The microsomal glucose-6-phosphatase enzyme of pancreatic islets

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Microsomal fractions isolated from pancreatic islet cells were shown to contain high specific glucose-6-phosphatase activity. The islet-cell glucose-6-phosphatase enzyme has the same M_r (36 500), similar immunological properties and kinetic characteristics to the hepatic microsomal glucose-6-phosphatase enzyme.

INTRODUCTION

The hepatic microsomal glucose-6-phosphatase enzyme (EC 3.1.3.9) catalyses a key step in the regulation of blood glucose concentrations (Ashmore & Weber, 1959), and it has more recently been suggested that glucose-6-phosphatase also plays a role in regulating hepatic cytosolic Ca^{2+} concentrations (Benedetti *et al.*, 1985, 1986, 1988). Glucose is the main physiological stimulus for insulin biosynthesis and secretion by pancreatic β -cells (Hedekov, 1980). Biosynthesis of insulin in islet cells is regulated by glucose (Ashcroft *et al.*, 1978; Welsh *et al.*, 1986), as is the secretion of insulin (Grodsky *et al.*, 1963; Coore & Randle, 1964; Ashcroft *et al.*, 1970; Hedekov, 1980; Berridge & Irvine, 1984; Montague *et al.*, 1985; Hellman, 1985). Insulin secretion is also regulated by cytoplasmic Ca^{2+} (Colca *et al.*, 1983a,b; Rorsman *et al.*, 1984; Hellman, 1985; Morgan *et al.*, 1987; Nilsson *et al.*, 1987) and glucose 6-phosphate concentrations (Wolf *et al.*, 1986a). Therefore, if the microsomal glucose-6-phosphatase enzyme was present in pancreatic islet cells, it would play an important role in the regulation of glucose-stimulated insulin release. There have been several reports of histochemical detection of glucose 6-phosphate hydrolysis in pancreatic islet β -cells (Lazarus, 1959a,b; Lazarus & Barden, 1965). However, the question of whether this is due to the presence of a specific microsomal glucose-6-phosphatase enzyme or to non-specific hydrolysis of glucose 6-phosphate has proved to be somewhat controversial. Low rates of glucose 6-phosphate hydrolysis have been measured in pancreatic islet cells (Ashcroft & Randle, 1968; Matschinsky & Ellerman, 1968; Taljedal, 1969; Hedekov & Capito, 1974; Wolf *et al.*, 1986b), but none of these reports established unequivocally that the activity measured was due to the presence of the microsomal glucose-6-phosphatase system. More recently it has been reported that there is no specific glucose-6-phosphatase enzyme activity in pancreatic islet cells (Giroix *et al.*, 1987). We demonstrated that an adult patient with type 1c glycogen-storage disease, which is caused by a defect of one of the translocase proteins of the hepatic microsomal glucose-6-phosphatase system (Waddell *et al.*, 1988), had an impaired insulin release in response to glucose (Burchell *et al.*, 1987), suggesting that the microsomal glucose-6-phosphatase system is present in pancreatic islet cells. We therefore used monospecific

antibodies to the hepatic microsomal glucose-6-phosphatase enzyme and a microassay method to characterize the glucose-6-phosphatase activity to determine whether the previously reported low rates of glucose 6-phosphate hydrolysis in islet cells are due to the presence of the microsomal glucose-6-phosphatase enzyme. The results described in the present paper demonstrate that the 36 500 Da microsomal glucose-6-phosphatase enzyme is present in microsomes (microsomal fractions) isolated from pancreatic cells and that the glucose-6-phosphatase activity is not low, but is several-fold higher than found in hepatic microsomes isolated from the same animals.

MATERIALS AND METHODS

Biotinylated donkey anti-sheep antibody and strept-avidin-linked peroxidase complex were purchased from Amersham International, Amersham, Bucks., U.K. 4-Chloro-1-naphthol, glucose 6-phosphate (monosodium salt), mannose 6-phosphate (monosodium salt) and prestained M_r markers were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Empigen BB was kindly given by Albright and Wilson, Whitehaven, Cumbria, U.K. Nitrocellulose was obtained from Schleicher and Schuell, Dassel, West Germany. Collagenase was from Boehringer Mannheim, Mannheim, West Germany, and Ficoll 400 was from Pharmacia, Uppsala, Sweden.

Preparation of microsomal fractions

Islets were prepared exactly as described in McDaniel *et al.* (1983), except that, in an attempt to decrease the losses of glucose-6-phosphatase activity, all steps were carried out at 4 °C and during the preparation the islets were not cultured for 24–48 h, but were subfractionated immediately. Microsomes were prepared from fresh livers, kidneys, whole pancreas and pancreatic islet cells from Wistar rats, Swiss mice, Cheviot sheep and White Lop-Eared rabbits as previously described (Burchell *et al.*, 1987) and assayed immediately for glucose-6-phosphatase activity.

Assays

Glucose-6-phosphatase and mannose-6-phosphatase activities were assayed in the microsomal fractions as

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Table 1. Rat microsomal glucose-6-phosphatase activity

Values represent means $\pm$ S.E.M. for five separate preparations each containing the pooled tissue from 20 Wistar rats which had been starved for 24 h. The average intactness of rat hepatic and pancreatic islet microsomal preparations was 97 % and 94 % respectively. Abbreviation: nd, not determined.

Tissue	Intact microsomes		Disrupted microsomes	
	$V_{\max.}$ ($\mu\text{mol/min per mg}$)	K_m (mM)	$V_{\max.}$ ($\mu\text{mol/min per mg}$)	K_m (mM)
Liver	0.18 ± 0.001	1.7 ± 0.03	0.52 ± 0.03	0.5 ± 0.08
Pancreas	nd	nd	0.04 ± 0.01	nd
Pancreatic islets	0.70 ± 0.1	3.5 ± 0.4	1.50 ± 0.5	0.7 ± 0.01

described in Burchell *et al.* (1988). The glucose 6-phosphate concentrations used for kinetic studies were 0.5, 1.0, 1.4, 2.0, 2.6 and 5.0 mM. Microsomes were disrupted with histone 2A as described by Blair & Burchell (1988). Glucose-6-phosphatase activity is expressed as $\mu\text{mol/min per mg}$ of microsomal protein. Non-specific hydrolysis of glucose 6-phosphate was assayed and corrected for as described by Burchell & Burchell (1980). Non-specific phosphatases and the specific microsomal glucose-6-phosphatase enzyme have different K_m values. Therefore non-specific phosphatase activity was measured at all the substrate concentrations used. In all cases the non-specific phosphatase activity was less than 3 % of the total glucose-6-phosphatase activity. Microsomes isolated from homogenates and assayed without further treatment are referred to as 'untreated microsomes'. These are heterogeneous preparations comprising intact vesicles (intact microsomes) in which the limiting membrane acts as a selective permeability barrier, and disrupted structures, in which selective permeability is lacking and the enzyme has free access to ionic substrates and inhibitors. The proportion of the two forms was estimated by assays of the low- K_m mannose-6-phosphatase activity, which is expressed only in disrupted structures (Arion *et al.*, 1976). All the data obtained from studies on untreated microsomes have been corrected for the contribution by disrupted structures; data after this correction are reported as referring to intact microsomes. This eliminates the large errors in activity measurements that occur if even a small proportion of the vesicles are disrupted. The activity of glucose-6-phosphatase in intact microsomes, and maximum reaction velocities ($V_{\max.}$) and Michaelis constants (K_m) were calculated as described by Burchell *et al.* (1988). K_{IES} and K_{IE} values were calculated as described in Engel (1981). Protein concentrations were measured by the method of Peterson (1977).

Immunoblot analysis

Sheep anti-(rat glucose-6-phosphatase) antiserum was prepared as described in Countaway *et al.* (1988). IgG was further purified by $(\text{NH}_4)_2\text{SO}_4$ fractionation (Fahey & Terry, 1978). SDS/polyacrylamide-gel electrophoresis was carried out in 7–16 % acrylamide gels as described by Laemmli (1970). Proteins separated on SDS/polyacrylamide gels were electrophoretically transferred to nitrocellulose as described by Towbin *et al.* (1979) in the presence of 1 % Empigen BB as described by Mandrell & Zollinger (1984). The Western blot was immunostained

with sheep anti-(rat glucose-6-phosphatase) IgG, and immunoreactive polypeptides were detected by using a biotin-streptavidin-peroxidase-linked detection system with 4-chloro-1-naphthol as the substrate (Domin *et al.*, 1984).

RESULTS AND DISCUSSION

The hepatic microsomal glucose-6-phosphatase is a multicomponent system comprising the glucose-6-phosphatase enzyme with its active site situated at the luminal surface of the endoplasmic-reticulum membrane, and a translocase, T_1 , which mediates the entry of glucose 6-phosphate into the luminal compartment. A second translocase, T_2 , mediates the equilibration of the product, phosphate. The mechanism of glucose permeation across the membrane has been designated T_3 (Arion *et al.*, 1980; Burchell *et al.*, 1987).

Glucose-6-phosphatase activity

To measure the activity of the microsomal glucose-6-phosphatase enzyme, it is necessary to disrupt the microsomal vesicles fully, to remove the rate limitations imposed on the system by the translocases. The kinetic constants of the glucose-6-phosphatase activity in fully disrupted microsomes isolated from rat pancreas, pancreatic islets and liver are shown in Table 1. The values for the kinetic constants of the liver glucose-6-phosphatase enzyme were very similar to previously reported values for starved-rat liver (Arion *et al.*, 1984; Lange *et al.*, 1986). The specific glucose-6-phosphatase enzyme activity of microsomes isolated from whole pancreas was, as expected, very low (see Table 1). However, the activity in disrupted islet microsomes was approx. 3 times higher than in liver. This finding was unexpected, as previous papers (e.g. Ashcroft & Randle, 1968; Matschinsky & Ellerman, 1968; Taljedal, 1969; Hedekov & Capito, 1974; Colilla *et al.*, 1975; Wolf *et al.*, 1986a; Giroix *et al.*, 1987) had all reported extremely low glucose-6-phosphatase activity in islet cells.

In the previous papers the intactness of the microsomes was not determined, nor were the microsomes disrupted to allow direct measurement of the activity of the glucose-6-phosphatase enzyme. The values of glucose-6-phosphatase activity in intact microsomes are a measure of the combined rates of the glucose-6-phosphatase enzyme and the translocases T_1 , T_2 and T_3 . Therefore one possible explanation of the extremely low glucose-6-

phosphatase activity found in the earlier studies was that in islet cells the transport capacity of T_1 , T_2 and/or T_3 is extremely low or in some cases perhaps totally absent. We therefore measured the glucose-6-phosphatase activity in intact microsomes isolated from rat pancreatic islets and liver (see Table 1). There is approx. 4 times more glucose-6-phosphatase activity in intact microsomes in islet cells than in the liver of the same animals (Table 1), which clearly shows that the transport capacity of the three translocases is not low. In liver, when the activity of the glucose-6-phosphatase enzyme is raised 3–4-fold, for example in streptozotocin-induced diabetes (Burchell & Cain, 1985), the K_m of the glucose-6-phosphatase activity in intact microsomes doubles. The K_m increases because the translocase T_1 , which has higher K_m than the glucose-6-phosphatase enzyme, becomes more rate-limiting (Arion *et al.*, 1980). Similarly the islet glucose-6-phosphatase activity is 3–4-fold higher than in the livers of the same animals, and the K_m of the glucose-6-phosphatase activity in intact microsomes isolated from islets is twice as high as in the liver (Table 1), showing that the kinetic constants of the translocase proteins are very similar in both tissues.

The activity of the hepatic glucose-6-phosphatase enzyme is very unstable, especially in disrupted microsomes (Burchell & Burchell, 1980; Arion *et al.*, 1984; Lange *et al.*, 1986; Burchell *et al.*, 1987; Blair & Burchell, 1988; Countaway *et al.*, 1988). The activity of the hepatic glucose-6-phosphatase enzyme also decreases rapidly in culture and can be inhibited by a variety of substances (for reviews see Ashmore & Weber, 1959; Nordlie & Sukalski, 1985). In the present study we found that the glucose-6-phosphatase activity in pancreatic islet cells was very unstable. To minimize any time-dependent losses in glucose-6-phosphatase activity, we did not culture the islet cells for 24–48 h, as in most of the previous studies, and all steps in the preparation were carried out at 4 °C. However, in studies designed to improve our methods of isolating islet cells, islets were prepared exactly as described in McDaniel *et al.* (1983), but the microsomes isolated from such cultured islets were devoid of all glucose-6-phosphatase activity. Indeed we found that culturing the islet cells for even 1 h caused substantial losses of glucose-6-phosphatase activity.

Loss of glucose-6-phosphatase activity during the preparation of pancreatic islet cells is therefore the most likely explanation of the low activity found in the earlier studies (e.g. Ashcroft & Randle, 1968; Matschinsky & Ellerman, 1968; Taljedal, 1969; Hedekov & Capito, 1974; Wolf *et al.*, 1986b). It is also presumably the reason why Ashcroft & Randle (1968) found no evidence of glucose 6-phosphate conversion into glucose in islet cells that were being incubated at 37 °C. The most recent paper (Giroix *et al.*, 1987) concluded that the low rates of glucose 6-phosphate hydrolysis that they measured in rat islet cells were due to non-specific phosphatases, and indeed the properties of the glucose-6-phosphatase activity which they reported suggest that they were indeed measuring only non-specific phosphatase activity. Unfortunately, the most likely reason why Giroix *et al.* (1987) found no glucose-6-phosphatase system in rat islets is that they had completely inactivated it. They cultured the islet cells and then sonicated them (sonication inactivates glucose-6-phosphatase enzyme) in the presence of 150 mM-Cl⁻, which inhibits the glucose-6-phosphatase enzyme (Colilla *et al.*, 1974). In addition,

Giroix *et al.* (1987) also reported extremely low values (0.00275 μ mol/min per mg of protein) for rat liver glucose-6-phosphatase activity, demonstrating that their procedures had inactivated both liver and islet-cell microsomal glucose-6-phosphatase enzymes.

The hepatic glucose-6-phosphatase system is inhibited by glucose, and Arion *et al.* (1980) reported K_{IES} and K_{IE} of 410 and 153 mM respectively for the glucose inhibition of rat hepatic microsomal glucose-6-phosphatase system in intact microsomes. In contrast, Randle & Ashcroft (1968) reported a K_{IES} of 38 mM and K_{IE} of 9 mM for the glucose inhibition of pancreatic islet-cell glucose-6-phosphatase activity, suggesting that the liver and pancreatic islet-cell enzymes have different properties. We therefore measured the glucose inhibition of the glucose-6-phosphatase system in microsomes isolated from pancreatic islet cells, and found a K_{IES} of 120 mM and K_{IE} of 116 mM, which are similar to the previously reported values for rat liver. Randle & Ashcroft (1968) found mannose-6-phosphatase activity in their islet-cell microsomes, which indicates that some of the microsomes in their preparations were disrupted. Disruption of the hepatic microsomal membrane alters the glucose inhibition of glucose-6-phosphatase activity in liver (Arion *et al.*, 1980), so partial disruption of the microsomes in the previous study is the probable reason for the differences seen in extents of glucose inhibition.

Comparison of the liver and pancreatic-islet glucose-6-phosphatase enzyme proteins

Microsomes isolated from rat liver, kidney and pancreatic-islet cells were subjected to immunoblot analysis using a sheep anti-(rat hepatic microsomal glucose-6-phosphatase) antiserum, which had previously been shown to be monospecific for the 36500 Da polypeptide which contains the active site of the rat hepatic glucose-6-phosphatase enzyme (Countaway *et al.*, 1988). Fig. 1 shows that rat liver, kidney and pancreatic-islet cells all contain the 36500 Da microsomal glucose-6-phosphatase protein, confirming that the microsomal glucose-6-phosphatase activity in pancreatic-islet cells (Table 1) is due to the presence of the liver-specific microsomal glucose-6-phosphatase enzyme. When the sheep anti-(rat hepatic glucose-6-phosphatase) antibody was shown to be monospecific, 50 μ g of rat hepatic microsomal protein was loaded on to the SDS/polyacrylamide gels, and only one broad band was immunoreactive (Countaway *et al.*, 1988). Subsequently, by loading less microsomal protein on gels we have shown that the band in fact comprises a major band of 36500 Da and a slightly smaller, minor, band. Both bands are specifically labelled with ³²P-labelled glucose-6-phosphate, therefore both bands contain the active site of the glucose-6-phosphatase enzyme. The glucose-6-phosphatase enzyme is a glycoprotein, and we believe that the smaller minor band contains less carbohydrate than the major band (I. D. Waddell & A. Burchell, unpublished work). In order to demonstrate clearly whether the islet glucose-6-phosphatase enzyme was also a doublet, we had to modify slightly the gradient of the polyacrylamide gels to obtain a better separation of the two bands. Fig. 1 shows that the islet glucose-6-phosphatase enzyme is also a doublet and that the ratio of the two bands of glucose-6-phosphatase in islets, liver and kidney is very similar.

Fig. 1. Immunoblot analysis of rat microsomal samples

Lane 1, Sigma prestained molecular-mass standards of 180 000, 116 000, 84 000, 58 000, 48 500, 36 500 and 26 600 Da; lane 2, starved-rat liver microsomes (10 µg); lane 3, starved-rat kidney microsomes (10 µg); lane 4, starved-rat pancreatic-islets microsomes (5 µg).

Species differences

We have shown that the same 36 500 Da microsomal glucose-6-phosphatase enzyme protein is present in rat, rabbit, mouse, guinea pig and sheep liver (Countaway *et al.*, 1988) and rat liver and pancreatic islet cells (Fig. 1). We have also demonstrated that the same glucose-6-phosphatase enzyme protein is present in islets isolated from mice, rabbits and sheep (results not shown). However, in contrast, it has been concluded (Taljedal, 1969; Giroix *et al.*, 1987) that islet glucose-6-phosphatase varies from species to species, and both report that mouse and rat islet glucose-6-phosphatase activities are different. We therefore also characterized starved-mouse pancreatic-islet and liver microsomal glucose-6-phosphatase activity. The mouse islet glucose-6-phosphatase enzyme had a V_{max} of 0.9 µmol/min per mg of microsomal protein and a K_m of 0.5 mM, and in intact

mouse islet microsomes the glucose-6-phosphatase system had a V_{max} of 0.4 µmol/min per mg of microsomal protein and a K_m of 2.1 mM, demonstrating that the rat (see Table 1) and mouse islet glucose-6-phosphatase systems have extremely similar properties. In addition, the mouse liver glucose-6-phosphatase enzyme activity (V_{max} 0.28 µmol/min per mg of microsomal protein and K_m 0.5 mM) and mouse liver intact microsomal glucose-6-phosphatase system activity (0.1 µmol/min per mg of microsomal protein and K_m 2.3 mM) were both 3–4-fold lower than that observed in islets isolated from the same animals, which is extremely similar to the ratio of rat liver and islet activities. We therefore conclude that the pancreatic-islet microsomal glucose-6-phosphatase system is very similar in a variety of mammalian species. In the previous studies (Taljedal, 1969; Giroix *et al.*, 1987) the microsomal intactness was not measured, and even the highest activities they reported were extremely low [e.g. Taljedal (1969) and Giroix *et al.* (1987) reported that mouse islet glucose-6-phosphatase activity was 0.006 and 0.001 µmol/min per mg of protein respectively]. Therefore the differences they found between mouse and rat islet activities most probably arose either from differences in the activities of non-specific phosphatases or from different degrees of microsomal intactness in their preparations, which both contained very inactivated and/or inhibited glucose-6-phosphatase.

Role of the glucose-6-phosphatase system in pancreatic islet cells

In Table 1 we report that the specific activity of the microsomal glucose-6-phosphatase enzyme is 3-fold higher in pancreatic islets than in liver. However, this must be regarded as a minimum value. The early histochemical studies showed that the only detectable glucose 6-phosphate hydrolysis in pancreatic islets was in the β -cells (Lazarus, 1959*a,b*; Lazarus & Barden, 1965). We prepared pancreatic islets which contain a heterogeneous population of cell types, of which only approx. 80% are β -cells in adult rats (Hellman, 1959; McDaniel *et al.*, 1983). In addition, islet-cell preparations isolated in this way are only about 80% pure (Lacy & Kostianovsky, 1967; McDaniel *et al.*, 1983), but a more reasonable estimate of the purity of our rapidly prepared islets is 50–60%. We prepared rat islet-cell microsomes as rapidly as possible (4–4½ h) in the cold to minimize time-dependent losses of glucose-6-phosphatase activity, but unfortunately it is extremely likely that we still lose some glucose-6-phosphatase activity. The yield of hepatic glucose-6-phosphatase activity in microsomal fractions was reported to be 87% by Hers *et al.* (1951). More recently yields of 70–80% are usually reported (for review see Nordlie, 1981) and in our laboratory we routinely obtain yields of 70–80%. The mean yield of islet glucose-6-phosphatase enzyme activity in the microsomal fractions of five separate preparations was 59.6% (range 53–70%). The yield of islet glucose-6-phosphatase was therefore about 10–15% lower than in liver preparations. β -Cells therefore probably have at least 10-fold higher microsomal glucose-6-phosphatase activity than that found in the liver of the same animals.

In the earlier studies, where low glucose-6-phosphatase activity was found in pancreatic islet cells, glucose-6-phosphatase activity was reported to be approximately the same as total hexokinase activity and/or glucokinase

Fig. 2. Proposed role of the microsomal glucose-6-phosphatase system in the regulation of glucose-stimulated insulin release from pancreatic-islet β -cells

Abbreviations: G-6-P, glucose-6-phosphate; enzyme, the glucose-6-phosphatase enzyme; IP_3 , inositol trisphosphate.

activity. For example, Matschinsky & Ellerman (1968) found that islet-cell glucose-6-phosphatase activity was approx. $\frac{1}{5}$ th of the hepatic glucose-6-phosphatase activity of the same animals and that islet-cell glucose-6-phosphatase activity was about equal to the sum of islet-cell hexokinase + glucokinase activities. Hexokinase and glucokinase are not extremely unstable enzymes, therefore the activities reported in the previous paper are presumably correct. The results reported in the present paper therefore mean that the glucose-6-phosphatase activity in islet cells is much greater than the total hexokinase activity. Glucose-6-phosphatase must therefore play a major role in the regulation of islet-cell glucose and glucose 6-phosphate concentrations.

The physiological significance of the presence of very high islet glucose-6-phosphatase enzyme activity and its probable roles in the regulation of islet carbohydrate metabolism, intracellular Ca^{2+} concentrations and glucose-stimulated release are briefly outlined below and in Fig. 2. For simplicity, only the situation of high blood glucose concentrations (in a non-diabetic) is discussed, and the role of the mitochondrial Ca^{2+} stores and effects of GTP concentrations are not mentioned.

High blood glucose concentrations are recognized by the β -cell (in an unknown manner) and activate two different major pathways, namely phospholipid hydrolysis and glucose metabolism, both of which affect Ca^{2+} concentrations by mechanisms dependent on the glucose-6-phosphatase system.

(1) Phospholipid hydrolysis, which leads to the release of *myo*-inositol 1,4,5-trisphosphate and arachidonic acid, both of which (in an unknown manner) induce the release of Ca^{2+} from the endoplasmic reticulum, leading to rise in cytoplasmic free Ca^{2+} concentrations, which in turn activate insulin secretion (Wolf *et al.* 1986a). The activity of the glucose-6-phosphatase system can affect this pathway in two ways. (a) It has been shown (Wolf *et al.* 1986a) that high concentrations of glucose 6-phosphate abolish *myo*-inositol 1,4,5-trisphosphate-induced Ca^{2+} release from the endoplasmic reticulum

in islet cells. (b) For inositol 1,4,5-trisphosphate or arachidonic acid to stimulate Ca^{2+} release from the endoplasmic reticulum, it is first essential for Ca^{2+} to be stored in the endoplasmic reticulum. Ca^{2+} is stored in a complex with P_i in the lumen of hepatic (and presumably islet) endoplasmic reticulum (Rasmussen, 1981). The P_i in the lumen of the endoplasmic reticulum is produced by the hydrolysis of glucose 6-phosphate by glucose-6-phosphatase (see Fig. 2).

(2) Glucose metabolism. Glucose entering the islet is converted into glucose 6-phosphate by glucokinase + hexokinase (previously thought to be the pace-setting enzymic step for glycolysis in islets). The glucose 6-phosphate can then be metabolized in a variety of ways in islets (see Fig. 2), e.g. via glycolysis to produce ATP and various metabolites (Hedeskov, 1980; Malaisse *et al.*, 1984). The presence of high glucose-6-phosphatase activity in islets means, however, that most of the glucose 6-phosphate will be taken into the lumen of the endoplasmic reticulum and hydrolysed to form phosphate and glucose (see Fig. 2), the phosphate released being either complexed with Ca^{2+} or released via T_2 . The glucose released from the lumen via T_3 could then be a substrate for islet hexokinase. The cycle between glucose and glucose 6-phosphate would ensure a continuous supply of free P_i inside the lumen of the endoplasmic reticulum, which could complex to Ca^{2+} to replenish the Ca^{2+} stores.

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REFERENCES

- Arion, W. J., Ballas, L. M., Lange, A. J. & Wallin, B. K. (1976) *J. Biol. Chem.* **251**, 4901–4907
- Arion, W. J., Lange, A. J., Walls, H. E. & Ballas, L. M. (1980) *J. Biol. Chem.* **255**, 10396–10406

- Arion, W. J., Burchell, B. & Burchell, A. (1984) *Biochem. J.* **220**, 835–842
- Ashcroft, S. J. H. & Randle, P. J. (1968) *Nature (London)* **219**, 857–858
- Ashcroft, S. J. H., Hedekov, C. J. & Randle, P. J. (1970) *Biochem. J.* **118**, 143–154
- Ashcroft, S. J. H., Bunce, J., Lowry, M., Hansen, S. E. & Hedekov, C. J. (1978) *Biochem. J.* **174**, 517–526
- Ashmore, J. & Weber, G. (1959) *Vitam. Horm. (N.Y.)* **18**, 91–133
- Benedetti, A., Fulceri, R. & Comporti, M. (1985) *Biochim. Biophys. Acta* **816**, 267–277
- Benedetti, A., Fulceri, R., Ferro, M. & Comporti, M. (1986) *Trends Biochem. Sci.* **10**, 284–285
- Benedetti, A., Fulceri, R., Romani, A. & Comporti, M. (1988) *J. Biol. Chem.* **263**, 3466–3473
- Berridge, M. J. & Irvine, R. F. (1984) *Nature (London)* **312**, 315–321
- Blair, J. & Burchell, A. (1988) *Biochim. Biophys. Acta* **964**, 161–167
- Burchell, A. & Burchell, B. (1980) *FEBS Lett.* **113**, 180–184
- Burchell, A. & Cain, D. I. (1985) *Diabetologia* **28**, 852–856
- Burchell, A., Jung, R. T., Lang, C. C., Bennet, W. & Shepherd, A. N. (1987) *Lancet* **i**, 1059–1062
- Burchell, A., Hume, R. & Burchell, B. (1988) *Clin. Chim. Acta* **173**, 183–192
- Colca, J. R., Kotagal, N., Lacy, P. E. & McDaniel, M. L. (1983a) *Biochem. J.* **212**, 113–121
- Colca, J. R., Kotagal, N., Lacy, P. E. & McDaniel, M. L. (1983b) *Biochem. Biophys. Acta* **729**, 176–184
- Colilla, W., Johnson, W. T. & Nordlie, R. C. (1974) *Biochim. Biophys. Acta* **364**, 78–87
- Colilla, W., Jorgenson, R. A. & Nordlie, R. C. (1975) *Biochim. Biophys. Acta* **377**, 117–125
- Coore, H. G. & Randle, P. J. (1964) *Biochem. J.* **93**, 66–78
- Countaway, J. L., Waddell, I. D., Burchell, A. & Arion, W. J. (1988) *J. Biol. Chem.* **263**, 2673–2678
- Domin, B. A., Serbjit-Singh, C. J. & Philpot, R. M. (1984) *Anal. Biochem.* **136**, 390–396
- Engel, P. C. (1981) *Enzyme Kinetics*, 2nd edn., pp. 26–36, Chapman and Hall, London
- Fahey, J. L. & Terry, E. W. (1978) *Handb. Exp. Immunol.* (3rd Ed.) pp. 7.3–7.10
- Giroix, M. H., Sener, A. & Malaisse W. J. (1987) *Mol. Cell. Endocrinol.* **49**, 219–225
- Grodsky, G. M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N. B. & Smith, D. F. (1963) *Am. J. Physiol.* **205**, 638–644
- Hedekov, C. J. (1980) *Physiol. Rev.* **60**, 442–509
- Hedekov, C. J. & Capito, K. (1974) *Biochem. J.* **140**, 423–433
- Hellman, B. (1959) *Acta Endocrinol. (Copenhagen)* **32**, 92–112
- Hellman, B. (1985) *Diabetologia* **28**, 494–501
- Hers, H. G., Berthet, J., Berthet, L. & de Duve, C. (1951) *Bull. Soc. Chim. Biol.* **33**, 21–41
- Lacy, P. E. & Kostianovsky, M. (1967) *Diabetes* **16**, 35–39
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lange, A. J., Arion, W. J., Burchell, A. & Burchell, B. (1986) *J. Biol. Chem.* **261**, 101–107
- Lazarus, S. S. (1959a) *Proc. Soc. Exp. Biol. Med.* **101**, 819–822
- Lazarus, S. S. (1959b) *Proc. Soc. Exp. Biol. Med.* **102**, 303–306
- Lazarus, S. S. & Barden, H. (1965) *Diabetes* **14**, 146–156
- Malaisse, W. J., Malaisse-Lagae, F. & Sener, A. (1984) *Experientia* **40**, 1035–1042
- Mandrell, R. E. & Zollinger, W. D. (1984) *J. Immunol. Methods* **67**, 1–11
- Matschinsky, F. M. & Ellerman, J. E. (1968) *J. Biol. Chem.* **243**, 2730–2736
- McDaniel, M. L., Colca, J. R., Kotagal, N. & Lacy, P. E. (1983) *Methods Enzymol.* **98**, 182–198
- Montague, W., Morgan, N. G., Rumford, G. M. & Prince, C. A. (1985) *Biochem. J.* **227**, 483–489
- Morgan, N. G., Rumford, G. M. & Montague, W. (1987) *Biochem. J.* **244**, 669–674
- Nilsson, T., Arkhammar, P., Hallberg, A., Hellman, B. & Berggren, P.-O. (1987) *Biochem. J.* **248**, 329–336
- Nordlie, R. C. (1981) in *The Regulation of Carbohydrate Formation and Utilization in Mammals* (Veneziale, C. M., ed.), pp. 291–341, University Park Press, Baltimore
- Nordlie, R. C. & Sukalski, K. A. (1985) *Curr. Top. Cell. Regul.* **8**, 33–117
- Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356
- Rasmussen, H. (1981) in *Calcium and cAMP as Synarchic Messengers* (Rasmussen, H., ed.), pp. 39–43, John Wiley and Sons, New York
- Rorsman, P., Abrahamsson, H., Gylfe, E. & Hellman, B. (1984) *FEBS Lett.* **170**, 196–200
- Taljedal, I. B. (1969) *Biochem. J.* **114**, 387–394
- Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
- Waddell, I. D., Lindsay, J. G. & Burchell, A. (1988) *FEBS Lett.* **229**, 179–182
- Welsh, M., Scherberg, N., Gilmore, R. & Steiner, D. F. (1986) *Biochem. J.* **235**, 459–467
- Wolf, B. A., Turk, J., Sharma, W. R. & McDaniel, M. L. (1986a) *J. Biol. Chem.* **261**, 3501–3511
- Wolf, B. A., Colca, J. R., Comens, P. G., Turk, J. & McDaniel, M. L. (1986b) *J. Biol. Chem.* **261**, 16284–16287