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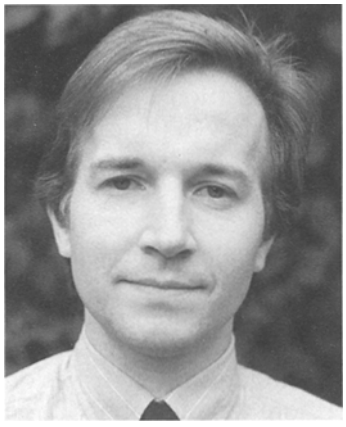
Emile Van Schaftingen
Minkowski Award, 1992, Prague



Emile Van Schaftingen studied medicine at the Université Catholique de Louvain from where he graduated with an M.D. degree in 1978. He started his research work under the supervision of Professor H.-G. Hers, and together with him and Louis Hue, discovered fructose 2,6-bisphosphate in 1980. Fructose 2,6-bisphosphate was central to his research during the proceeding years and was the subject of his doctoral thesis (Thèse d' Agrégation) in 1985. After a sabbatical stay at NIH in the laboratory of B. de Crombrugghe, he returned to Brussels where he now heads a research group at the International Institute of Cellular and Molecular Pathology. His interest has now moved mainly to the regulatory protein of glucokinase, which he discovered in 1988. He is tenured investigator of the National Fund for Scientific Research and teaches biochemistry at the Université Catholique de Louvain.

Short-term regulation of glucokinase

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Summary The activity of liver glucokinase is controlled in the short term by the concentration of its substrate glucose and by a regulatory protein, which acts as a competitive inhibitor with respect to glucose. In mammalian species, the effect of this protein is modulated by fructose 6-phosphate, which reinforces the inhibition, and by fructose 1-phosphate which antagonizes it. In the rat, the regulatory protein is found in the two tissues that express glucokinase, i.e., the liver and the pancreatic islets. Of particular interest is the fact that the regulatory protein is absent from the liver in those species that have no hepatic glucokinase. These results indicate that the two proteins form a functional unit. The regulatory protein appears in rat liver before birth, whereas glucokinase is only synthesized after 15 days of extrauterine life. The concentration of regulatory protein in the liver of the adult rat decreases by about 50 % during starvation and in diabetes mellitus. Under these conditions, the difference between the concentrations of regulatory protein and glucokinase remains constant at about 0.4–0.5 nmol/g. [Diabetologia (1994) 37 [Suppl 2]: S43–S47]

Key words Glucokinase, fructose, fructose 1-phosphate, glycolysis, regulatory protein.

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Abbreviations: Fructose 1-P, fructose 1-phosphate; DEAE, diethylaminoethyl; glucose 6-P, glucose 6-phosphate.

Glucose phosphorylation in animal tissues is catalysed by four different hexokinases [1]. Hexokinase I, II and III display a low K_m for glucose and are inhibited by micromolar concentrations of glucose 6-P, which acts as a feedback inhibitor. Hexokinase IV, more commonly called glucokinase, dis-

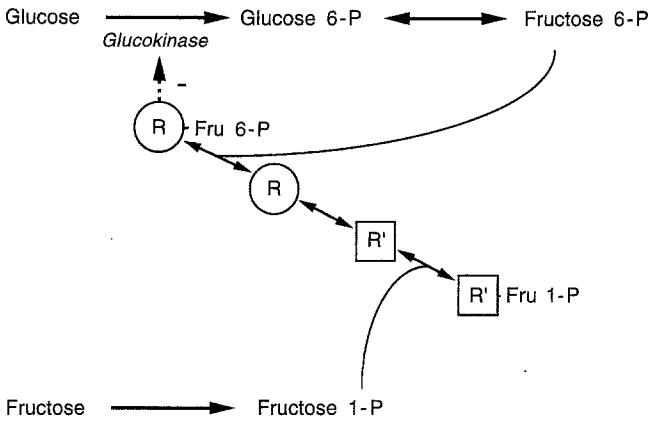

Fig. 1. Regulation of glucokinase by a fructose 1-P and fructose 6-P-sensitive protein. R and R' represent two different conformations of the regulatory protein

plays a much lower affinity for glucose, and is not inhibited by physiological concentrations of glucose 6-P [2–4]. The activity of glucokinase is therefore mainly controlled by the concentration of its substrate.

Glucokinase is expressed in the parenchymal cells of the liver and beta cells of pancreatic islets, two cell types that play a central role in glucose homeostasis by adjusting their physiological activity to changes in the level of the glycaemia. Both types of cell express GLUT 2 [5], a very active glucose transporter capable of maintaining the intracellular glucose concentration at a level close to that found in the plasma. Glucokinase therefore catalyses the rate-limiting step of glucose metabolism and therefore can play the role of a glucose "receptor". The most telling demonstration of the importance of glucokinase in glucose homeostasis was the recent demonstration that mutations in the gene of this enzyme are responsible for about 50 % of the cases of MODY (maturity onset diabetes of the young) [4, 6–7].

Compared to the low K_m hexokinases, glucokinase has a much lower molecular mass (50 compared to 100 kDa). In addition, its saturation curve is sigmoidal [8], which increases the sensitivity of this enzyme to changes in the blood glucose concentration and makes it a more suitable glucose "receptor". In contrast, low K_m hexokinases display a hyperbolic saturation curve. Another major difference is that, unlike low K_m hexokinases, glucokinase is inhibited by long chain acyl-CoAs and by a regulatory protein.

Dawson and Hales reported in 1968 [9] that palmitoyl-CoA inhibits glucokinase competitively with respect to glucose, and that the inhibition is reversible upon dilution or addition of albumin. This indicated that it was not due to protein denaturation, as often occurs with detergents. Further studies by Tippett and Neet [10] extended this inhibition to all long chain acyl-CoAs and provided evidence for the fact that these compounds did not act as micelles but in their free form. In theory, inhibition by long chain acyl-CoAs could provide an explanation for the inhibition that fatty acids exert on glycolysis. However, Hue and co-workers [11] have shown that addition of palmitate to suspensions of isolated hepatocytes decreases by about two-fold the rate of detritiation of [3-^{3}H] glucose but not the rate of detritiation of [2-^{3}H]glucose, indicating that fatty acids act by inhibiting 6-phosphofructo 1-kinase but not glucokinase. Accordingly, fatty acids induce a decrease in the concentration of fructose 2,6-bisphosphate, a powerful stimulator of phosphofructokinase. These, and other results, suggest that the concentration of free long chain acyl-CoAs is probably too low in the cytosol to modulate the activity of glucokinase.

Discovery and properties of the regulatory protein

Glucokinase is also inhibited by a regulatory protein [12]. The seminal observation that led to this finding was the fact that fructose stimulates the detritiation of [2-^{3}H] glucose (and therefore the phosphorylation of glucose) in isolated hepatocytes [13]. Further work showed that the effect was observed at subsaturating concentrations of glucose but not at saturating concentrations of this sugar. Furthermore, it was also found that in addition to fructose, two other compounds (sorbitol and D-glyceraldehyde) that gave rise to fructose 1-P, also stimulated the phosphorylation of glucose [14]. These results suggested that fructose 1-P was a positive effector of glucokinase. Accordingly, fructose 1-P stimulated the activity of glucokinase in liver extracts. It was, however, without effect on the enzyme that had been purified by chromatography on an anion-exchanger [15]. This type of chromatography was found to separate glucokinase from a fructose 6-P and fructose 1-P-sensitive protein, which inhibits glucokinase by forming an inactive complex with this enzyme. Fructose 6-P favours the binding of the regulatory protein to glucokinase and, in so doing, reinforces the inhibition [15]. Fructose 1-P has the opposite effects. Both fructose-phosphates act by binding to the regulatory protein, presumably to two different conformations (Fig. 1).

As mentioned above, the regulatory protein does not act on other mammalian hexokinases. It has no effect on *Saccharomyces cerevisiae* hexokinases A and B, and yeast glucokinase, as could be tested on crude extracts of double-mutants kindly provided by D. Fraenkel (Harvard University, Boston, Mass., USA). It does not appear to affect the activity of glucose 6-P or of several other enzymes of carbohydrate metabolism in liver (Vandercammen A, Van Schaftingen E, unpublished results). The regulatory protein appears therefore to act specifically on glucokinase.

Table 1. Occurrence of the regulatory protein in the liver of different species and in different rat tissues. The regulatory protein was assayed as described previously [18]. Its presence in islets is inferred from the fact that glucokinse is inhibited in tissue extracts in a fructose 1-P sensitive manner

Tissue	Glucokinase	Regulatory protein	Sensitivity to phosphate esters
Liver			
Man, Rat, Mouse, Guinea Pig, Rabbit, Pig	+	+	+
Goat, Beef, Cat	–	–	
Chicken	–	–	
Xenopus, Toad, Turtle	+	+	–
Trout	–	–	
Brain, muscle, heart, spleen, kidney			
Rat	–	–	
Pancreatic islets			
Rat	+	(+)	(+)

The regulatory protein has been purified to homogeneity [16] from rat liver and shown to be a polypeptide of circa 62 kDa by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. Its cDNA has recently been cloned; the deduced amino acid sequence does not show substantial homology with other protein sequences found in data banks [17].

Expression of the regulatory protein in different tissues

In the rat, glucokinase is present only in the liver and in pancreatic islets. It was therefore of interest to know if this was also the case for the regulatory protein. Extracts of different tissues of fed rats were therefore chromatographed on DEAE-Sepharose and the resulting fractions tested for their effect on purified liver glucokinase. With this procedure, the liver was found to contain approximately 140 U/of regulatory protein per gram [18]. In contrast, brain, skeletal muscle, spleen and kidney did not contain detectable amounts of regulatory protein (Table 1). Western blots performed with an antibody raised against homogeneous rat liver regulatory protein confirmed these results.

In islets, the presence of regulatory protein is inferred from the following observations: 1) islet glucokinase is sensitive to inhibition by rat liver regulatory protein; 2) glucokinase activity is stimulated by fructose 1-P islet extracts; 3) when added to islet homogenates, liver glucokinase is (partly) inhibited in the absence, but not in the presence of fructose 1-P; 4) D-glyceraldehyde is partly converted to fructose 1-P in islets and stimulates the detritiation of [5-^{3}H] glucose in intact islets [19].

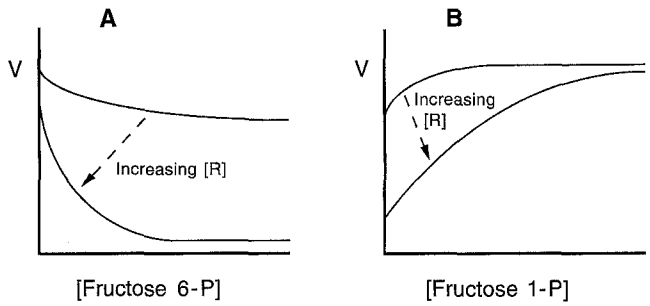

Fig. 2 A, B. Effect of the concentration of regulatory protein (R) on the activity of glucokinase measured in the presence of increasing concentrations of fructose 6-P (**A**) or of fructose 1-P (**B**). A fixed concentration of fructose 6-P is supposed to be present in (**B**)

Table 1 also shows that the regulatory protein is expressed in the liver of all species that have glucokinase. These include some (but not all) mammalian species, as well as amphibians and reptiles. The fact that glucokinase is absent from the liver of some species does not detract from the importance of this enzyme for other species. Thus, the presence of glucokinase is presumably not needed in the liver of ruminants, because glucose is fermented in the rumen, or in cats, because feline species are strictly carnivorous and, therefore, do not eat much glucose.

The regulatory protein present in the liver of mammalian species has the same properties as the rat protein. The protein found in amphibians and reptiles is similar to the rat liver protein in its molecular mass, its chromatographic behaviour and the fact that it inhibits glucokinase competitively vs glucose. It differs, however, in not being sensitive to fructose 6-P and fructose 1-P. Cloning of the cDNA encoding the *Xenopus* liver protein has recently shown that it is homologous to the mammalian protein (Veiga-da-Cunha M, Watelet N, Detheux M, Van Schaftingen E, unpublished results).

The fact that the regulatory protein and glucokinase are expressed in the same tissues further indicates that these two proteins form a functional unit.

Regulation of the expression of the regulatory protein

As mentioned above, the regulatory protein behaves as a competitive inhibitor vs glucose. Changes in the degree of expression of this protein could therefore modulate the affinity of glucokinase for its substrate. A detailed kinetic investigation [20] has also shown that an increase in the concentration of regulatory protein also causes an increase in the apparent affinity for the inhibitor fructose 6-P and a decrease in the apparent affinity for the deinhibitor, fructose 1-P (Fig. 2). It was therefore of interest to test the effect of various conditions known to affect the expression

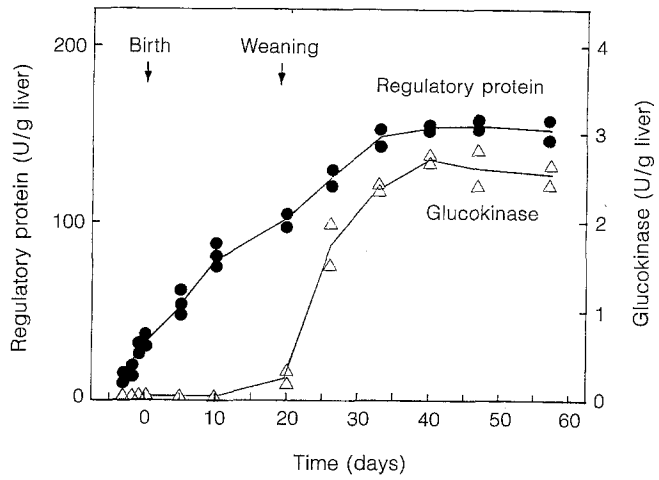

Fig. 3. Time-course of the changes in the concentration of regulatory protein and in the activity of glucokinase in rat liver during the perinatal period. From [18], with permission

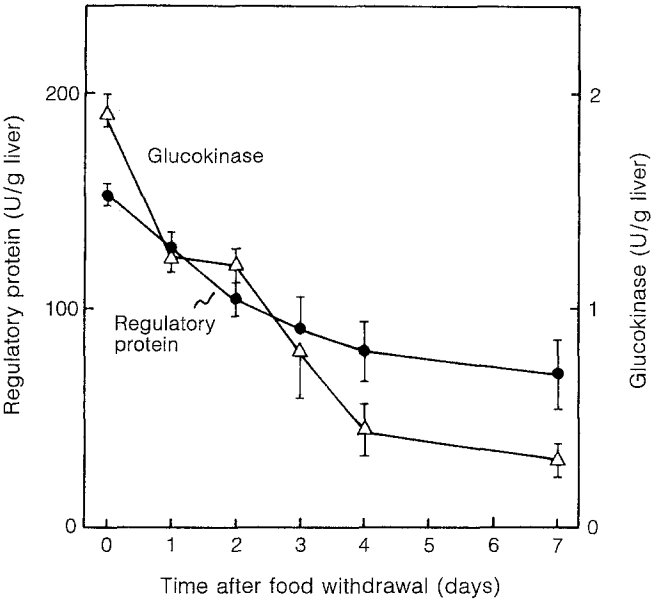

Fig. 4. Concentration of regulatory protein and activity of glucokinase in rat liver during starvation. From [18] with permission

of glucokinase on the expression of the regulatory protein.

As shown in Figure 3, glucokinase appears in rat liver between day 10 and day 20 of extrauterine life, and its activity then increases rapidly to reach the adult level after 30 days [2]. In contrast the regulatory protein is detected in the liver as early as day 18 of gestation. Its concentration increases during the first weeks of life to reach the adult level at about the same time as glucokinase [18]. Of particular interest is the fact that the concentration of regulatory protein is about 60 % of the adult level when glucokinase starts to appear.

Table 2. Effect of streptozotocin diabetes and of insulin on the concentration of regulatory protein and the activity of glucokinase in rat liver. Modified from previously published data [18]

		Glucokinase	Regulatory protein
	n	(U/g liver)	(U/g liver)
Healthy control rats, fed	19	1.86 ± 0.25	142.9 ± 11.8
Diabetic – 4 days after injection of streptozotocin	9	0.37 ± 0.14[b]	82.4 ± 12.1[b]
– 7 days after injection of streptozotocin	3	0.15 ± 0.04[b]	66.3 ± 1.4[b]
Diabetic (4 days), insulin treated for 3 days[a]	5	2.25 ± 0.15[b,c]	117.8 ± 20.4[b]

[a] Lente insulin (3 IU/100 g body weight) was injected subcutaneously once daily. [b] Significantly different from control ($p < 0.005$, unpaired t-test). [c] Significantly different from 4-day diabetic ($p < 0.005$) and from 7-day diabetic ($p < 0.02$)

As shown in Figure 4 and Table 2, the activity of glucokinase decreases in the liver of the adult rat upon starvation or after induction of diabetes, to reach 10–20 % of the adult level after 7 days [18]. The concentration of regulatory protein also decreases but more slowly and to a much lesser extent, being about 40–50 % of the control after 1 week. Both glucokinase and regulatory protein increase upon refeeding of starved rats or after treatment of diabetic rats with insulin [18].

Table 3 lists the molar concentrations of regulatory protein and of glucokinase in the liver under four different conditions. To calculate these concentrations, it was assumed that pure rat liver regulatory protein has a specific "activity" of 3200 U/mg protein (Vandercammen A, unpublished results) and that the molecular mass of the protein is 62 kDa. For glucokinase, the calculations were based on a specific activity of 180 U/mg protein and a molecular mass of 52 kDa [21]. It appears from Table 3 that the ratio of the concentrations of the two proteins varies over a wide range of values, being equal to about 3 in the liver of the adult fed rat, and infinite in the perinatal period. In contrast, the difference between the concentrations of the two proteins is constant.

The important changes in the ratio of the two proteins could argue against the regulatory protein being exclusively involved in the control of glucokinase activity. It must, however, be remembered that the association-dissociation phenomenon is central in the regulation of glucokinase by its regulatory protein. The latter inhibits glucokinase by forming a complex with this enzyme, and glucokinase must dissociate from this complex to become active again; furthermore, the allosteric effectors fructose 6-P, fructose 1-P and inorganic phosphate act by modulating the affinity of the regulatory protein for glucokinase. In this type of mechanism, the concentration of

Table 3. Molar concentrations of regulatory protein and of glucokinase in rat liver under various experimental conditions which are calculated as described in the text from previously presented data [18]

	Regulatory protein (nmol/g)	Glucokinase (nmol/g)	Reg. protein / Glucokinase	Reg. protein – Glucokinase (nmol/g)
Adult – fed	0.71	0.19	3.7	0.52
– fasted (4 days)	0.40	0.05	8	0.35
– diabetic (4 days)	0.41	0.04	10.2	0.37
Pup (10 days)	0.40	0.00	∞	0.40

free regulatory protein is what determines the sensitivity of glucokinase to glucose and to allosteric effectors. By keeping an excess of regulatory protein of about 0.4 nmol/g under all conditions, the sensitivity of glucokinase to its substrate and effectors is kept constant despite wide changes in the V_{max} of this enzyme.

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