Hypothesis

A PROPOSED MODEL FOR THE REGULATION OF PHOSPHOFRUCTOKINASE AND FRUCTOSE 1,6-BISPHOSPHATASE BASED ON THEIR RECIPROCAL ANOMERIC SPECIFICITIES

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1. Introduction

An often overlooked aspect of carbohydrate metabolism is that most carbohydrates in solution are mixtures of several constitutional and configurational isomers (acyclic forms and anomers), each present in different concentrations and capable of displaying a different affinity and reactivity for enzyme catalytic and allosteric sites (anomeric specificity). In this paper, the possibility is considered that this characteristic isomerism of carbohydrates might provide the basis for regulation of the enzymes that metabolize them, using the example of the phosphofructokinase/fructose 1,6-bisphosphatase enzyme pair. Several investigators have pointed out the possibility that anomeric specificity may play a regulatory role [1–5]; however, until now no mechanism has been proposed to show how this regulation may be achieved.

The basis for the regulatory mechanism proposed in this communication is the recent harvest of data concerning the anomeric equilibria of carbohydrates in solution (reviewed in [6,7]) and the anomeric specificity of the enzymes of glucose metabolism (reviewed in [2,4]). D-Fructose 6-phosphate (F6P) and D-fructose 1,6-bisphosphate (FBP) have been shown by ^{13}C NMR spectroscopy to be equilibrated mixtures in aqueous solution (fig.1), composed of approx. 20% α-anomer, 80% β-anomer, and 2–4% keto form [8–10]. The possibility that approx. 1% of the hydrated keto form may be present has been considered [10] but is not supported by recent work [11] as well as earlier studies [12]. The rate constants for the spontaneous $\alpha \rightarrow \beta$ anomerization of F6P and FBP at 25°C have been reported to be 1.6 s^{-1} and 0.55–8.1 s^{-1}, respectively [10,13,14].

The enzymes that act on F6P and FBP, namely phosphofructokinase (PFK) and fructose 1,6-bisphosphatase (FBPase), have been shown to have reciprocal anomeric specificities for their substrates. Thus PFK from rabbit muscle has been shown to be specific for βF6P [1,14–17], as are probably the enzyme from microbial sources [18,19]. FBPase from rabbit liver has been shown to be specific for αFBP [20,21]. The error in the report [22] that bovine liver FBPase is specific for βFBP has been recently pointed out [21].

2. Assumptions and supporting evidence

Along with the confirmed results mentioned above, we are making the following assumptions in the conception of the proposed regulatory model. Supporting evidence for each assumption is discussed below.

1. The transit time of F6P and FBP between PFK and FBPase in vivo is small compared to the spontaneous rates of anomerization of these ketose phosphates.

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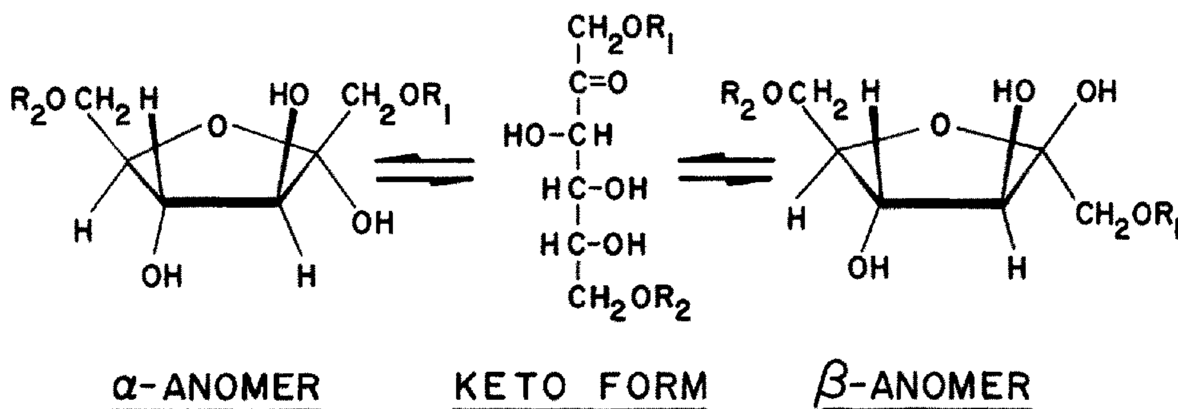

Fig.1. Tautomeric (cyclic/acyclic) and anomeric (α- and β-cyclic) forms of D-fructose 6-phosphate and D-fructose 1,6-bisphosphate.

2. The rates of F6P phosphorylation and/or FBP hydrolysis in vivo are much greater than the spontaneous anomerization rates of F6P and FBP.

3. The transit time of F6P and FBP between PFK and FBPase is much shorter than the transit time of these sugar phosphates between the latter two enzymes and any enzyme with anomerase activity.

4. PFK is inhibited by αF6P.

5. FBPase is inhibited by βFBP.

6. PFK is allosterically activated by αFBP.

7. FBPase is allosterically activated by βF6P.

In support of the first assumption is the evidence that PFK and FBPase bind specifically to each other in vitro [23–28] to form an enzyme complex. Based on this evidence, we can envision that the active-sites of both enzymes are as close as 100 Å. Taking the diffusion coefficient within the enzyme complex to be 10^{-8} cm^2/s [29], then the transit time of F6P and FBP between active sites would be more than 10^3 times shorter than the half-life of their spontaneous anomerization.

The second assumption is supported by calculations based on the values for the rates of spontaneous anomerization of F6P and FBP in vitro [13,14] and the concentrations of these sugar phosphates and activities of PFK and FBPase in vivo [29,30]. The rates of anomerization reported at 25°C were arbitrarily multiplied by 3 to obtain the approximate rate at 37°C. These calculations show one or both of the enzymatic rates to be 10–15-fold faster than the rates of ketose phosphate anomerization. Similar supporting calculations have been presented by Bloxham and York [31]. Such estimates may be conservative since Frey et al. [21] have suggested that the in vivo rates of anomerization of ketose phosphates are probably lower than their in vitro rates [10,13,14] because of enzymatic binding and sequestering of keto forms which are obligatory intermediates in the anomerization process.

Potentially challenging the second assumption would be the action of anomerases (mutarotases), enzymes capable of catalyzing the anomerization process. Of the enzymes endowed with such activity, only two have been shown to act on F6P or FBP in

vitro [4]. These are yeast phosphoglucose isomerase (PGI) [32] and yeast aldolase (ALD) [33]. Muscle aldolase [13], on the other hand, lacks anomerase activity. Thus it would seem possible, especially in the case of yeast, that the anomerase activity of PGI or ALD would invalidate assumption number two. However, this would only be true if PGI and ALD were a part of the PFK–FBPase complex. It is for this reason that assumption number three is advanced and supported.

In support of the third assumption is the evidence that PFK and FBPase exist together in an enzyme complex (vide supra) and that other enzymes, including aldolase, show no evidence of participation in this complex [28]. A recent study [34] on the adsorption of skeletal muscle glycolytic enzymes onto muscle structural proteins revealed PFK to be almost completely bound, whereas PGI and ALD to have much lower affinities as well as different responses to the presence of calcium. Unfortunately, the FBPase affinity or its effect on PFK activity were not reported in that system.

Based on the above findings, which are probably true for most eukaryotic cells, one can assume the PFK–FBPase complex is adsorbed onto a surface in vivo. As such the enzyme pair becomes analogous to immobilized, coupled enzymes. In such immobilized systems there is ample evidence [29] that substrate and product concentrations within the boundary of the adsorbed complex are maintained.at a level far from their equilibrium concentrations outside the boundary (e.g., in the cytosol). Thus, even if one assumes that PGI and ALD do have anomerase activity in vivo, they would have negligible effect on the anomerization of F6P and FBP inside the complex as long as they are not components thereof.

The fourth assumption is supported by the finding that 2,5-anhydro-D-glucitol 6-phosphate, an analogue of αF6P, is a good inhibitor of PFK [1,18]. Similarly, the fifth and sixth assumptions are supported by the findings that analogues of βFBP are inhibitors of FBPase and those of αFBP are activators of PFK [4,35,36].

Additional evidence supporting the sixth assumption is the recent study of the mechanism of activation of PFK [37] by FBP. If the enzyme is activated by αFBP only, its activation rate should equal the spontaneous rate of anomerization of $\beta \rightarrow \alpha$ FBP since PFK produces βFBP only. The finding [37] that the half-life for FBP-mediated activation of PFK is approx. 0.4 s at 25°C is remarkably similar to the half-life of the $\beta \rightarrow \alpha$ anomerization of FBP (0.43 s [10]).

The seventh assumption has yet to be experimentally tested. Experiments, in our laboratory, are underway to verify this assumption. Finally, it should be noted that assumptions number 6 and 7 are not essential for the operation of the proposed model; however they allow it to function at an even higher level of efficiency.

3. The model (fig.2)

It is proposed that the anomeric specificities of PFK and FBPase create a nonequilibrium distribution of the anomers of F6P and FBP in the PFK–FBPase complex. This anomeric nonequilibrium acts as a signal for the rapid communication of information between the two enzymes. It is further proposed that the anomeric specificity of the catalytic and allosteric sites of PFK and FBPase renders them signal receivers for this information and acts to coordinate their activities. The basis for anomeric signal reception at the catalytic sites is the competitive inhibition of one enzyme by the product of the other. The basis of modulation at the allosteric sites is the activation of one enzyme by the substrate of the other.

The result of this anomeric signaling and receiving is an antagonism of the activity of one enzyme with respect to the other. This antagonism of activity constitutes a substrate-level regulation that acts to:

(i) Inhibit futile cycling.

(ii) Amplify the stimulation or inhibition of each enzyme of the pair by its allosteric modulators, such as AMP and citrate, thus enhancing the behavior of this enzyme pair as an electronic flip-flop device.

(iii) Coordinate optimally the activities of PFK and FBPase with other glycolytic enzymes such as pyruvate kinase if these other enzymes exist in the same complex as PFK and FBPase.

The corollaries described below follow from the application of this model.

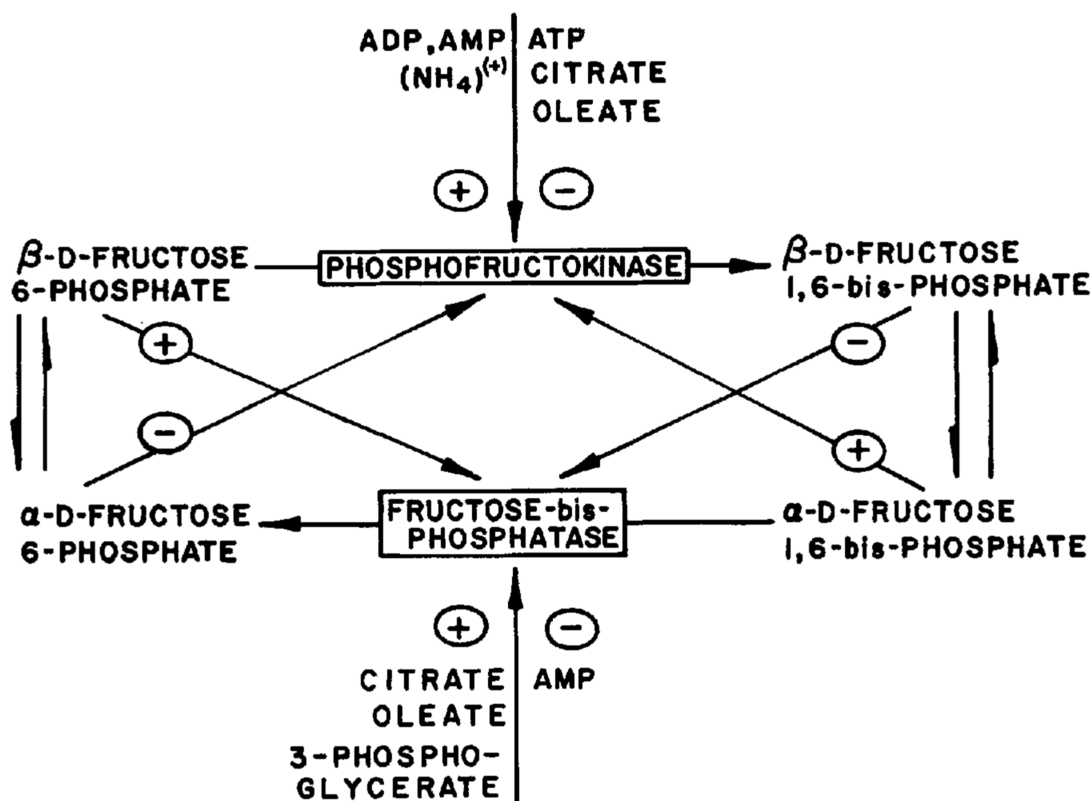

Fig.2. Proposed mechanism for the regulation of phosphofructokinase and fructose 1,6-bisphosphatase activities through reciprocal anomeric specificities at their catalytic and allosteric sites.

Corollary 1

External stimulation of PFK (e.g., by AMP or a pulse of βF6P) leads to decreased activity of FBPase by:

(i) Competitive inhibition at the FBPase catalytic site by βFBP.
(ii) Utilization of βF6P, the allosteric activator of FBPase.

Stimulation of PFK is enhanced by a decrease in concentration of its catalytic inhibitor αF6P.

Corollary 2

External stimulation of FBPase (e.g., by citrate or a pulse of αFBP) leads to decreased activity of PFK by:

(i) Competitive inhibition at the PFK catalytic site by αF6P.
(ii) Utilization of αFBP, the allosteric activator of PFK.

Stimulation of FBPase is enhanced by decrease in concentration of its catalytic inhibitor βFBP.

Corollaries 3 and 4

External inhibition of PFK (e.g., by citrate) or external inhibition of FBPase (e.g., by AMP) leads to activation of the antagonistic enzyme by elimination of competitive inhibition at its catalytic site and increased production of its allosteric activator.

Corollaries 5 and 6

When neither enzyme is stimulated or both are equally stimulated, the anomeric equilibria of their substrates and products tend to inhibit both enzymes.

4. Further evidence for the model

Our proposal that anomeric specificities of PFK and FBPase can produce a nonequilibrium distribution of the anomers of F6P and FBP is supported by a computer simulation study [38] based on the in vitro measurements of anomer concentrations and anomerization rates [8–10,13,14] corrected to 37°C, and in vivo measurements of F6P and FBP concentrations as well as PFK and FBPase activities [29,30]. In this study, we [38] have shown that the extent of perturbation from anomeric equilibrium is dependent on the activities of PFK and FBPase as well as the total amount of fructose phosphate present in the system. For example, in rat liver (pH 7.4, 37°C), for which PFK activity is 3.3 μmol/min/g wet wt and FBPase activity 15 μmol/min/g wet wt [30] and the total equilibrated fructose phosphate 40 nmol/g wet wt [29], the β/α ratio is 2.2 for F6P and 86 for FBP, instead of the equilibrium ratio of 4.0 [9]. The experimental confirmation of the results of these simulations has to await the surmounting of certain technical difficulties.

The reciprocal anomeric specificities of PFK and FBPase that lead to the proposed antagonism of the two enzyme activities (fig.2) is consistent with the reciprocal activator–inhibitor relationships of the extensive list of allosteric modulators of the two enzymes [39,40] as well as the reciprocal oscillatory behavior of F6P and FBP [41].

As to whether the proposed mechanism (fig.2) is indeed operative in vivo, we can state that the effects expected to result from anomeric regulation are consistent with the experimental data. For example:

1. A pulse of βFBP, produced by stimulation of PFK, serves to coordinate optimally the activity of that enzyme with those of aldolase and pyruvate kinase; since aldolase requires βFBP as substrate [13,33,42] and pyruvate kinase requires it as allosteric activator [3].

2. The presence of FBPase in skeletal muscle does enhance the stimulatory effect of AMP on PFK [43]. We would explain this observation, however, not in terms of the effects of futile cycling as proposed [43], but in terms of the anomeric non-equilibrium state of the fructose phosphates and its amplification of PFK activity (fig.2).

3. Our model is consistent with the observation that low levels of FBP both prevent as well as reverse the inactivation of PFK by FBPase [28].

Finally, significant futile cycling remains to be established [44,45]. The isotope studies consistent with high levels of futile cycling [46] have recently been given other explanations [44,45,47–49].

5. Significance of the model

Previously, nucleotides such as ATP, AMP, and cAMP as well as other metabolites such as citrate and oleate have been shown to exert a reciprocal regulation at the allosteric sites of PFK and FBPase. We have shown that the anomeric specificities of these enzymes may serve as the basis for a similar reciprocal regulation at both the catalytic and allosteric sites. Such a regulation would be another example of the kind of efficient, self-regulatory mechanism characteristic of the living state.

It is of interest to assess the contribution of the proposed anomeric regulation to the total modulation of PFK and FBPase activities. Computer simulations [38] show that futile cycling by these enzymes may be reduced to levels as low as 37% of those without the inclusion of anomeric regulation. These studies not withstanding, it seems likely that anomeric regulation must play a role secondary to the regulation by nucleotides and the other allosteric effectors. However, it is conceivable that, prior to the evolution of allosteric sites, the proposed anomeric regulation provided a mechanism for the suppression of futile cycling

Finally, it should be noted that the proposed anomeric regulation might be operative at other antagonistic enzyme pairs (e.g., glucokinase/glucose 6-phosphatase).

6. Conclusions

In summary, we have proposed a model (fig.2) for the anomeric regulation of phosphofructokinase and fructose 1,6-bisphosphatase in which each enzyme is allosterically activated by the substrate, and competitively inhibited by the product of the other (phos-

phofructokinase acts on and produces the β-forms whereas fructose 1,6-bisphosphatase acts on and produces the α-forms of the fructose phosphates). The proposed mechanism enhances the action of this enzyme pair as a 'metabolic flip–flop regulatory mechanism', thus suppressing futile cycling. It is supposed to operate simultaneously with other known modulators of these two enzymes (e.g., adenine nucleotides, citrate) and might have played a more important role prior to the evolution of the allosteric sites of these ligands. The model provides an alternative explanation for the results of many studies of the two enzymes in vitro and in vivo.

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References

[1] Koerner, T. A. W., Younathan, E. S., Ashour, A. E. and Voll, R. J. (1974) J. Biol. Chem. 249, 5749–5754.
[2] Wurster, B. and Hess, B. (1974) FEBS Lett. 40 S, 112–118.
[3] Wurster, B., Hess, B., Koerner, T. A. W., Voll, R. J. and Younathan, E. S. (1976) FEBS Lett. 63, 17–21.
[4] Benkovic, S. J. and Schray, K. J. (1976) Adv. Enzymol. 44, 139–164.
[5] Hess, B. (1976) Biochem. Soc. Trans. 4, 1045.
[6] Angyal, S. J. (1969) Angew. Chem., Int. Ed. Engl. 8, 157–166.
[7] Gray, G. R. (1976) Acc. Chem. Res. 9, 418–424.
[8] Benkovic, S. J., Engle, J. L. and Mildvan, A. S. (1972) Biochem. Biophys. Res. Commun. 47, 852–858.
[9] Koerner, T. A. W., Cary, L. W., Bhacca, N. S. and Younathan, E. S. (1973) Biochem. Biophys. Res. Commun. 51, 543–549.
[10] Midelfort, C. F., Gupta, R. K. and Rose, I. A. (1976) Biochemistry 15, 2178–2185.
[11] Angyal, S. J., Bethell, G. S., Cowley, D. E. and Pickles, V. A. (1976) Aust. J. Chem. 29, 1239–1247.
[12] Bell, R. P. (1966) Advan. Phys. Org. Chem. 4, 1–29.
[13] Wurster, B. and Hess, B. (1973) Biochem. Biophys. Res. Commun. 55, 985–992.
[14] Wurster, B. and Hess, B. (1974) FEBS Lett. 38, 257–260.
[15] Koerner, T. A. W., Ashour, A. E., Voll, R. J. and Younathan, E. S. (1973) Fed. Proc. Fed. Am. Soc. Exp. Biol. 32, 668.
[16] Fishbein, R., Benkovic, P. A., Schray, K. J., Siewers, I. J., Steffens, J. J. and Benkovic, S. J. (1974) J. Biol. Chem. 249, 6047–6051.
[17] Bar-Tana, J. and Cleland, W. W. (1974) J. Biol. Chem. 249, 1263–1270.
[18] Koerner, T. A. W., Reeves, R. E., Voll, R. J. and Younathan, E. S. (1977) Fed. Proc. Fed. Am. Soc. Exp. Biol. 36, 858.
[19] Sols, A. and Salas, M. L. (1966) Methods Enzymol. 9, 436–442.
[20] Benkovic, P. A., Bullard, W. P., DeMaine, M., Fishbein, R. Schray, K. J., Steffens, J. J. and Benkovic, S. J. (1974) J. Biol. Chem. 249, 930–931.
[21] Frey, W. A., Fishbein, R., DeMaine, M. M. and Benkovic, S. J. (1977) Biochemistry 16, 2479–2484.
[22] Marcus, C. J. (1976) J. Biol. Chem. 251, 2963–2966.
[23] El-Badry, A. M., Otani, A. and Mansour, T. E. (1973) J. Biol. Chem. 248, 557–563.
[24] Uyeda, K. and Luby, L. J. (1974) J. Biol. Chem. 249, 4562–4570.
[25] Kono, N. and Uyeda, K. (1974) J. Biol. Chem. 249, 1490–1496.
[26] Pogell, B. M., Tanaka, A. and Siddons, R. C. (1968) J. Biol. Chem. 243, 1356–1367.
[27] Carlson, C. W., Baxter, R. C., Ulm, E. H. and Pogell, B. M. (1973) J. Biol. Chem. 248, 5555–5561.
[28] Proffitt, R. T., Sankaran, L. and Pogell, B. M. (1976) Biochemistry 15, 2918–2925.
[29] Ottaway, J. H. and Mowbray, J. (1977) Curr. Top. Cell. Reg. 12, 107–208.
[30] Scrutton, M. C. and Utter, M. F. (1968) Ann. Rev. Biochem. 37, 249–302.
[31] Bloxham, D. P. and York, D. A. (1976) Biochem. Soc. Trans. 4, 989–993.
[32] Wurster, B. and Hess, B. (1973) Z. Physiol. Chem. 354, 407–420.
[33] Schray, K. J., Fishbein, R., Bullard, W. P. and Benkovic, S. J. (1975) J. Biol. Chem. 250, 4883–4887.
[34] Clarke, F. M. and Masters, C. J. (1975) Biochim. Biophys. Acta 381, 37–46.
[35] Benkovic, S. J., Kleinschuster, J. J., DeMaine, M. M., Fishbein, R. and Schray, K. J. (1973) Abst. IXth Int. Cong. Biochem. Stockholm, p. 376.
[36] Benkovic, S. J., Kleinschuster, J. J., DeMaine, M. M. and Siewers, I. J. (1971) Biochemistry 10, 4881–4887.
[37] Hood, K. and Hollaway, M. R. (1976) FEBS Lett. 68, 8–14.
[38] Voll, R. J., Koerner, T. A. W. and Younathan, E. S. (1976) unpublished.
[39] Mansour, T. E. (1972) Curr. Topics Cell. Reg. 5, 1–46.
[40] Pontremoli, S. and Horecker, B. L. (1970) Curr. Top. Cell. Reg. 2, 173–199.
[41] Boiteux, A. and Hess, B. (1974) Faraday Symp. Chem. Soc. 9, 202–214.
[42] Rose, I. A. and O'Connell, E. L. (1977) J. Biol. Chem. 252, 479–482.

[43] Newsholme, E. A. and Crabtree, B. (1970) FEBS Lett. 7, 195–198.
[44] Katz, J. and Rognstad, R. (1976) Curr. Top. Cell. Reg. 10, 237–289.
[45] Hue, L. (1976) Biochem. Soc. Trans. 4, 994–998.
[46] Clark, D. G., Rognstad, R. and Katz, J. (1973) Biochem. Biophys. Res. Commun. 54, 1141–1148.
[47] Hue, L. and Hers, H. G. (1974) Biochem. Biophys. Res. Commun. 58, 532–539.
[48] Katz, J., Wals, P. A., Golden, S. and Rognstad, R. (1975) Eur. J. Biochem. 60, 91–101.
[49] Clark, D. G., Lee, D., Rognstad, R. and Katz, J. (1975) Biochem. Biophys. Res. Commun. 67, 212–219.