Diabetologia (1993) 36: 581–588

Diabetologia

Review

Glycolysis revisited*

E. Van Schaftingen

Laboratoire de Chimie Physiologique, International Institute of Cellular and Molecular Pathology, and Université Catholique de Louvain, Brussels, Belgium

Summary. Glycolysis is usually considered as a paradigm metabolic pathway, due to the fact that it is present in most organisms, and also because it is the pathway by which an important nutrient, glucose, is consumed. Far from being completely understood, the regulation of this pathway witnessed several important progresses during the last few years. One of these is the discovery of fructose 2,6-bisphosphate, a potent stimulator of phosphofructokinase and inhibitor of fructose-1,6-bisphosphatase. Originally found in the liver during the course of a study on the mechanism by which glucagon acts on gluconeogenesis, this compound is now recognized as a major element in the control of glycolysis and/or gluconeogenesis in many cell types and in various organisms. The other finding is that of a regulatory protein that modulates the activity of glucokinase, the enzyme that phosphorylates glucose in the liver and in the beta cells of pancreatic islets.

Key words: Glycolysis, gluconeogenesis, fructose 2,6-bisphosphate, phosphofructokinase, fructose-1,6-bisphosphatase, glucokinase, fructose, maturity onset diabetes of the young.

The central role of glycolysis

In the first four decades of the 20th century, the pioneering work of such famous names as Harden, Young, Embden, Meyerhof and Warburg led to the identification of the intermediates of glycolysis and of the enzymes catalysing its different steps [1]. Glycolysis thus became the first metabolic pathway to be deciphered, which is one of the reasons for its fame. Another reason is that it is present in almost all cell types, except for a few bacteria that developed alternative means of fermenting glucose. The ubiquity of glycolysis reflects the important role played by glucose in cell physiology. Glucose is unique in that its metabolism can furnish ATP even in the absence of O_2. Furthermore, this hexose is an essential substrate for tissues such as the brain and the retina, as well as for mammalian erythrocytes. Finally, the most prevalent metabolic disease, diabetes mellitus, is to a large extent, a perturbation of glucose metabolism.

During the last 40 years the interest of investigators in the field of metabolism has focused on the regulation of biochemical pathways. Control enzymes have been identified and their properties investigated. The importance of covalent modification has been recognized and the existence of second messengers has been established. The purpose of this review is to describe two discoveries pertaining to the regulation of glycolysis. The first is that of fructose 2,6-bisphosphate, which is now known to play a central role in the regulation of glycolysis in most eukaryotic cells, and the second is that of a regulatory protein that affects the activity of glucokinase, an enzyme present in the liver and in pancreatic islets of mammals.

Fructose 2,6-bisphosphate

Discovery

The liver plays an important role in the control of glycaemia. Following a carbohydrate rich diet, this organ is able to consume glucose, which is first stored as glycogen and secondarily metabolized to glycolytic products and lipids. More importantly, the liver is able to secrete glucose during fasting [reviewed in 2]. This glucose is formed by depolymerization of glycogen, or by gluconeogenesis from various compounds such as lactate, pyruvate, glycerol and several amino acids. Gluconeogenesis, which is

* Presented as the Minkowski Lecture, EASD Meeting Prague, Czechoslovakia 1992

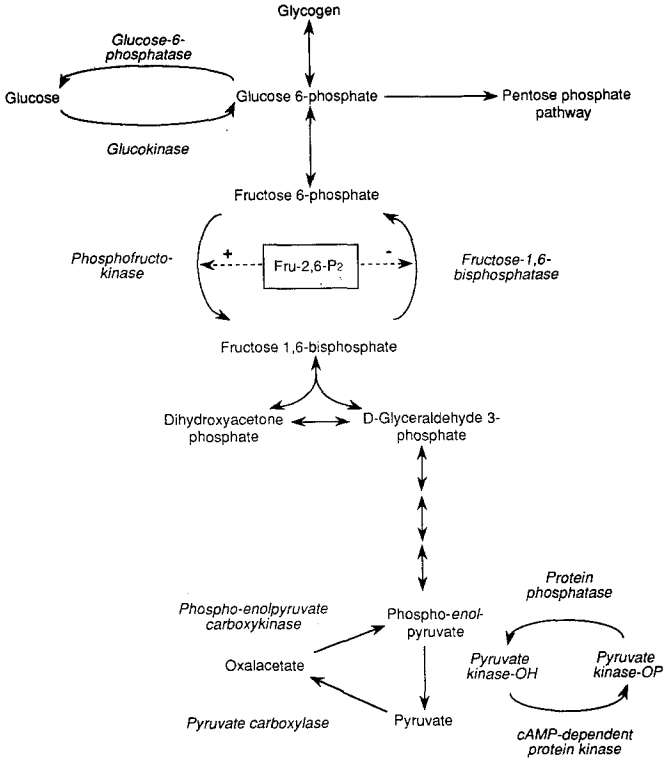

Fig. 1. The pathways of glycolysis and gluconeogenesis, and their regulation by fructose 2,6-bisphosphate. For the sake of clarity, co-substrates have not been indicated

the reverse of glycolysis, is different at only three levels (Fig. 1). These are: 1) the interconversion between glucose and glucose 6-phosphate, catalysed by glucokinase in the glycolytic direction, and by glucose 6-phosphatase in the gluconeogenic direction; 2) the interconversion between fructose 6-phosphate and fructose 1,6-bisphosphate, operated by phosphofructokinase (PFK 1) and by fructose-1,6-bisphosphatase (FBPase 1); 3) the interconversion between phosphoenolpyruvate and pyruvate, implicating one enzyme, pyruvate kinase, in the glycolytic direction and two enzymes, pyruvate carboxylase and phosphoenolpyruvate carboxykinase, in the gluconeogenic direction. Each of these seven enzymes catalyses a unidirectional reaction and is therefore a potential target for flux control.

Glucagon is a pancreatic hormone, a major effect of which is to stimulate the release of glucose by the liver. It not only promotes glycogen breakdown, but also stimulates gluconeogenesis and inhibits glycolysis, as was recognized in the early 1960s [3]. Like the effect on glycogen metabolism, the effect on gluconeogenesis is mediated by cyclic AMP [4], but its precise mechanism remained elusive for a number of years. Measurements of the concentrations of metabolites identified the interconversions between pyruvate and phosphoenolpyruvate, and between fructose 1,6-bisphosphate and fructose 6-phosphate as the targets for the regulation by glucagon [3, 5, 6]. The discovery that liver pyruvate kinase was an interconvertible enzyme, which was inactivated upon phosphorylation by cAMP-dependent protein kinase offered an explanation for the first crossover [reviewed in 7]. This inactivation causes an inhibition of the conversion of phosphoenolpyruvate to pyruvate, which, in turn, raises the concentration of phosphoenolpyruvate and favours its conversion to fructose 1,6-bisphosphate (Fig. 1).

Flux measurements using isotopically labelled substrates indicated that glucagon also caused in the intact cell a decrease in the flow of metabolites through phosphofructokinase [8–11]. The possibility that this enzyme was interconvertible, was seriously considered when in 1979, several groups of investigators showed that the activity of phosphofructokinase, when measured at subsaturating concentration of fructose 6-phosphate, was decreased in extracts of liver cells that had been exposed to glucagon [12–15]. No effect was observed at saturating concentrations of fructose 6-phosphate, indicating that treatment with the hormone only changed the affinity of the enzyme for its substrate.

It turned out, however, that the change in the kinetic properties induced by the glucagon treatment disappeared if the extracts were carefully filtered on Sephadex G-25 or if the enzyme was partially purified [11], two processes that separate proteins from the small molecules present in the extracts. Gel filtration actually decreased the apparent affinity for fructose 6-phosphate of the phosphofructokinase present in control extracts, without significantly affecting the kinetics of the enzyme present in extracts of cells treated with glucagon. In addition, protein-free extracts of control liver cells were found to stimulate phosphofructokinase, whereas similar extracts prepared from cells treated with glucagon exerted no stimulation. These results indicated that the effect of glucagon to reduce the activity of phosphofructokinase was mediated by the decrease in the concentration of a low-molecular-weight effector of this enzyme [11]. Work from the laboratory of Pilkis [16] also indicated that the change in the kinetic properties of phosphofructokinase was not due to a covalent modification and also disclosed a low-molecular-weight stimulator. Furuya and Uyeda [17] also found evidence of the presence of an "activation factor" in liver.

The new effector of phosphofructokinase was characterized in our laboratory as an extremely acid-labile diphosphoric ester, and eventually identified as fructose 2,6-bisphosphate on the basis that it was quantitatively converted to stoichiometric amounts of fructose 6-phosphate and inorganic phosphate upon incubation under mild acid conditions. Furthermore, acid hydrolysis caused the appearance of a reducing power that was exactly accounted for by the liberated fructose 6-phosphate. This indicated that fructose 6-phosphate was bound to a second molecule of phosphate in the intact stimulator of phosphofructokinase [18].

That no other chemical group entered in the native structure was proved by the demonstration that an effector having all the properties of the natural one was formed upon incubation of fructose 6-phosphate in the presence of concentrated phosphoric acid at 0 °C [19]. Fructose 2,6-bisphosphate was then synthesized [20–22] in millimolar amounts from fructose 1,6-bisphosphate and its properties could be further investigated.

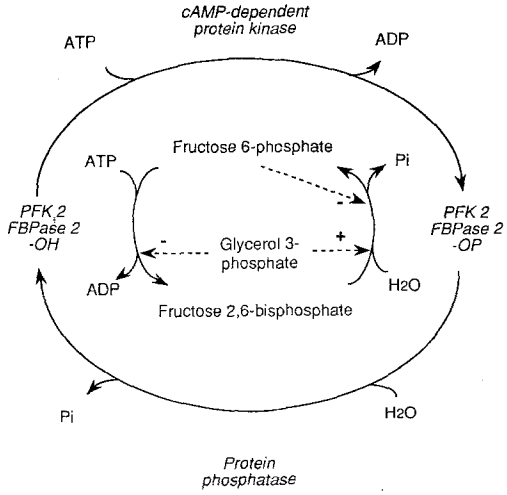

Fig. 2. Regulation of the biosynthesis and biodegradation of fructose 2,6-bisphosphate in the liver. PFK2, Phosphofructokinase 2; FBPase 2, fructose-2-6-bisphosphatase

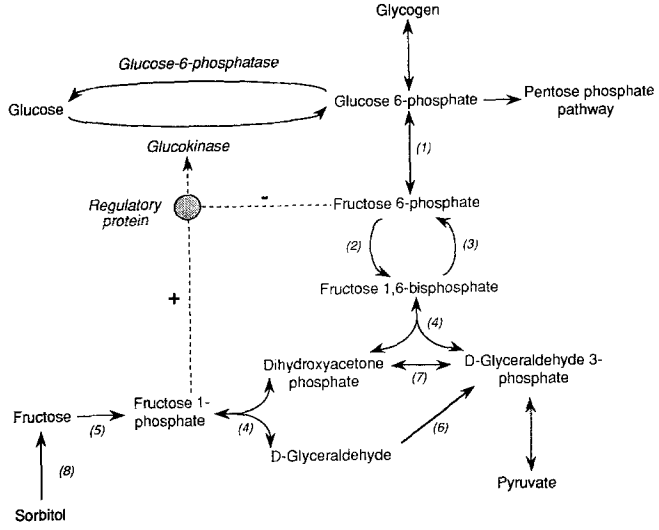

Fig. 3. Metabolism of glucose, fructose, D-glyceraldehyde and sorbitol in the liver. Numbers between parentheses indicate the reactions catalysed by the following enzymes: (1) glucose 6-phosphate isomerase; (2) phosphofructokinase; (3) fructose-1,6-bisphosphatase; (4) aldolase; (5) fructokinase; (6) triokinase; (7) triose-phosphate isomerase; (8) sorbitol dehydrogenase

Roles

Fructose 2,6-bisphosphate is the most potent stimulator of phosphofructokinase not only from liver, but also from all animal tissues [reviewed in 23–27]. These enzymes are characteristically inhibited by high concentrations of ATP and display a sigmoidal saturation curve for their other substrate, fructose 6-phosphate. At micromolar or submicromolar concentrations, fructose 2,6-bisphosphate stimulates the activity by antagonizing the inhibition exerted by ATP and increasing the affinity for fructose 6-phosphate.

The diphosphate sugar is also a positive effector for phosphofructokinases from yeast and from other fungi [reviewed in 26]. In plants, the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate is catalysed not only by the classic phosphofructokinase that uses ATP as a phosphate donor, but also by PPi-PFK, which uses inorganic pyrophosphate (PPi) instead of ATP. As originally shown by Sabularse and Anderson [28], plant PPi-PFK is greatly stimulated by fructose 2,6-bisphosphate. The remarkable sensitivity of the potato tuber enzyme to this effector permitted the development of an assay allowing one to measure less than 1 picomole of this effector [29].

Fructose 2,6-bisphosphate is also an inhibitor of fructose-1,6-bisphosphatase from animal tissues and from fungi, as well as of the cytosolic enzyme from plants [reviewed in 23–27]. The inhibition is stronger at low concentrations of fructose 1,6-bisphosphate than at high concentrations. Furthermore fructose 2,6-bisphosphate changes the shape of the saturation curve from a hyperbola to a sigmoid, and acts synergically with the allosteric effector AMP.

Through its opposite effects on phosphofructokinase and fructose 1,6-bisphosphatase (Fig. 1), the novel effector can act as a switch: when its concentration is elevated, it favours glycolysis and inhibits gluconeogenesis. Conversely, when its concentration is low, glycolysis is turned off and gluconeogenesis proceeds without inhibition.

Biosynthesis and biodegradation, and their control by hormones and metabolites

Fructose 2,6-bisphosphate is formed from fructose 6-phosphate and ATP by a special phosphofructokinase called phosphofructokinase 2 (PFK 2) and hydrolysed to fructose 6-phosphate and inorganic phosphate by fructose-2,6-bisphosphatase (FBPase 2) [reviewed in 2, 23–26, 30–33]. The two enzymes are part of a single bifunctional protein, several isozymes of which have now been described. The liver isozyme, the first to be studied, is a substrate for cyclic AMP-dependent protein kinase (Fig. 2). Its phosphorylation causes the inactivation of PFK 2 and the activation of FBPase 2 and, therefore, the disappearance of fructose 2,6-bisphosphate, as observed when liver cells are treated with glucagon or other agents that cause an increase in the concentration of cAMP. The two activities of the bifunctional enzymes are also controlled by the concentration of some metabolites, principally fructose 6-phosphate, a substrate for PFK 2 and a powerful inhibitor for FBPase 2, and glycerol 3-phosphate, an inhibitor of PFK 2 and a stimulator for FBPase 2. The regulatory role of fructose 6-phosphate is best illustrated by the effect of glucose, which increases the concentrations of fructose 6-phosphate and of fructose 2,6-bisphosphate in the liver, and hence increases glycolysis and inhibits gluconeogenesis [2]. Glucose also causes an increase in the concentration of fructose 2,6-bisphosphate in other cells, including the beta cells of pancreatic islets [34].

In streptozotocin-diabetes, the concentration of fructose 2,6-bisphosphate in the liver is low, in accordance with the elevated gluconeogenic flux. The low concentration of the bisphosphate ester is presumably the result of the elevated glucagon concentration and the low insulin concentration in the plasma. These hormonal changes are expected to cause not only an increase in the phosphorylation

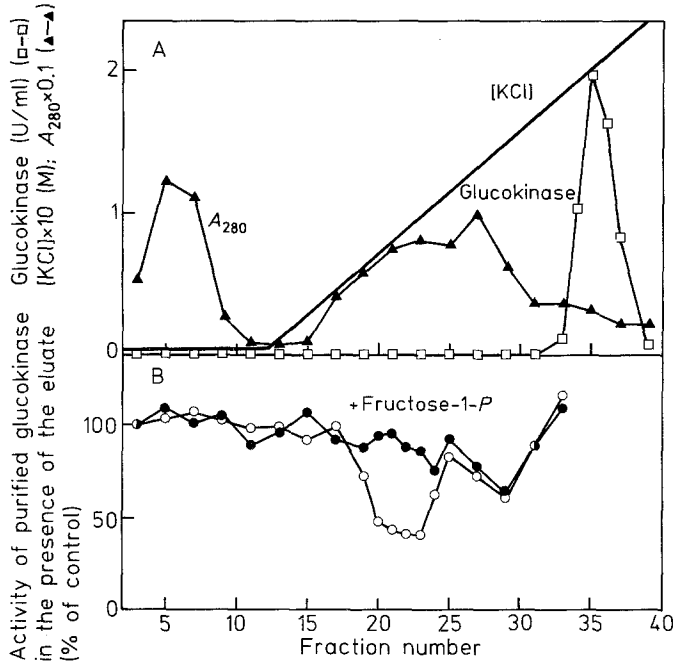

Fig. 4 A, B. Separation of glucokinase from a fructose 1-phosphate sensitive factor. A liver fraction prepared by precipitation with polyethyleneglycol was applied to an anion-exchange column, which was developed with a KCl gradient. **A** shows the activity of glucokinase measured at 50 mmol/l glucose. **B** shows measurements of the activity of purified glucokinase in the presence of 25 µl of the fractions with (●) or without (○) 1 mmol/l fructose 1-phosphate. From [46] with permission

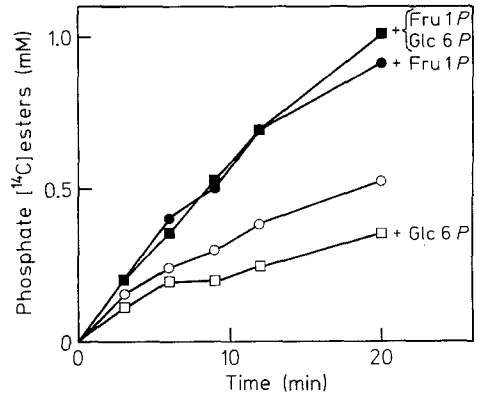

Fig. 5. Effects of fructose 6-phosphate and of fructose 1-phosphate on the activity of glucokinase in the presence of regulatory protein. Partially purified glucokinase was measured in the presence of 5 mmol/l glucose, 1 mmol/l ATPMg, 3 U of regulatory protein per ml, and the indicated concentrations of fructose 1-phosphate and fructose 6-phosphate. From [46], with permission

state of PFK2/FBPase 2, but also reduce the expression of glucokinase (see below) and hence decrease the glucose 6-phosphate and fructose 6-phosphate concentration [35].

Importance and pathophysiological implications

Fructose 2,6-bisphosphate is now recognized as a major control element for switching from glycolysis to gluconeogenesis after glucagon treatment [2]. It has also been established that it plays an important role in the control of glycolysis in widely different cell types, including mammalian cells, *Saccharomyces cerevisiae* and the protozoon *Trypanosoma brucei* [26]. Fructose 2,6-bisphosphate is, like cyclic AMP, a signal molecule, which is formed from ubiquitous compounds by a highly regulated enzyme. It is not itself on a major metabolic pathway, but is used as an integrator of metabolism. Originally, cyclic AMP was probably a "hunger signal", which signified the lack of glucose [36]. By contrast, fructose 2,6-bisphosphate signifies that glucose is abundant and can be used by glycolysis, and that gluconeogenesis must be stopped [23].

The regulatory protein of glucokinase

Glucokinase, a special form of hexokinase

The phosphorylation of glucose in animal tissues is catalysed by four different hexokinases (EC 2.7.1.1). Hexokinase I, II and III display a high affinity for glucose ($K_m < 0.2$ mmol/l) and are inhibited by concentrations of glucose 6-phosphate in the physiological range [reviewed in 37]. Their activity can therefore be adjusted to the rate at which glucose 6-phosphate is utilized by tissues.

The fourth hexokinase, glucokinase [reviewed in 38] has distinctive kinetic properties: it displays a sigmoidal saturation curve for glucose [39], and its half saturating concentration of substrate ($S_{0.5}$) is about 10 mmol/l, a value slightly above the concentration of glucose in the plasma of normal subjects. Since glucokinase is not inhibited by micromolar concentrations of glucose 6-phosphate, it is generally accepted that the activity of this enzyme is controlled on a short-term basis by the concentration of glucose. Accordingly glucokinase is present only in two cell types that have to respond physiologically to changes in the glucose concentration. These cell types are the parenchymal cells of the liver and the beta cell of pancreatic islets [reviewed in 38, 40, 41].

The glucokinase gene comprises two different promoters, separated by at least 10 kilobase pairs. The most upstream of the two promoters is used in the pancreas and the other in the liver and the two resulting mRNAs differ at their 5' end. The deduced sequence of the predominant islet enzyme differs from the liver isozyme by less than 10 amino acids at the N terminus. It is not known if this difference has any effect on the physiological function of glucokinase [reviewed in 42].

Effect of fructose on the phosphorylation of glucose in isolated hepatocytes

The rate of glucose phosphorylation can be monitored in cell suspensions by the release of tritium from [2-^{3}H] water [43]. In 1979, Clark and co-workers [44] found that the detritiation of glucose was stimulated about three-fold when 2.5 mmol/l fructose was added to a suspension of isolated hepatocytes. This effect remained without explanation for about 10 years.

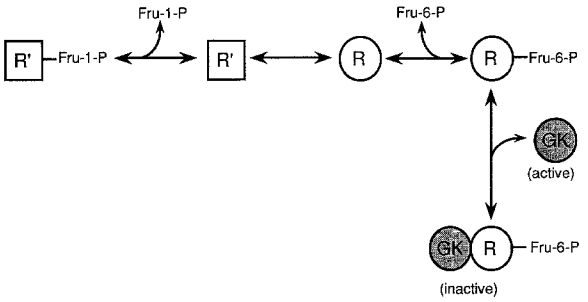

Fig. 6. Model for the action of the regulatory protein on glucokinase (GK). Fru-6-P, Fructose 6-phosphate; Fru-1-P, fructose 1-phosphate. See section *mechanism of action* for details

When this problem was reinvestigated [43], it was found that fructose increased the apparent affinity of glucokinase for its sugar substrate, the $S_{0.5}$ for glucose decreasing from 15–20 mmol/l to 9–10 mmol/l. Furthermore, the ketohexose was found to act at very low concentrations, a half-maximal effect being reached at ≈ 50 µmol/l. The effect of fructose was almost maximal 5 min after its addition, and it was readily lost when the cells were washed free of the ketose. These results indicated that the increase in glucokinase activity was not due to synthesis of new protein but to a more rapid process, such as a change in the concentration of an allosteric effector or in the extent of a covalent modification [45].

It was also found that sorbitol, at similar concentrations as fructose, and D-glyceraldehyde at about 10-fold higher concentrations, stimulated detritiation whereas dihydroxyacetone or glycerol had no such effect [43]. This suggested that the stimulation resulted from the formation of a metabolite that could be produced from fructose, sorbitol or D-glyceraldehyde, although not from dihydroxyacetone or glycerol. As can be deduced from Figure 3, the best candidate was fructose 1-phosphate, an intermediate of fructose and sorbitol metabolism, which can also be formed from D-glyceraldehyde and dihydroxyacetonephosphate by reversal of the reaction catalysed by aldolase.

Discovery of the regulatory protein

Fructose 1-phosphate was found to stimulate glucokinase in cell-free extracts but had no effect on this enzyme when it had been partially purified by anion-exchange chromatography [46]. These results indicated that, in order to be sensitive to fructose 1-phosphate, glucokinase required the presence of a substance from which it was separated during purification. Accordingly, we found that the fractions eluted from an anion-exchange column at approximately 80 mmol/l KCl contained an inhibitor of glucokinase, whose effect was cancelled by fructose 1-phosphate (Fig. 4).

The inhibitor was a protein, as indicated by its heat- and trypsin-sensitivity and by its molecular mass of ≈ 60 kDa. Its effect was largely dependent on the presence of fructose 6-phosphate, which, by itself, did not inhibit glucokinase. Figure 5 illustrates the fact that the inhibition exerted by fructose 6-phosphate is partial and that it is antagonized by fructose 1-phosphate, which acts competitively vs fructose 6-phosphate. Other experiments showed that the regulatory protein inhibited glucokinase competitively with respect to glucose [46].

The regulatory protein was purified to homogeneity by a procedure involving several chromatographic steps and identified as a 62 kDa polypeptide. Since the molecular mass of the native protein is ≈ 60 kDa, these results indicated that the regulatory protein is a monomer [47].

Mechanism of action

It was conceivable that the regulatory protein inhibited glucokinase indirectly, for example by catalysing the conversion of fructose 6-phosphate to an hypothetical inhibitor. The fact that no inhibitor was formed when regulatory protein was incubated with fructose 6-phosphate eliminated this possibility. Furthermore, the regulatory protein did not appear to cause a stable modification in the kinetic properties of glucokinase, i.e. a modification that would persist after separation of the two proteins [47]. This indicated that the regulatory protein was not acting as a modifying enzyme, that would catalyse a covalent modification.

The possibility that the regulatory protein inhibited glucokinase merely by forming a complex with this enzyme was tested by measuring the apparent molecular mass of glucokinase by centrifugation in sucrose gradient. In the absence of regulatory protein, the mass of glucokinase was about 55 kDa and this value was unchanged when glucokinase was centrifuged together with regulatory protein in the absence of fructose 6-phosphate. However, it almost doubled in the simultaneous presence of regulatory protein and fructose 6-phosphate, although not when fructose 1-phosphate was also included. Conversely glucokinase caused an increase in the apparent molecular mass of the regulatory protein in the presence of fructose 6-phosphate. These results indicated that fructose 6-phosphate promoted the formation of a complex between glucokinase and regulatory protein, and that fructose 1-phosphate inhibited the formation of this complex [47].

The next problem was to understand how these phosphate esters could affect the association of the two proteins. Since neither fructose 6-phosphate nor fructose 1-phosphate affected the kinetic properties of glucokinase in the absence of regulatory protein, they most likely acted by binding to the latter. Accordingly the regulatory protein was found to bind up to about 1 mol of fructose 1-phosphate or of sorbitol 6-phosphate (an inhibitor that is about 5 times more potent than fructose 6-phosphate) per mol [48]. The binding of fructose 1-phosphate and of sorbitol 6-phosphate are mutually competitive, suggesting that both types of effectors bind to one single site.

On the basis of these results the model shown in Figure 6 was proposed. According to this model, the regulatory protein exists under two different conformations, one (R) which can bind glucokinase and inhibit this enzyme, and the other (R'), which cannot. Fructose 6-phosphate

binds to conformation R, and fructose 1-phosphate, to conformation R'. In the absence of inhibitor and activator, the ratio R/R' is equal to 1/15, accounting for the modest inhibition exerted by the regulatory protein in the absence of effector [46, 49]. Fructose 6-phosphate increases this ratio, thus reinforcing the inhibition exerted by the regulatory protein. Fructose 1-phosphate antagonizes the fructose 6-phosphate effect by trapping the regulatory protein in the R' conformation.

The model shown in Figure 6 is reminiscent of that proposed for the regulation of cyclic AMP-dependent protein kinase by its regulatory subunit [reviewed in 50]. In both cases, the free catalytic subunit is active and loses activity upon association to the regulatory subunit, the effect of ligands such as fructose 1-phosphate or cyclic AMP being to prevent the association. One difference between the two systems is that the holoenzyme is an heterotetramer in the case of cyclic AMP dependent protein kinase and a heterodimer in the case of glucokinase. Another difference is that no ligand is required to promote the association in the case of cyclic AMP-dependent protein kinase. Finally, the association constant of the glucokinase/regulatory protein complex is lower than that of protein kinase, explaining that the regulatory protein easily dissociates from glucokinase following homogenization of the liver [46].

Kinetic studies fully supported the model shown in Figure 6 [51]. In addition they showed that the regulatory protein binds to glucokinase at a site distinct from the catalytic site, despite the fact that it inhibits glucokinase competitively with respect to glucose [49].

Physiological implications

The liver cell contains under all conditions at least 10 μmol/l fructose 6-phosphate. Since in the absence of fructose, it does not contain fructose 1-phosphate [52], glucokinase is inhibited by the regulatory protein. This explains that, in intact cells, the apparent affinity of glucokinase is lower than that of the pure enzyme. The fact that fructose and other compounds that give rise to fructose 1-phosphate restore the affinity to the expected level is in accordance with this interpretation [45, 52]. This type of regulation not only occurs in isolated hepatocytes, but also in vivo [53]. In anaesthetized rats, the rate of glucose phosphorylation, as measured by the detritiation of [2-^{3}H] glucose administered via the portal vein, amounts to $\approx 0.6\ \mu mol \cdot min^{-1} \cdot g^{-1}$, i.e. about 40 % of the glucokinase activity that can be measured in a cell-free extract at the concentration of glucose present in the liver. Fructose, when injected in a peripheral vein, stimulated this rate up to about 2.5-fold in fed and overnight fasted rats, a maximal effect being observed at a dose of 50 mg/kg. Fructose was also active when administered by intragastric infusion, a dose of 20 mg/kg causing the rate to increase twofold. When extrapolated to man, this dose of fructose corresponds to only between 1 and 2 g of pure fructose.

The novel mode of regulation of glucokinase accounts for the fact that fructose administration causes a moderate hypoglycaemia in anaesthetised rats or in perfused livers [54, 55], and for the fact that fructose increases the conversion of radiolabelled glucose to glycogen [44, 56]. Since fructose is present in most foods of vegetable origin that also contain glucose, it can play the role of a signal that stimulates the consumption of alimentary glucose by the liver.

The regulatory protein in pancreatic islets

In pancreatic beta cells, glucokinase plays an important role in the control of the glycolytic flux and, consequently, in the control of insulin secretion [57]. The following data indicate that the regulatory protein is present also in this cell type [58]: 1) glucokinase from islet tissue is inhibited by the regulatory protein from rat liver; 2) in islet extracts, the rate of glucose phosphorylation is stimulated by fructose 1-phosphate; 3) in intact islets, the rate of detritiation of [5-^{3}H]glucose is stimulated by D-glyceraldehyde, which causes a significant increase in the intracellular concentration of fructose 1-phosphate. Fructose is, however, unable to stimulate the phosphorylation of glucose, because fructokinase activity is extremely low in this tissue. The physiological role of the regulatory protein in islets remains unknown.

Is the regulatory protein implicated in the development of diabetes?

One rare subtype of non-insulin-dependent diabetes, maturity-onset diabetes of the young (MODY) is distinctive clinically because it has an early onset and is consistently inherited as an autosomal dominant trait [59]. It was recently shown that in some families, MODY is linked with the glucokinase gene [60]. Further analysis showed that, in those families, missense or non-sense mutations were present in the glucokinase gene and cosegregated with MODY [61, 62]. This clearly indicated that decreased expression of functional glucokinase in the liver and in the beta cells is implicated in this disease. We speculate that mutations in the gene encoding the regulatory protein could also be responsible for diabetes, if they result in the synthesis of an "hyperactive" protein. The mode of inheritance of this type of diabetes is expected to be also dominant.

Acknowledgements. I am greatly indebted to my teachers Professors H.-G. Hers and L. Hue for their help and encouragement. Work in this laboratory is supported by the Fonds de la Recherche Scientifique Médicale and by the Prime Minister's Office, Science Policy Programming. I am Maître de Recherches of the Fonds National de la Recherche Scientifique.

References

1. Fruton JS (1972) Molecules and life. Historical essay on the interplay between chemistry and biology. Wiley, New York
2. Hers HG, Hue L (1983) Gluconeogenesis and related aspects of glycolysis. Ann Rev Biochem 52: 617–653
3. Schimassek H, Mitzkat HJ (1963) Über eine spezifische Wirkung des Glucagon auf die Embden-Meyerhof-Kette in der Leber. Biochem Z 337: 510–518

4. Exton JH, Mallette LE, Jefferson LS et al. (1970) The hormonal control of hepatic gluconeogenesis. Rec Prog Horm Res 26: 411–461
5. Exton JH, Park CR (1969) Control of gluconeogenesis in liver. III. Effects of L-lactate, pyruvate, fructose, glucagon, epinephrine and adenosine 3, 5 monophosphate on gluconeogenic intermediates in the perfused rat liver. J Biol Chem 244: 1424–1433
6. Williamson JR, Browning ET, Thurman RG, Scholz R (1969) Inhibition of glucagon effects in perfused rat liver by (+) decanoylcarnitine. J Biol Chem 244: 5055–5064
7. Engström L (1978) The regulation of liver pyruvate kinase by phosphorylation-dephosphorylation. Curr Top Cell Regul 13: 29–51
8. Clark MG, Kneer NM, Bosch AL, Lardy HA (1974) The fructose, 1,6-diphosphatase-phosphofructokinase substrate cycle. A site of regulation of hepatic gluconeogenesis by glucagon. J Biol Chem 249: 5695–5703
9. Rognstad R, Katz J (1976) Effects of hormones and of ethanol on the fructose 6-P-fructose 1,6-P_2 futile cycle during gluconeogenesis in the liver. Arch Biochem Biophys 177: 337–345
10. Van Schaftingen E, Hue L, Hers HG (1980) Study of the fructose 6-phosphate/fructose 1,6-bisphosphate cycle in the liver in vivo. Biochem J 192: 263–271
11. Van Schaftingen E, Hue L, Hers HG (1980) Control of the fructose 6-phosphate/fructose 1,6-bisphosphate cycle in isolated hepatocytes by glucose and glucagon. Role of a low-molecular-weight stimulator of phosphofructokinase. Biochem J 192: 887–895
12. Castaño JG, Nieto A, Feliu JE (1979) Inactivation of phosphofructokinase by glucagon in rat hepatocytes. J Biol Chem 254: 5576–5579
13. Pilkis SJ, Schlumpf J, Pilkis J, Claus TH (1979) Regulation of phosphofructokinase activity by glucagon in isolated rat hepatocytes. Biochem Biophys Res Commun 88: 960–967
14. Kagimoto T, Uyeda K (1979) Hormone-stimulated phosphorylation of liver phosphofructokinase in vivo. J Biol Chem 254: 5584–5587
15. Clarke SD, Watkins PA, Lane MD (1979) Acute control of fatty acid synthesis by cAMP in the chick liver cell: possible site of inhibition of citrate formation. J Lipid Res 20: 974–985
16. Claus TH, Schlumpf JR, El-Maghrabi MR, Pilkis J, Pilkis SJ (1980) Mechanism of action of glucagon on hepatocyte phosphofructokinase activity. Proc Natl Acad Sci USA 77: 6501–6505
17. Furuya E, Uyeda K (1980) Regulation of phosphofructokinase by a new mechanism. An activation factor binding to the phosphorylated enzyme. J Biol Chem 255: 11656–11659
18. Van Schaftingen E, Hue L, Hers HG (1980) Fructose 2,6-bisphosphate, the probable structure of the glucose- and glucagon-sensitive stimulator of phosphofructokinase. Biochem J 192: 897–901
19. Van Schaftingen E, Hers HG (1980) Synthesis of a stimulator of phosphofructokinase, most likely fructose 2,6-bisphosphate, from phosphoric acid and fructose 6-phosphoric acid. Biochem Biophys Res Commun 96: 1524–1531
20. Pilkis SJ, El-Maghrabi MR, Pilkis J, Claus TH, Cumming DA (1981) Fructose 2,6-bisphosphate. A new activator of phosphofructokinase. J Biol Chem 256: 3171–3174
21. Uyeda K, Furuya E, Sherry AD (1981) The structure of "activation factor" for phosphofructokinase. J Biol Chem 256: 8679–8684
22. Van Schaftingen E, Hers HG (1981) Formation of fructose 2,6-bisphosphate from fructose 1,6-bisphosphate by intramolecular cyclisation followed by alkaline hydrolysis. Eur J Biochem 117: 319–323
23. Hers HG, Van Schaftingen E (1982) Fructose 2,6-bisphosphate 2 years after its discovery. Biochem J 206: 1–12
24. Claus TH, El-Maghrabi MR, Regen DM et al. (1984) The role of fructose 2,6-bisphosphate in the regulation of carbohydrate metabolism. Curr Top Cell Reg 23: 57–86
25. Hue L, Rider MH (1987) Role of fructose 2,6-bisphosphate in the control of glycolysis in mammalian tissues. Biochem J 245: 313–324
26. Van Schaftingen E (1987) Fructose 2,6-bisphosphate. Adv Enzymol 59: 315–395
27. Kemp RG, Marcus F (1990) Effects of fructose-2,6-bisphosphate on 6-phosphofructo-1-kinase and fructose-1,6-bisphosphatase. In: Pilkis SJ (ed) Fructose-2,6-bisphosphate. CRC Press, Boca Raton, pp 17–38
28. Sabularse DC, Anderson RL (1981) D-Fructose-2,6-bisphosphate: a naturally occurring activator for inorganic pyrophosphate: D-fructose-6-phosphate 1-phosphotransferase in plants. Biochem Biophys Res Commun 103: 848–855
29. Van Schaftingen E, Lederer B, Bartrons R, Hers HG (1982) A kinetic study of pyrophosphate: fructose 6-phosphate phosphotransferase from potato tubers. Application to a microassay of fructose 2,6-bisphosphate. Eur J Biochem 129: 191–195
30. Pilkis SJ, El-Maghrabi MR, Claus TH (1988) Hormonal regulation of hepatic gluconeogenesis and glycolysis. Ann Rev Biochem 57: 755–783
31. El-Maghrabi MR, Colosia A, Tauler A, Kurland I, Pilkis SJ (1990) Liver 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. In: Pilkis SJ (ed) Fructose-2,6-bisphosphate. CRC Press, Boca Raton, pp 87–124
32. Hue L, Rider M, Rousseau GG (1990) Fructose-2,6-bisphosphate in extra-hepatic tissues. In: Pilkis SJ (ed) Fructose-2,6-bisphosphate. CRC Press, Boca Raton, pp 173–192
33. Rousseau GG, Hue L (1993) Mammalian 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: a bifunctional enzyme that controls glycolysis. Progr Nucleic Acid Res Mol Biol 45: 99–127
34. Sener A, Van Schaftingen E, Van De Winkel M et al. (1984) Effects of glucose and glucagon on the fructose 2,6-bisphosphate content of pancreatic islets and purified pancreatic β-cells. Biochem J 221: 759–764
35. Van Schaftingen E (1990) Role of fructose-2,6-bisphosphate in the regulation of hepatic carbohydrate metabolism. In: Pilkis SJ (ed) Fructose-2,6-bisphosphate. CRC Press, Boca Raton, pp 65–85
36. Stryer L (1988) Biochemistry, 3rd edn W. H. Freeman, San Francisco, p 983
37. Colowick SP (1973) The hexokinases. In: Boyer P (ed) The enzymes, 3rd edn, vol 9. Academic Press, New York, pp 1–48
38. Weinhouse S (1976) Regulation of glucokinase in the liver. Curr Top Cell Regul, 11: 1–50
39. Storer AC, Cornish-Bowden A (1976) Kinetics of rat liver glucokinase. Co-operative interactions with glucose at physiologically significant concentrations. Biochem J 159: 7–14
40. Meglasson MD, Matschinsky FM (1986) Pancreatic islet glucose metabolism and regulation of insulin secretion. Diabet Metab Rev 2: 163–214
41. Lenzen S, Panten U (1988) Signal recognition by pancreatic β-cells. Biochem Pharmacol 37: 371–378
42. Magnuson MA (1990) Glucokinase gene structure. Functional implications of molecular genetic studies. Diabetes 39: 523–527
43. Katz J, Rognstad R (1975) Futile cycles in the metabolism of glucose. Curr Top Cell Regul 10: 237–289
44. Clark DG, Filsell, OH, Topping DL (1979) Effects of fructose concentration on carbohydrate metabolism, heat production and substrate cycling in isolated rat hepatocytes. Biochem J 184: 501–507
45. Van Schaftingen E, Vandercammen A (1989) Stimulation of glucose phosphorylation by fructose in isolated rat hepatocytes. Eur J Biochem 179: 173–177
46. Van Schaftingen E (1989) A protein from rat liver confers to glucokinase the property of being antagonistically regulated by fructose 6-phosphate and fructose 1-phosphate. Eur J Biochem 179: 179–184
47. Vandercammen A, Van Schaftingen E (1990) The mechanism by which rat liver glucokinase is inhibited by the regulatory protein. Eur J Biochem 191: 483–489
48. Vandercammen A, Detheux M, Van Schaftingen E (1992) Binding of sorbitol 6-phosphate and of fructose 1-phosphate to the regulatory protein of liver glucokinase. Biochem J 286: 253–256

49. Vandercammen A, Van Schaftingen E (1991) Competitive inhibition of liver glucokinase by its regulatory protein. Eur J Biochem 200: 545–551
50. Beebe SJ, Corbin JD (1986) Cyclic nucleotide-dependent protein kinases. In: Boyer PD, Krebs EG (eds) The enzymes, 3rd edn, vol 17. Academic Press, Orlando, pp 43–111
51. Detheux M, Vandercammen A, Van Schaftingen E (1991) Effectors of the regulatory protein acting on liver glucokinase: a kinetic investigation. Eur J Biochem 200: 553–561
52. Davies DR, Detheux M, Van Schaftingen E (1990) Fructose 1-phosphate and the regulation of glucokinase activity in isolated hepatocytes. Eur J Biochem 192: 283–289
53. Van Schaftingen E, Davies DR (1991) Fructose administration stimulates glucose phosphorylation in the liver of anesthetized rats. FASEB J 5: 326–330
54. Topping DL, Mayes PA (1971) The concentrations of fructose, glucose and lactate in the splanchnic blood vessels of rats absorbing fructose. Nutr Metab 13: 331–338
55. Topping DL, Mayes PA (1976) Comparative effects of fructose and glucose on the lipid and carbohydrate metabolism of perfused rat liver. Br J Nutr 36: 113–126
56. Seglen PO (1974) Autoregulation of glycolysis, respiration, gluconeogenesis and glycogen synthesis in isolated parenchymal rat liver cells under aerobic and anaerobic conditions. Biochem Biophys Acta 338: 317–336
57. Matschinsky FM (1990) Glucokinase as glucose sensor and metabolic signal generator in pancreatic β-cells and hepatocytes. Diabetes 39: 647–652
58. Malaisse WJ, Malaisse-Lagae F, Davies DR et al. (1990) Regulation of glucokinase by a fructose-1-phosphate-sensitive protein in pancreatic islets. Eur J Biochem 190: 539–545
59. Fajans SS (1989) Maturity-onset diabetes of the young (MODY). Diabetes Metab Rev 5: 579–606
60. Froguel Ph, Vaxillaire M, Sun F et al. (1992) Close linkage of glucokinase locus on chomososme 7p to early-onset non-insulin-dependent diabetes mellitus. Nature 356: 162–164
61. Vionnet N, Stoffel M, Takeda J et al. (1992) Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. Nature 356: 721–722
62. Stoffel M, Froguel Ph, Takeda J et al. (1992) Human glucokinase gene: isolation, characterization, and identification of two missense mutations linked to early-onset non-insulin-dependent (type 2) diabetes mellitus. Proc Natl Acad Sci USA 89: 7698–7702

Dr. E. Van Schaftingen
Laboratoire de Chimie Physiologique
UCL 7539
Avenue Hippocrate 75
B-1200 Brussels
Belgium