Volume 255, number 1, 175-178 FEBS 07594 September 1989

Presence of fructokinase in pancreatic islets

Willy J. Malaisse, Francine Malaisse-Lagae, Dewi R. Davies* and Emile Van Schaftingen*

Laboratory of Experimental Medicine, Brussels Free University, Brussels and **Laboratoire de Chimie Physiologique, Université Catholique de Louvain, Louvain and International Institute of Cellular and Molecular Pathology, Brussels, Belgium*

Received 26 July 1989

Homogenates of rat pancreatic islets that had been heated for 5 min at 70°C to inactive hexokinases, catalyzed the ATP-dependent phosphorylation of D-fructose. This reaction was dependent on the presence of K^+ and was inhibited by D-tagatose although not by D-glucose or D-glucose 6-phosphate. The phosphorylation product was identified as fructose 1-phosphate through its conversion to a bisphosphate ester by *Clostridium difficile* fructose 1-phosphate kinase. These findings allowed the conclusion that fructokinase (ketohexokinase) was responsible for this process. Similar results were observed with tumoral insulin-producing cells (*RIN*m5F line). Fructokinase may account for a large share of fructose phosphorylation in intact islets, particularly in the presence of D-glucose.

Pancreatic islet; Fructokinase; (Ketohexokinase, Tumoral islet cell)

1. INTRODUCTION

Although unable to stimulate insulin secretion by itself, fructose is known to augment the release of insulin induced by glucose or other secretagogues [1–4]. According to the fuel hypothesis, the effect of nutrient secretagogues is related to the rate at which they are metabolized in islet cells [5]. Fructose is indeed metabolized in pancreatic islets, though at a maximal rate roughly 5-fold lower than that of D-glucose [2–4,6].

Fructose is phosphorylated to fructose 6-phosphate by hexokinases and to fructose 1-phosphate by ketohexokinase, also called fructokinase, which is present in liver, kidney and the intestinal mucosa (reviewed in [7]). Mammalian hexokinases display a much greater affinity for glucose than for the ketose and are inhibited by glucose 6-phosphate [8]. In addition to fructose, fructokinase phosphorylates, with lower efficiency, a series of other ketohexoses [9]. Fructose phosphorylation in pancreatic islet homogenates is inhibited to an extent of about 90% by glucose, mannose or glucose 6-phosphate, indicating that the low-K_m hexokinases are mainly responsible for this process [10]. However, these data do not exclude the possibility that a small percentage of this phosphorylating activity is contributed by fructokinase.

In the present study, advantage was taken of the relative heat stability of fructokinase to demonstrate its presence in islets derived from rat pancreas.

Correspondence address: W.J. Malaisse, Laboratory of Experimental Medicine, Brussels Free University, Brussels, Belgium

2. MATERIALS AND METHODS

Radioactive products were purchased from Amersham Corp. (Buckinghamshire, UK). Fructose 1-phosphate kinase from *Clostridium difficile* was purified about 150-fold by a procedure involving heat treatment, precipitation with polyethyleneglycol and DEAE-Trisacryl chromatography (Davies, Delmée and Van Schaftingen, in preparation).

For the measurement of fructokinase activity, pancreatic islets isolated from fed albino rats [11] or tumoral islet cells of the *RIN*m5F line [12] were sonicated in a Hepes-NaOH buffer (25 mM, pH 7.4) containing EDTA (0.1 mM) and $MgCl_2$ (10.0 mM). When required, this homogenate was heated for 5 min at 70°C. Aliquots (25 μl) containing 130 ± 15 islet equivalents) of the homogenate were added to 75 μl of a reaction mixture to yield the same concentration of Hepes, EDTA and $MgCl_2$ as mentioned above, ATP (5 mM), and, as required, KCl

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

(100 mM), D-glucose 6-phosphate (1.0 mM), D-tagatose, and either D-glucose or D-fructose (mixed with a tracer amount of D-[U-^{14}C]glucose or D-[U-^{14}C]fructose, respectively). After 60 min incubation at 37°C, labelled hexose-phosphates were separated by anion exchange chromatography [13]. All readings were corrected for the blank value found in the absence of islet homogenate, and results expressed as the mean (± SE) together with the number of individual measurements (n).

For the identification of the fructose-phosphate produced by islet ketohexokinase, a heated homogenate was incubated for 0 or 2 h in a final volume of 100 μl and in the presence of the same concentrations of Hepes, pH 7.1, EDTA, ATP and $MgCl_2$ as above, together with 50 μm D-[U-^{14}C]fructose (1.3 μCi). At the times indicated the reaction was stopped by the addition of 400 μl of ethanol. The samples were diluted with 1.5 vol of water and 1 ml of this mixture was applied to a Dowex AG 1 X8 (Cl form, 200–400 mesh) column (0.5 × 4 cm). The column was washed successively with 4 ml of water, 5 ml of 150 mM NaCl to elute the monophosphates esters and 5 ml of 400 mM NaCl to eluate the biphosphate esters. The fractions containing fructose-monophosphates were pooled. Portions (250 μl) of the pool were incubated for 30 min and at 30°C with 40 mM Hepes, pH 7.1, 1 mM $MgCl_2$ and, where indicated, 2 mM ATP Mg and 6 mU fructose-1-phosphate kinase in a final volume of 500 μl. The mixture was then diluted with 3 vols of water and 1.0 ml was immediately applied to a Dowex AG 1 X8 column, which was processed as described above.

3. RESULTS

In the presence of 100 mM KCl, the rate of phosphorylation of D-[U-^{14}C]glucose (50 μM) was close to 1.0 pmol/min per islet in unheated homogenates and decreased to 0.3 ± 0.2 fmol/min per islet after heating for 5 min at 70°C, indicating that hexokinases had been substantially destroyed. All further experiments were performed with heated homogenates.

In a series of 5 experiments, the phosphorylation of D-[U-^{14}C]fructose (50 μM) measured in the presence of 100 mM KCl, averaged 19.8 ± 4.0 fmol/min per islet, i.e. about 2% of the value found with 50 μM glucose in unheated homogenates. The reaction velocity measured at increasing concentrations of fructose (0.05, 0.40 and 2.05 mM) indicated a K_m value of 0.8 ± 0.2 mM. In sharp contrast to the results obtained with unheated homogenates [10], the phosphorylation of [D-U-^{14}C]fructose (50 μM) by heated homogenates was unaffected by either D-glucose 6-phosphate (1.0 mM) or D-glucose (1.0–5.0 mM), and was almost entirely dependent on the presence of KCl (table 1). D-tagatose caused a concentration-dependent inhibition of the phosphorylation of D-[U-^{14}C]fructose with a K_i of about 4.5 mM as indicated by Dixon plot analysis.

Table 1

Effect of several agents upon the phosphorylation of D-fructose by islet homogenates heated for 5 min at 70°C

Agent	Phosphorylation rate[a]
Nil	100.0
D-Glucose 6-phosphate (1.0 mM)	100.3 ± 2.5
D-Glucose (1.0 mM)	100.1 ± 0.4
No KCl	2.1 ± 0.9
D-Tagatose (0.35 mM)	89.0 ± 1.0
D-Tagatose (2.0 mM)	67.9 ± 1.2

[a] Mean values are derived from 3 or more measurements and expressed relative to the paired control value found with D-[U-^{14}C]fructose (50 μM) in the presence of KCl (100 mM)

*RIN*m5F cells were also tested for the presence of fructokinase. Heated homogenates catalyzed the phosphorylation of D-[U-^{14}C]fructose at a rate of

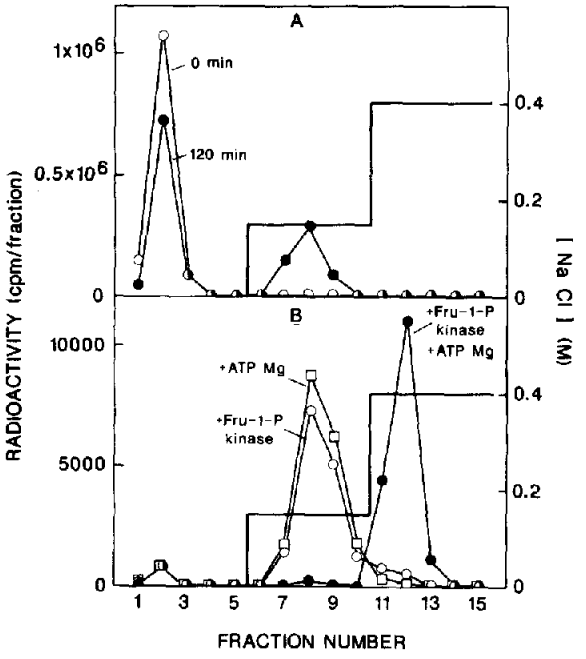

Fig.1. Identification of the product of the reaction catalyzed by islet fructokinase. (A) A heat-treated extract of pancreatic islets was incubated with [U-^{14}C]fructose and ATP-Mg for 0 (○) or 120 (●) min and the product of the reaction was isolated by chromatography on Dowex AG-1 X8 (B). Fractions 7–9 of column A containing the radioactive fructose-phosphate were pooled. Portions of the pool were incubated for 30 min at 30°C with ATP-Mg and *Clostridium difficile* fructose 1-phosphate kinase either seperately or together and rechromatographed.

7.16 ± 0.42 fmol/min per 10^3 cells. Here again the phosphorylation of fructose by the heated homogenates greatly exceeded that found with D-[U-^{14}C]glucose (50 μM), was not inhibited by 1.0 mM D-glucose or 1.0 mM D-glucose 6-phosphate or a combination of the two, but was suppressed in the absence of KCl.

Fig.1 shows that the product of the reaction catalyzed by islet fructokinase eluted from a Dowex column at 150 mM NaCl, a concentration known to elute the hexose monophosphate although not the hexose-bisphosphates [14]. Reaction of the hexose monophosphate with ATP Mg and *Clostridium difficile* fructose 1-phosphate kinase resulted in the almost complete conversion of the product to a compound eluting with the bisphosphate esters at a concentration of 400 mM NaCl. It has been checked that the preparation of fructose 1-phosphate kinase did not phosphorylate D-fructose 6-phosphate under the same experimental conditions.

4. DISCUSSION

The present study indicates that the pancreatic islets contain, in addition to hexokinase, a more heat-stable enzyme that catalyses the phosphorylation of fructose. This enzyme displays a K_m of 0.8 mM for the ketose, is not inhibited by glucose or glucose 6-phosphate, is dependent on the presence of potassium ions for its activity, and is inhibited by tagatose, all properties reminiscent of hepatic fructokinase [9,15]. Proof of the identity of the islet enzyme as fructokinase was brought by the demonstration that the reaction product is fructose 1-phosphate, not fructose 6-phosphate.

From the present measurements of both phosphorylation rates recorded in heated islet homogenates incubated with 50 μM D-[U-^{14}C]fructose and apparent affinity of islet fructokinase for the ketohexose, it can be calculated that the maximal velocity of fructokinase would be close to 0.2–0.3 pmol/min per islet. This value represents less than 10% of the maximal rate of D-fructose phosphorylation as catalyzed by the islet hexokinase in unheated homogenates [10]. However, in intact islets exposed to a high concentration of D-[5-^{3}H]fructose (33.0 mM) in the absence of glucose, the rate of tritiated water generation does not exceed about 0.3 pmol/min per islet [4]. Since fructokinase, in contrast to hexokinase, is not inhibited by glucose 6-phosphate or glucose, it might be expected to fully express its activity in intact cells. The participation of fructokinase to ketohexose phosphorylation in intact islet cells may account, in part at least, for the fact that fructose metabolism in pancreatic islets is less severely inhibited ($\leq$36%) by the presence of glucose [3,6,10] than expected from the respective affinities of hexokinase for glucose and fructose [8,10].

In the liver, fructose 1-phosphate is split by aldolase B to dihydroxyacetone phosphate and D-glyceraldehyde. The latter enters the glycolytic-gluconeogenic pathway after phosphorylation by triokinase [7]. The fact that islets catalyse the formation of tritiated water from D-[5-^{3}H]fructose even in the presence of glucose suggests that the same sequence of reactions could occur in the endocrine tissue. The release of tritium from the fifth carbon of the ketose is indeed thought to occur in the course of triose phosphate isomerisation [16]. Triokinase has been shown to occur in these endocrine cells [17].

Fructose 1-phosphate has been shown to relieve the inhibition that a regulatory protein exerts on liver glucokinase in the presence of fructose 6-phosphate [18]. Because of this property, fructose 1-phosphate is thought to mediate the effect of fructose to stimulate 2–4-fold the phosphorylation of glucose [19]. Since the islets also contain glucokinase [reviewed in 20] and are capable of synthesising fructose 1-phosphate, a similar mechanism could occur in that tissue [21]. However, for unknown reasons, fructose, if anything, barely stimulates glucose metabolism in islets [3,4,6,10].

Acknowledgements: The authors are indebted to M. Mahy for technical assistance and C. Demesmaeker for secretarial help. This work was supported by grants from the Belgian Foundation for Scientific Medical Research, National Institute of Health (Grant DK 9235) and Belgian State (Prime Minister's office) Science Policy Programming. E.V.S. is Chercheur qualifié of the Belgian Fonds National de la Recherche Scientifique.

REFERENCES

[1] Ashcroft, S.J.H., Bassett, J.M. and Randle, P.J. (1972) Diabetes 21 (suppl. 2), 538–545.
[2] Malaisse, W.J., Sener, A. and Mahy, M. (1974) Eur. J. Biochem. 47, 365–370.

[3] Zawalich, W.S., Rognstad, R., Pagliara, A.S. and Matschinsky, F.M. (1977) J. Biol. Chem. 252, 8519–8523.
[4] Sener, A. and Malaisse, W.J. (1988) Arch. Biochem. Biophys. 261, 16–26.
[5] Malaisse, W.J., Sener, A., Herchuelz, A. and Hutton, J.C. (1979) Metabolism 28, 373–386.
[6] Capito, K., Hedeskov, C.J., Landt, J. and Thams, P. (1984) Acta Diabetol. Lat. 21, 365–374.
[7] Van den Berghe, G. (1986) Prog. Biochem. Pharmacol. 21, 1–32.
[8] Colowick, S.P. (1973) in: The Enzymes (Boyer, P.D., ed.) 3d edn 9, 1–48.
[9] Raushel, F.M. and Cleland, W.W. (1977) Biochemistry 16, 2169–2175.
[10] Sener, A., Giroix, M.-H. and Malaisse, W.J. (1984) Eur. J. Biochem. 144, 223–226.
[11] Malaisse-Lagae, F. and Malaisse, W.J. (1984) in: Methods in Diabetes Research (Larner, R. and Pohl, S.L., eds) pp. 147–152, Wiley, New York.
[12] Giroix, M.-H., Malaisse-Lagae, F., Sener, A. and Malaisse, W.J. (1985) Mol. Cell. Biochem. 66, 61–64.
[13] Giroix, M.-H., Sener, A., Pipeleers, D.G. and Malaisse, W.J. (1984) Biochem. J. 223, 447–453.
[14] Van Schaftingen, E., Davies, D.R. and Hers, H.G. (1982) Eur. J. Biochem. 124, 143–149.
[15] Hers, H.G. (1952) Biochem. Biophys. Acta 8, 416–422.
[16] Katz, J. and Rognstad, R. (1975) Curr. Top. Cell. Regul. 10, 237–289.
[17] Mac Donald, M.J. (1989) Arch. Biochem. Biophys. 270, 15–22.
[18] Van Schaftingen, E. (1989) Eur. J. Biochem. 179, 179–184.
[19] Van Schaftingen, E. and Vandercammen, A. (1989) Eur. J. Biochem. 179, 173–177.
[20] Lenzen, S. and Panten, U. (1988) Biochem. Pharmacol. 37, 371–378.
[21] Malaisse, W.J., Malaisse-Lagae, F., Rasschaert, J., Zähner, D., Sener, A., Davies, D.R. and Van Schaftingen, E. (1989) Biochem. Soc. Trans., in press.