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The control of glycolysis and gluconeogenesis by protein phosphorylation

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Fructose 2,6-bisphosphate has been discovered as a potent stimulator of liver phosphofructokinase. It is also an inhibitor of fructose 1,6-biphosphatase and a stimulator of PP_i:fructose 6-phosphate phosphotransferase from higher plants. It is formed from fructose 6-phosphate and ATP by a 6-phosphofructo 2-kinase and hydrolysed by a fructose 2,6-bisphosphatase. These two enzymes have very similar physicochemical properties and could not be separated from each other. They are substrates for cyclic-AMP-dependent protein kinase, which inactivates the first enzyme and activates the second.

Introduction

Five liver enzymes belonging either to glycolysis or to gluconeogenesis are substrates for cyclic-AMP-dependent protein kinase. One of them is pyruvate kinase, the inactivation of which by phosphorylation causes the arrest of glycolysis and the stimulation of glyconeogenesis; little information has been published on that enzyme since its properties were reviewed by Engström (1978), and I shall therefore not include it in this paper. The four other phosphorylatable enzymes of glycolysis or gluconeogenesis are related to the fructose 6-phosphate–fructose 1,6-bisphosphate interconversion. Indeed, both 6-phosphofructo 1-kinase (also called phosphofructokinase 1 or PFK 1) and fructose 1,6-bisphosphatase (at least the rat liver enzyme) are substrates for cyclic-AMP-dependent protein kinase (Claus *et al.* 1980; Riou *et al.* 1977). However, their catalytic properties are not or only little affected by phosphorylation, but are essentially controlled by the concentration of a series of effectors of low molecular mass. Among these effectors, the most important are fructose 2,6-bisphosphate and AMP, which play the major regulatory role under aerobic and anaerobic conditions respectively. Fructose 2,6-bisphosphate was discovered only $2\frac{1}{2}$ years ago (Van Schaftingen 1980*a*, *b*) and for this reason will be the main subject of this paper. It is formed by a 6-phosphofructo 2-kinase (also called phosphofructokinase 2 or PFK 2) and degraded by fructose 2,6-biphosphatase; PFK 2 and fructose 2,6-bisphosphatase are interconvertible by phosphorylation and dephosphorylation. Fructose 2,6-bisphosphate has been the subject of several recent reviews (Hers & Van Schaftingen 1982; Hers *et al.* 1982; Pilkis *et al.* 1982; Uyeda *et al.* 1982) in which additional information and references to original work can be found.

The discovery of fructose 2,6-bisphosphate

In the spring of 1980, it was recognized in this laboratory that incubation of isolated hepatocytes in the presence of high concentrations of glucose causes the formation of a low-molecular-mass stimulator of phosphofructokinase that was then rapidly destroyed upon addition of glucagon. This stimulator was identified as fructose 2,6-bisphosphate from the following properties. It had a molecular mass, estimated by gel filtration, similar to that of fructose

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1,6-bisphosphate; it was extremely acid-labile, was destroyed by alkaline phosphatase and was not adsorbed by charcoal. It was precipitated by barium and upon anion exchange chromatography was eluted in close association with fructose 1,6-bisphosphate. Limited acid hydrolysis in the presence of 0.01 M HCl resulted in the quantitative conversion of the stimulator to a mixture of fructose 6-phosphate and P_i. An equivalent amount of reducing group was formed in parallel, indicating that the stimulator consisted of a fructose 6-phosphate moiety linked to phosphate by its anomeric carbon as in fructose 2,6-bisphosphate (figure 1). Furthermore, the stimulator could be synthesized from phosphoric acid and fructose 6-phosphoric acid in the cold, allowing the conclusion that no constituent other than fructose 6-phosphate and phosphate enter into its structure.

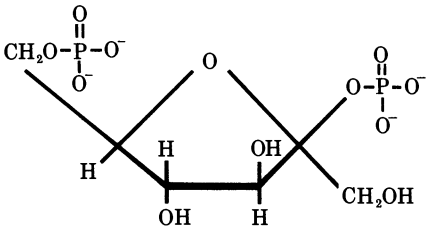

FIGURE 1. β-Fructose 2,6-bisphosphate.

Fructose 2,6-bisphosphate could be synthesized on a millimolar scale from fructose 1,6-bisphosphate, thanks to the procedure developed by Pontis & Fischer (1963) for the conversion of fructose 1-phosphate into fructose 2-phosphate. The synthetic fructoside, which had identical chemical and biological properties to the natural compound, was shown by n.m.r. analysis to be the β anomer of fructose 2,6-bisphosphate.

BIOLOGICAL EFFECTS OF FRUCTOSE 2,6-BISPHOSPHATE

The stimulation of phosphofructokinase

The activity of phosphofructokinase is regulated by the concentration of several metabolites, most notably the two substrates, fructose 6-phosphate and ATP. The saturation curve for fructose 6-phosphate is sigmoidal; this curve is shifted to the left in the presence of positive effectors, among which fructose 1,6-bisphosphate, the product of the reaction, and AMP are currently considered to play the major role. The negative effectors ATP and citrate have the opposite effect. The saturation curve for ATP is characterized by inhibition by excess substrate. This inhibition is released by the positive effectors and by larger concentrations of fructose 6-phosphate, whereas it is intensified by the negative effectors.

As illustrated in figure 2, fructose 2,6-bisphosphate has the properties of a potent positive effector of liver phosphofructokinase. It is apparent from the same figure that, under conditions in which no activity could be detected in the absence of fructose 2,6-bisphosphate, the efficiency of the stimulator was greatest at high fructose 6-phosphate and low ATP concentrations. The positive effect of fructose 2,6-bisphosphate was also greatly synergistic with that of AMP (not shown).

It is of interest to compare the sensitivity of phosphofructokinase to fructose 2,6-bisphosphate with that to fructose 1,6-bisphosphate, which has often been considered to play a major role in the control of phosphofructokinase activity in the liver and other tissues. When assayed in

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similar conditions, the same stimulation could be obtained with fructose 2,6-bisphosphate at 1,000-fold lower concentration than fructose 1,6-bisphosphate. At higher concentrations, fructose 1,6-bisphosphate became inhibitory. Because the two fructose bisphosphates are usually present in the liver at a similar concentration, there is little doubt that fructose 2,6-bisphosphate, rather than fructose 1,6-bisphosphate is the major regulator of phosphofructokinase in the liver, and most likely in other tissues also.

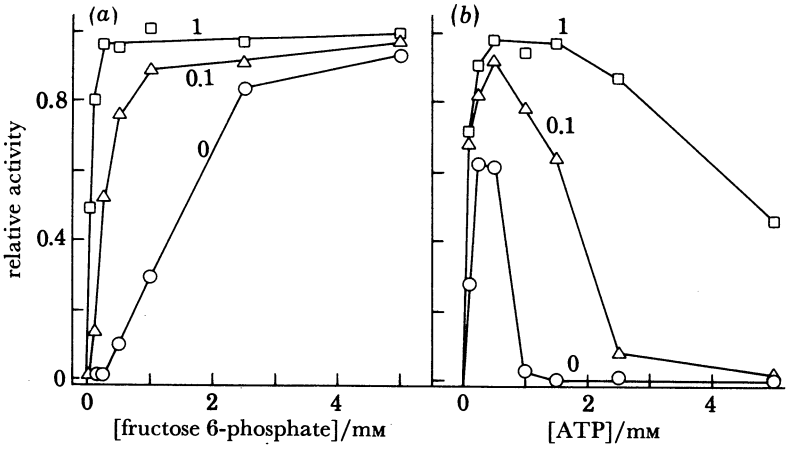

FIGURE 2. Effect of fructose 2,6-bisphosphate on (*a*) the affinity of liver phosphofructokinase for fructose 6-phosphate and (*b*) the inhibition of the enzyme by ATP. Numbers on the curves are fructose 2,6-bisphosphate concentrations in micromoles per litre. In (*a*) the ATP concentration was 1.5 mM; or in (*b*) the fructose 6-phosphate concentration was 0.25 mM. (From Van Schaftingen *et al.* (1981).)

Fructose 2,6-bisphosphate is also known to stimulate phosphofructokinases from all mammalian tissues that were tested, as well as phosphofructokinase from yeast and other fungi, and the plastid phosphofructokinase from higher plants.

Inhibition of fructose 1,6-*bisphosphatase by fructose* 2,6-*bisphosphate*

In liver and several other tissues, the activity of phosphofructokinase is opposed by that of fructose 1,6-bisphosphatase. It is therefore not surprising that, as illustrated in figure 3, this enzyme is inhibited by fructose 2,6-bisphosphate. The fact that this inhibition is much stronger at low than at high concentrations of substrate, together with the obvious structural similarity

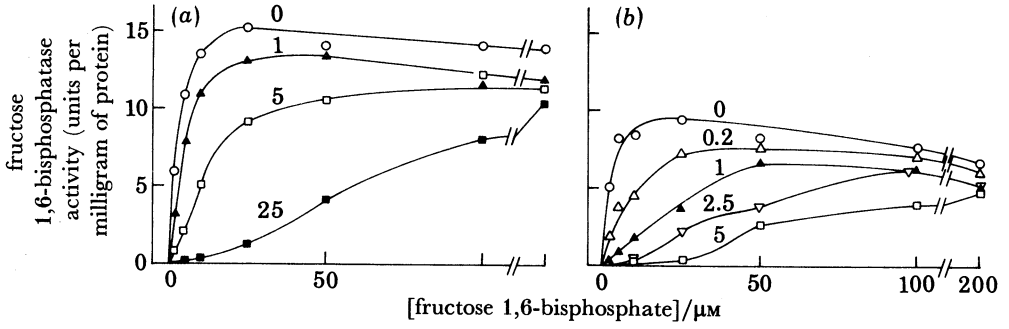

FIGURE 3. Inhibition of fructose 1,6-bisphosphatase by fructose 2,6-bisphosphate at various concentrations of substrate in the absence (*a*) and in the presence (*b*) of 25 μM AMP. Numbers on the curves are fructose 2,6-bisphosphate concentrations in micromoles per litre. (From Van Schaftingen & Hers (1981).)

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between the two fructose bisphosphates, suggests that the inhibition would be competitive with the substrate. However, several properties of this inhibition are against this simple interpretation. Firstly, the inhibition by fructose 2,6-bisphosphate is synergistic with that by AMP, another potent inhibitor of the enzyme known to decrease V_{max} with no effect on K_m. Secondly, in contrast to the effect of AMP, fructose 2,6-bisphosphate changes the kinetics for fructose 1,6-bisphosphate from almost hyperbolic to sigmoidal, indicating an allosteric type of interaction. Thirdly, the binding of fructose 2,6-bisphosphate to the enzyme is affected by a series of conditions that have no effect on the affinity for the substrate (for a review see Hers & Hue 1983). Fructose 2,6-bisphosphate is also a potent inhibitor of fructose 1,6-bisphosphatase from skeletal muscle, yeast and higher plants.

Stimulation of PP_i: *fructose* 6-*phosphate* 1-*phosphotransferase*

PP_i-phosphofructokinase is widespread in higher plants; its activity is greatly increased by very low concentrations of fructose 2,6-bisphosphate. A sensitive assay procedure, allowing the determination of fructose 2,6-bisphosphate in amounts smaller than 1 pmol, is based on this property (Van Schaftingen *et al.* 1982).

Biosynthesis and biodegradation of fructose 2,6-bisphosphate

The mechanism by which fructose 2,6-bisphosphate is synthesized and degraded in the liver is summarized in figure 4. Fructose 2,6-bisphosphate is formed by transfer of the γ-phosphoryl group of ATP onto carbon 2 of fructose 6-phosphate, catalysed by phosphofructokinase 2. It is hydrolysed back to fructose 6-phosphate and P_i by a specific fructose 2,6-bisphosphatase. Remarkably, phosphofructokinase 2 and fructose 2,6-bisphosphatase could not be separated by procedures based on the size, charge or hydrophobicity of the proteins. This indicates that the two enzymes are not only very similar in their physico-chemical properties but also that they might be part of a single multifunctional protein. Both enzymes are substrates for cyclic AMP-dependent protein kinase and, as a result of phosphorylation, the activity of phosphofructokinase 2 is diminished and that of fructose 2,6-bisphosphatase is increased about fourfold.

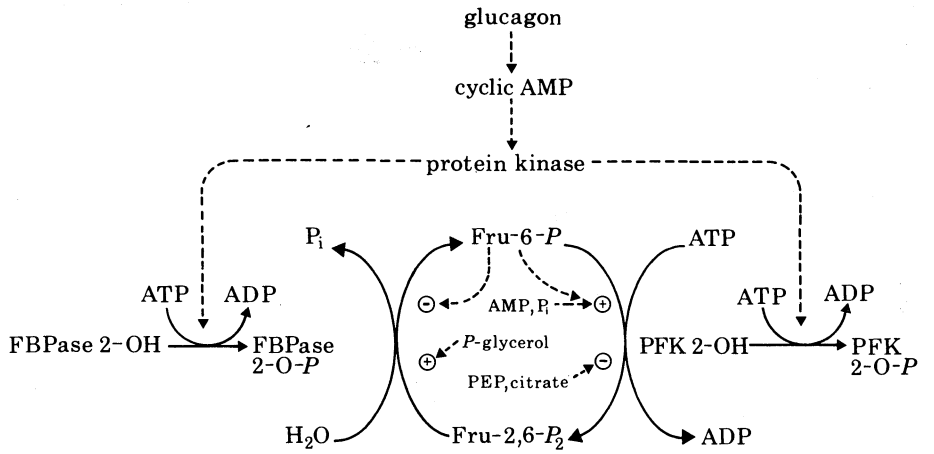

FIGURE 4. Biosynthesis and biodegradation of fructose 2,6-bisphosphate in the liver and their control of glucagon and metabolites. Abbreviations: FBPase 2, fructose 2,6-bisphosphatase, PEP, phosphoenolpyruvate, *P*-glycerol, glycerol 3-phosphate. (From Hers & Van Schaftingen (1982).)

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The activity of the two enzymes is also controlled by the concentration of various metabolites, the most important being fructose 6-phosphate, which is the substrate of phosphofructokinase 2 and a strong inhibitor of fructose 2,6-bisphosphatase.

The concentration of hexose 6-phosphates is greatly increased when glycogen breakdown is stimulated, as for instance by glucagon or by α-adrenergic agents and vasopressin. Glucagon, however, by its action on phosphofructokinase 2 and fructose 2,6-bisphosphatase, prevents the rise in fructose 2,6-bisphosphate concentration and favours glucose formation from both glycogen and gluconeogenesis. In contrast, the α-agonists and vasopressin do not modify the concentration of cyclic AMP and are therefore without effect on the activity of phosphofructokinase 2 and fructose 2,6-bisphosphatase. They therefore cause an increase in the concentration of fructose 2,6-bisphosphate and a stimulation of glycolysis. A rise in the concentration of fructose 6-phosphate is also the most likely explanation for the effect of a high concentration of glucose in increasing the concentration of fructose 2,6-bisphosphate in isolated hepatocytes.

Conclusion

Fructose 2,6-bisphosphate is a newly discovered regulatory molecule present in animals, higher plants and fungi. Its main role appears to be the control of the fructose 6-phosphate–fructose 1,6-bisphosphate interconversion. It may be considered as a signal of metabolism in the sense defined by Stryer (1981) in that it is formed from ubiquitous molecules but it is not itself part of any important metabolic interconversion; it is an integrator of metabolism and not a biosynthetic precursor or intermediate in energy production. In contrast to cyclic AMP, which has been called a 'hunger signal', fructose 2,6-bisphosphate signifies that glucose is abundant and can be utilized, whereas gluconeogenesis can be stopped.

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Discussion

P. COHEN (*Department of Biochemistry, University of Dundee, U.K.*). El-Maghrabi *et al.* (*J. biol. Chem.* **257**, 7603–7607 (1982); *Biochem. biophys. Res. Commun.* **106**, 798–803 (1982)) have reported that PFK 2 and fructose 2,6-bisphosphatase are both located on a single polypeptide chain. Would Professor Hers comment on this please?

H.-G. HERS. The data presented by El-Maghrabi *et al.* confirm and reinforce our initial observation (Van Schaftingen *et al.*, *Biochem. Biophys. Res. Commun.* **103**, 362–368 (1981); see also Van Schaftingen *et al.*, *Eur. J. Biochem.* **124**, 143–149 (1982)) that fructose 2,6-bisphosphatase copurifies with PFK 2 in the course of all fractionation procedures that were used. This type of observation is a good indication, although not a proof, that the two enzymes are located on a single polypeptide chain; they could as well be very similar polypeptides, originating for instance by mutation of a common ancestor gene.

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