. The preparation was free from contamination by enzymes which might interfere with the kinetic measurements (e.g. hexokinase, fructokinase, ketohexokinase or ketose 1-*P* aldolase).

A typical purification procedure, starting from 900 g frozen yeast cells, yielded 20 mg phosphofructokinase protein (Table I). Protein was determined according to the method of Lowry et al. [19] with bovine serum albumin as standard. Enzyme activity (fructose 1,6-P_2-formation) was measured by monitoring the oxidation of NADH in an Eppendorf photometer with recorder in a coupled assay with auxiliary enzymes (aldolase, triosephosphate isomerase and glycerophosphate dehydrogenase). All enzymes were dialyzed for 2 h against 50 mM Tris-HCl (pH 7.4) at 4°C before use in order to remove NH_4^+ and fructose 6-*P*. The standard reaction mixture (3 ml) contained 50 mM Tris-HCl, 20 mM KCl, 2 mM NADH and 0.06 ml dialyzed enzymes. Fructose 6-*P* and $MgATP^{2-}$ as well as the effectors, D-fructose and fructose 1-*P*, were added. $MgATP^{2-}$ concentrations were made by mixing 2 mM $MgCl_2$ and 1 mM Na_2ATP. The concentrations of $MgATP^{2-}$ and also ATP^{4-} were calculated from the stability constant (20 000 M^{-1}) for the $MgATP^{2-}$ complex [20—22]. Although the pH optimum of the purified enzyme was found at pH 6.9 in all kinetic studies a pH of 7.4 was used, because this was the optimum for the coupled assay system. The reaction was started by the addition of 0.01 ml enzyme suspension (30 ng/ml). The reaction velocity was expressed in enzyme units (μmol fructose 1,6-P_2/min) at 25°C. The initial velocities were estimated after Neuer [23] and all kinetic data computed by the method of Cleland [24]. Nucleotides, fructose 1-*P*, fructose 6-*P* and the auxiliary enzymes aldolase, triosephosphatisomerase and glycerophosphat dehydrogenase were purchased

TABLE I

PURIFICATION OF YEAST PHOSPHOFRUCTOKINASE

Preparation steps	Total protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Purification
French press	60 416	14 500	0.24	1
Ammonium sulfate 30%	35 370	11 318	0.32	1.3
Protaminesulfate	15 378	9 842	0.64	2.6
Ammonium sulfate 55%	3 500	8 039	2.30	9.6
Cibacron Blue	290	5 645	19.40	80.8
Sepharose 6-B	85	3 842	45.20	188.3
Ammonium sulfate 37%	20	1 440	72.00	300.0

from Boehringer, Mannheim Corporation. Buffers and other reagents (p.a. or highest purity grade) were products of Merck, Darmstadt. Sepharose 6-B and Sephadex were obtained from Pharmacia, Uppsala, Cibacron Blue F 3G-A was a kind gift from Ciba Corporation, Basel. D-Fructose and fructose 1-*P* were found pure by thin layer chromatography.

Results

Substrate specificity

The different ability of baker's and brewer's yeast to produce glycolytic oscillations may reside in differences in the sensitivity of phosphofructokinase to effectors which could be detected by comparison of the enzymes from both sources. The michaelis constants for the substrates fructose 6-*P* (0.26 mM) and $MgATP^{2-}$ (0.025 mM) of our enzyme do not differ distinctly from those reported by other authors for the protein from baker's yeast [14,25—27]. However, there is a significant difference in the phosphorylation of fructose 1-*P* instead of fructose 6-*P* as substrates. Our enzyme preparation phosphorylated fructose 1-*P* at a maximum rate of 3% compared to fructose 6-*P* (K_m fructose 1-*P* 2.5 mM) whereas the enzyme from baker's yeast showed only 0.5% [27] and the rabbit muscle one 5% [28] under the same conditions. Since there is no catalyst for the transformation of fructose 1-*P* to fructose 6-*P* as Uyeda [28] has shown for the rabbit muscle enzyme, phosphofructokinase has to catalyze the phosphorylation of fructose 1-*P* to fructose 1-P_2 as well. The low phorphorylation rate of fructose 1-*P* indicates a lower affinity to a common binding site for fructose 1-*P* and fructose 6-*P*.

Influence of D-fructose

As shown in Fig. 1, phosphofructokinase activity is stimulated in the presence of 0.1 mM $MgATP^{2-}$ and fructose 6-*P* from 0.2 to 1.0 mM by D-fructose

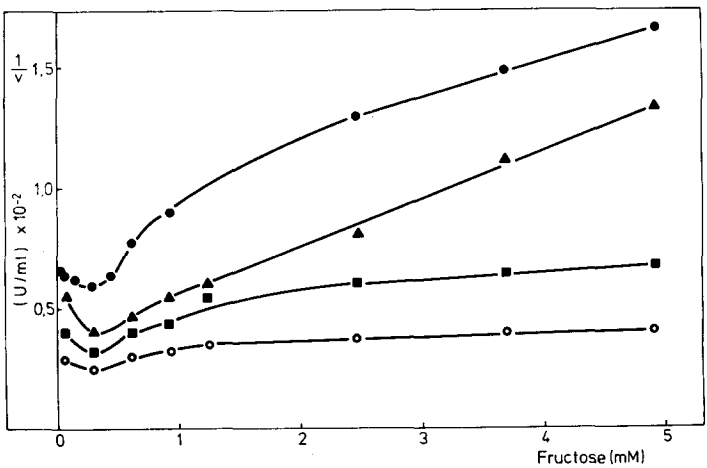

Fig. 1. Influence of D-fructose. Plot of the reciprocal activity of phosphofructokinase showing the influence of D-fructose in the presence of (●) 0.2; (▲) 0.3; (■) 0.5 and (○) 1.0 mM fructose 6-*P*. The concentration of $MgATP^{2-}$ was constant at 0.1 mM.

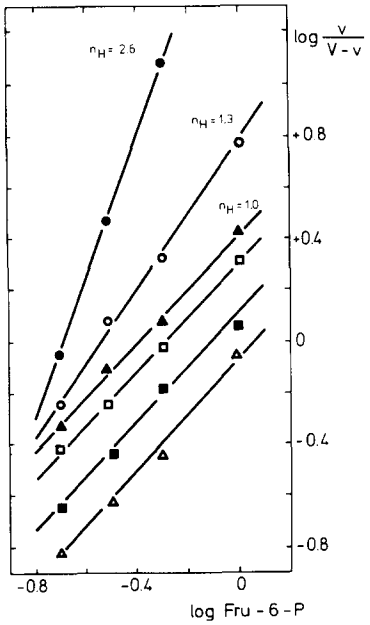

Fig. 2. Hill plot of the phosphofructokinase initial velocities in dependence of added D-fructose concentrations as (●) 0.0; (○) 0.3; (▲) 0.6; (□) 1.2; (■) 3.7 and (△) 5.0 mM. $MgATP^{2-}$ concentration was 0.1 mM. The resulting Hill coefficients are indicated in the graph.

up to 0.3 mM, whereas at concentrations higher than 0.5 mM, D-fructose starts to inhibit competitively with regard to the substrate fructose 6-*P* (Fig. 5A). Therefore, increasing fructose 6-*P* concentrations up to 1.0 mM can overcome inhibition and a slight stimulation persists. In order to test, whether or not D-fructose interacts directly with the fructose 6-*P* site, the data of the initial velocities determined with several fixed effector concentrations (0.0—5.0 mM) and variable fructose 6-*P* (0.2—1.0 mM) were plotted in a Hill plot (Fig. 2). There is a distinctly higher Hill coefficient ($n_H = 2.6$) in the absence of D-fructose, which was lost with increasing concentrations. The Hill coefficient decreased to $n_H = 1$ with D-fructose higher than 0.3 mM, further increase beyond 0.5 mM was without influence on the Hill coefficient.

Influence of fructose 1-P

Fig. 3 illustrates the complex influence of fructose 1-*P* on fructose 6-*P* phosphorylation. At low fructose 6-*P* concentrations (0.05 mM) fructose 1-*P* up to 1 mM stimulates the enzyme activity, whereas at higher fructose 6-*P* concentrations (0.5 mM) stimulation is lost. Conversely, at high fructose 1-*P* concentrations (2.0 to 3.5 mM) and fructose 6-*P* concentration of 0.05—0.1 mM the phosphorylation rate is lowered. This inhibition is released either by higher substrate concentrations ([fructose 6-*P*] = 0.5 mM) or by further increase of fructose 1-*P*, the latter of which seems to be caused by phosphorylation of this component. Beyond fructose 6-*P* concentrations of 0.5 mM, fructose 1-*P* is without any influence.

Inhibition by fructose 1-*P* is competitive with regard to fructose 6-*P* (Fig.

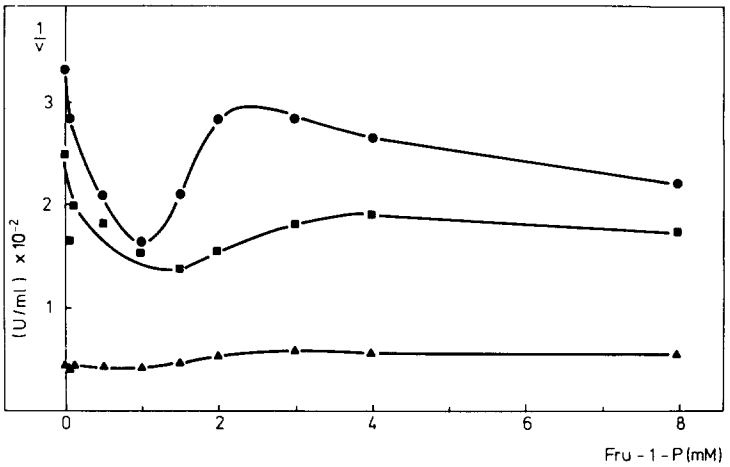

Fig. 3. Influence of fructose 1-*P*. Plot of the reciprocal activity of phosphofructokinase showing the influence of fructose 1-*P* in the presence of (●) 0.05; (■) 0.1 and (▲) 0.5 mM fructose 6-*P*. The concentration of $MgATP^{2-}$ was constant at 0.1 mM.

5C; K_i = 2.5 mM). Since apparently both molecules bind to the same site, it is probable that the different configuration of the effector fructose 1-*P* would influence the protein conformation too. This assumption is supported by the data summarized in Fig. 4, where fructose 1-*P* was kept constant at several concentrations and fructose 6-*P* was varied. The slopes show a decreasing Hill coefficient at inhibitionary fructose 1-*P* concentrations (n_H = 1.3 to n_H = 0.9) which is similar to the influence of D-fructose shown above. At fructose 6-*P* 0.05 mM fructose 1-*P* seems to decrease the Hill coefficient even below 0.9.

Reaction mechanism

The mechanism of yeast phosphofructokinase with fructose 6-*P* and $MgATP^{2-}$ as substrates was supposed to be Ping Pong Bi Bi [29]. The use of

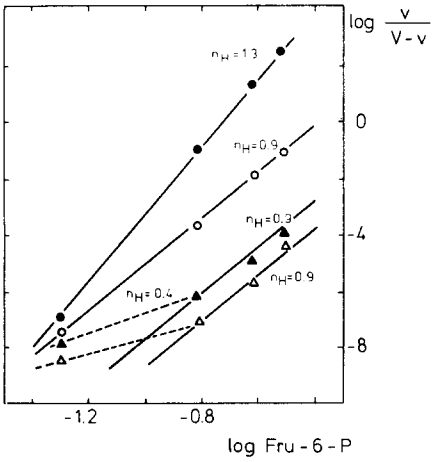

Fig. 4. Hill plot of the phosphofructokinase initial velocities in dependence of fructose 1-*P* concentration (●) 0.0; (▲) 2.0; (△) 4.0 and (○) 10.0 mM. $MgATP^{2-}$ concentration was constant at 0.1 mM. The resulting Hill coefficients are indicated in the graph.

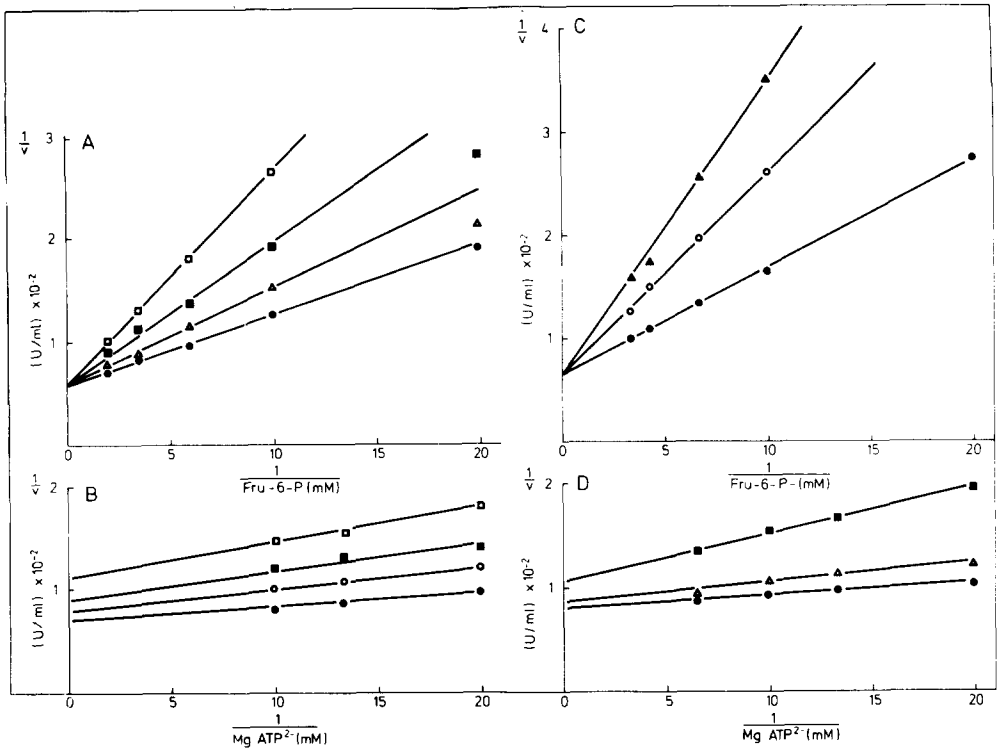

Fig. 5. Double reciprocal plot of the initial velocity of phosphofructokinase. (A) with varied fructose 6-*P* at fixed D-fructose concentrations as follows (●) 0.0; (△) 2.0; (■) 4.0 and (□) 8.0 mM. $MgATP^{2-}$ 0.1 mM. (B) with varied $MgATP^{2-}$ in the presence of (●) 0.0; (○) 1.0; (■) 4.0 and (□) 8.0 mM D-fructose. Fructose 6-*P* 0.3 mM. (C) with variable fructose 6-*P* in the presence of (●) 0.0; (○) 1.5 and (▲) 3.0 mM fructose $MgATP^{2-}$ 0.1 mM. (D) with varied $MgATP^{2-}$ in the presence of (●) 0.0; (△) 2.0 and (■) 4.0 mM fructose 1-1-*P*. Fructose 6-*P* constant at 0.1 mM.

competitive substrate inhibitors is one of the most direct procedures to discriminate between a sequential and a Ping Pong reaction mechanism [30]. As mentioned above, both D-fructose and fructose 1-*P* compete for the fructose 6-*P*-binding site under inhibitory conditions (Fig. 5C). This inhibition of both effectors is non-competitive vs. $MgATP^{2-}$ (Fig. 5B, D). This indicates that the kinetics of yeast phosphofructokinase follow a sequential mechanism consistent with recent results for animal phosphofructokinase [2]. In order to detect whether the fructose 6-*P* and $MgATP^{2-}$ binding is random or ordered, ATP^{4-} and both effectors D-fructose and fructose 1-*P* were used as inhibitors with regard to the substrates. The data for the inhibition with ATP^{4-} indicate competitive inhibition with regard to $MgATP^{2-}$ (Fig. 6A). It can even be shown that ATP^{4-} inhibits fructose 6-*P* binding (Fig. 6B) and both D-fructose and fructose 1-*P* $MgATP^{2-}$-binding (Fig. 5B, D) in a non-competitive manner. These results give evidence for a sequential reaction mechanism, in which the addition of fructose 6-*P* and $MgATP^{2-}$ to the enzyme proceeds randomly.

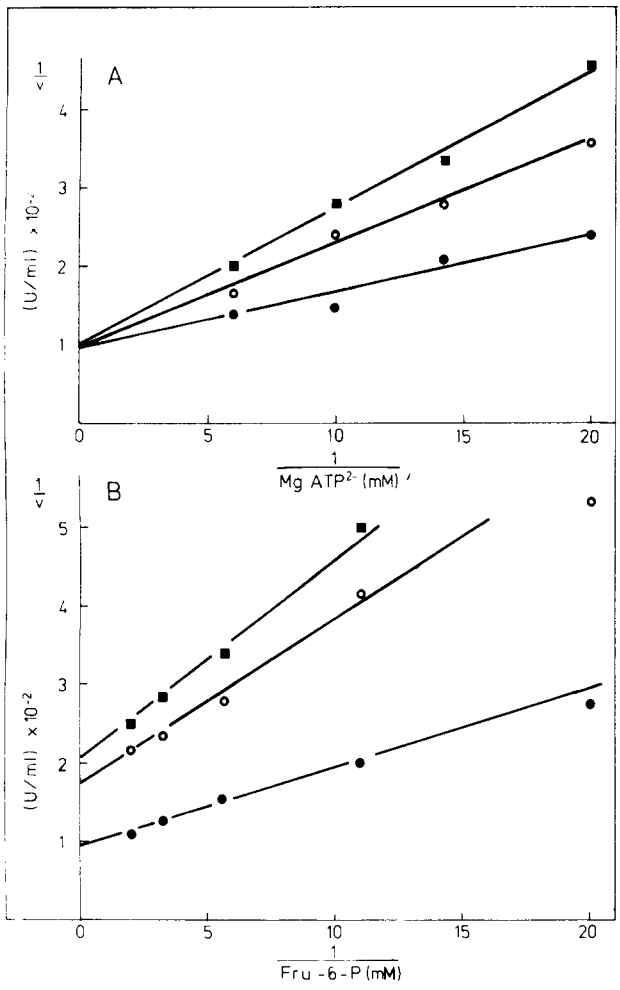

Fig. 6. Double reciprocal plot of the initial velocity of phosphofructokinase: (A) with varied $MgATP^{2-}$ concentrations in the presence of (●) 0.0; (○) 0.05 and (■) 0.1 mM ATP^{2-}. Fructose 6-*P* constant at 0.3 mM. (B) with varied fructose 6-*P* and fixed ATP^{4-} concentrations at (●) 0.0; (○) 0.05 and (■) 0.1 mM. $MgATP^{2-}$ constant at 0.1 mM. Concentrations of $MgATP^{2-}$ and ATP^{4-} were determined by using a stability constant of 20 000 M^{-1} for the $MgATP^{2-}$ complex.

Discussion

The results indicate that the binding of D-fructose and fructose 1-*P* affects the interactions of yeast phosphofructokinase with its substrates. Both effectors bind at the fructose 6-*P* site and can stimulate or inhibit fructose 6-*P* phosphorylation. There seems to be no difficulty in understanding the binding of D-fructose, if the sugar is in the β-furanose form, since phosphofructokinase can only phosphorylate this epimer of fructose 6-*P* [31]. The configuration of the C_2 atom of the hexosephosphate appears to be responsible for substrate-protein binding [32], a prerequisite for the phosphorylation. It is easy to assume that the configurations of β-fructo-furanose 6-*P* and β-D-fructose at the C_2 atom are

identical. Therefore, D-fructose can bind to the fructose 6-*P*-binding site as well as fructose 1-*P*.

Determination of the reaction mechanism with D-fructose and fructose 1-*P* showed that the mode of substrate binding to the enzyme is not altered by the influence of either.

In contrast to the well known effectors of phosphofructokinase $MgATP^{2-}$ and AMP, the increase and decrease of enzyme affinity for fructose 6-*P* by D-fructose and fructose 1-*P* is accompanied by alteration of the Hill coefficient for fructose 6-*P*. In order to discuss the action of D-fructose and fructose 1-*P* on yeast phosphofructokinase, different models to explain non-hyperbolic kinetics of 2 substrate reactions had to be considered. On one hand the concept of cooperative interactions of enzyme oligomers was shown to explain deviation from hyperbolic kinetics completely. On the other hand, sigmoidity may be also explained in terms of a steady-state system [33—35]. Although other models might fit the data as well, it seems to be justified to discuss the influence of both effectors, D-fructose and fructose 1-*P*, in terms of the two-state Monod model as mentioned by Smith et al. [26], since phosphofructokinase is well known to be an allosteric enzyme with cooperative interaction between enzyme subunits [2]. Phosphofructokinase is proposed to exist in two conformations R and T, which are in equilibrium. Fructose 6-*P* binds with high affinity only to the predominant R form. If the effector now binds preferably also at the fructose 6-*P* site of the R conformation, then at low substrate and effector concentrations stimulation results from shifting the equilibrium from the T to the R conformation. In contrast, at high effector concentrations competitive inhibition with regard to the substrate fructose 6-*P* is obtained, which now can also influence the R-T equilibrium. This shift is reflected by a lowered cooperativity expressed by a decrease of the Hill coefficients at higher effector concentrations (D-fructose 0.3 mM, $n_H = 2.6—1.0$; fructose 1-*P* 2.0 mM, $n_H = 1.3—0.9$). This decrease of Hill coefficient corresponds even to transition from non hyperbolic to hyperbolic kinetics. This means with respect to glycolytic oscillations that the catalytic activity of phosphofructokinase in the presence of D-fructose and fructose 1-*P* is the same, only if the changed enzyme affinity caused by the lowered cooperativity is compensated by altered substrate and effector concentrations. Therefore, the modulated enzyme should be more sensitive against changes in fructose 6-*P* and $MgATP^{2-}$ as well as against the positive effectors AMP and fructose 1,6-P_2.

It should be mentioned the proposed existence of rapid equilibrium and cooperative interactions between enzyme subunits can only be confirmed by direct physico-chemical measurement, which is beyond the scope of this work. Therefore, it should be remembered that sigmoidity is not necessarily dependent on cooperative interactions. Other models, dealing with steady state kinetics [33—35] may also explain the observed kinetic data from phosphofructokinase.

Nevertheless, D-fructose and fructose 1-*P* change the affinity of phosphofructokinase against substrates and effectors like AMP, fructose 1,6-P_2 and $MgATP^{2-}$, too. On the other hand, glycolytic oscillations are thought to be generated by a gradual increase and decrease of phosphofructokinase activity, which itself is caused by temporal accumulation of substrate and effectors. This

means that with a modulated phosphofructokinase enzyme, (a) higher concentrations of substrates and effectors are needed, in order to get an equal flux rate through the phosphofructokinase reaction, if yeast cells are fed with D-fructose instead of D-glucose; (b) the time scale for periodic phosphofructokinase activity might be altered resulting in a different period length of NADH oscillations. The data obtained from in vivo experiments [8,9,11,37] give good evidence for the assumption that D-fructose or one of its metabolites can interact directly with phosphofructokinase and change the period length of the NADH oscillation with respect to glucose.

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References

1 Passoneau, J.V. and Lowry, O.H. (1962) Biochem. Biophys. Res. Commun. 7, 10—15
2 Hofmann, E. (1976) Rev. Physiol. Biochem. Pharmacol. 75, 1—68
3 Ghosh, A. and Chance, B. (1964) Biochem. Biophys. Res. Commun. 16, 174—181
4 Frenkel, R. (1968) Arch. Biochem. Biophys. 125, 166—174
5 Betz, A. and Moore, C. (1967) Arch. Biochem. Biophys. 120, 268—273
6 Hess, B. and Boiteux, A. (1971) Annu. Rev. Biochem. 40, 237—258
7 Hess, B. and Boiteux, A. (1968) Hoppe Seyler's Z. Physiol. Chem. 349, 1574—1574
8 Pye, K. (1969) Can. J. Bot. 47, 271—285
9 Becker, J.U. and Betz, A. (1972) Biochim. Biophys. Acta 274, 584—597
10 Kreuzberg, K. (1977) Doctoral Thesis, Bonn
11 Betz, A., Kreuzberg, K. and Röttger, U. (1974) Abstracts 9th FEBS Meeting, Budapest, 53
12 Kreuzberg, K., Richter, O., Martin, W. and Betz, A. (1977) J. interdiscpl. Cycle Res. 8, 135—146
13 Betz, A., Röttger, U. and Kreuzberg, K. (1975) Arch. Microbiol. 103, 123—126
14 Tamaki, N. and Hess, B. (1975) Hoppe Seyler's Z. Physiol. Chem. 356, 399—415
15 Nissler, K., Friedrich, S. and Hofmann, E. (1972) Acta Biol. Med. Germ. 28, 739—749
16 Diezel, W., Böhme, H.J. Nissler, K., Freyer, R., Heilmann, W., Kopperschläger, W. and Hofmann, E. (1973) Eur. J. Biochem. 38, 479—488
17 Atzpodien, W. and Bode, H. (1970) Eur. J. Biochem. 12, 126—132
18 Böhme, H.J., Schellenberger, W., Retterath, P., Hofmann, E. and Misselwitz, R. (1975) Acta Biol. Med. Germ. 34, 1447—1451
19 Lowry, O.H., Rosebrough, N.J., Farr, A.D. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
20 Akkerman, J.W.N., Gorter, G., Sixma, J.J. and Gerhard, E.J. (1974) Biochim. Biophys. Acta 370, 113—119
21 Mavis, R.D. and Stellwagen, E. (1970) J. Biol. Chem. 245, 674—680
22 O'Sullivan, W.J. and Perrin, D.D. (1964) Biochemistry 3, 18—26
23 Neuer, H. (1974) Z. Anal. Chem. 269, 200—205
24 Cleland, W.W. (1967) Adv. Enzymol. 29, 1—32
25 Lindell, T.J. and Stellwagen, E. (1968) J. Biol. Chem. 243, 907—912
26 Freyer, R., Kubel, M. and Hofmann, E. (1970) Eur. J. Biochem. 17, 378—385
27 Sols, A. and Salas, M.L. (1966) Methods Enzymol. 9, 436—442
28 Uyeda, K. (1972) J. Biol. Chem. 247, 1692—1698
29 Salsas, M.L., Salas, J. and Sols. A. (1968) Biochem. Biophys. Res. Commun. 31, 461—466
30 Fromm, H.J. (1975) in Molecular Biology, Biochemistry and Biophysics, Springer Verlag, Berlin
31 Koerner, T.A.W., Younathan, E.S., Ashow, A.L. and Voll, R.J. (1974) J. Biol. Chem. 249, 5749—5774
32 Vinuela, E., Salsas, M.L. and Sols, A. (1968) Biochem. Biophys. Res. Commun. 12, 140—145
33 Frieden, C. (1970) J. Biol. Chem. 245, 5788—5799
34 Sweeny, J.R. and Fisher, J.R. (1968) Biochemistry 7, 561—565
35 Ferdinand, W. (1966) Biochem. J. 98, 278—283
36 Smith, G.D., Roberts, D.V. and Kuchel, P.W. (1970) Biochim. Biophys. Acta 317, 197—202