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Anomeric specificity of hexokinase and glucokinase activities in liver and insulin-producing cells

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Conflicting data have been reported concerning the anomeric specificity of glucokinase. In the present study, liver hexokinase (K_m for D-glucose 0.4mM) displayed a higher affinity for but lower $V_{max.}$ with α- than with β-D-glucose. The velocity of the reaction catalysed by liver glucokinase was higher with β- than with α-D-glucose, whatever the glucose concentration. The apparent K_m of glucokinase was somewhat lower, however, with α- than with β-D-glucose. Comparable results were obtained for the high-K_m glucokinase-like enzymic activity present in normal pancreatic islets or insulin-producing tumoral cells. These results suggest that the anomeric specificity of glucokinase cannot account for the higher rate of glycolysis found in islets exposed to α- as distinct from β-D-glucose.

The fine regulation of glucose metabolism in intact cells may depend among other things on the anomeric specificity of certain enzymes (Malaisse *et al.*, 1983). Mammalian hexokinase was recently found to display a greater affinity for but lower maximal velocity with α- than with β-D-glucose (Malaisse *et al.*, 1985*b*), in mirror image of the properties of yeast hexokinase (M. Salas *et al.*, 1965; Bailey *et al.*, 1968; Wurster & Hess, 1973). Conflicting data were reported, however, concerning the anomeric specificity of glucokinase. J. Salas *et al.* (1965) first reported that, at a high concentration (50mM), α- and β-D-glucose are phosphorylated at the same rate by rabbit liver glucokinase. Okuda *et al.* (1978), however, reported that, in the 5–25mM range, α-D-glucose, as well as α-D-mannose (Miwa *et al.*, 1983), is phosphorylated at a higher rate than is the corresponding β-anomer by rat liver glucokinase. In that study, the α/β ratio in reaction velocity was much higher at low (e.g. 5mM) than at high (e.g. 25mM) glucose concentrations, suggesting a major difference in affinity but no difference in maximal velocity (Miwa *et al.*, 1983). Yet, with glucokinase prepared from either rat livers or radiation-induced insulinomas, Meglasson & Matschinsky (1983) reported that the maximal velocity is 15–20% lower but the affinity twice as high for α- than for β-D-glucose. As a result of these two differences, the rate of glucose phosphorylation was somewhat higher with α- than with β-D-glucose in a restricted range of concentrations between 2 and 11mM, with the opposite situation at higher glucose concentrations (40–80mM). Because the anomeric specificity of glucokinase may be relevant to our understanding of the anomeric specificity of glucose metabolism in intact cells, e.g. in pancreatic islets (Malaisse *et al.*, 1976*a*), and in view of the conflicting data mentioned above, in the present study we have re-investigated the anomeric specificity of both hexokinase-like and glucokinase-like enzymic activities in liver, pancreatic islets and insulin-producing-tumoral-cell homogenates. Our study takes advantage of the parallel use of radioisotopic and non-isotopic procedures in assessing the anomeric specificity of the glucose-phosphorylating enzymes.

Materials and methods

The anomers of D-glucose were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Crystalline α- and β-D-[U-^{14}C]glucose were prepared from D-[U-^{14}C]glucose (3.9mCi/mmol; New England Nuclear, Boston, MA, U.S.A.) by the procedure of Miwa *et al.* (1975), and their anomeric purity was assessed by the β-D-glucose oxidase method (Okuda & Miwa, 1973). Glucose 6-phosphate, $NADP^+$, ATP and glucose -6-phosphate dehydrogenase (EC 1.1.1.49) were purchased from Boehringer Pharma (Mannheim, Germany).

Liver and normal pancreatic islets were removed from adult rats that had been given free access to food. Insulin-producing tumoral cells were grown, harvested and counted as previously described (Giroix *et al.*, 1985*a*). These tissues were homogenized in 50mM-Hepes/NaOH buffer, pH7.4, containing KCl (150mM), $MgCl_2$ (5mM), EDTA (1mM), cysteine (2mM) and bovine albumin (0.2mg/ml). The liver samples were homogenized in Potter–Elvehjem tubes at an initial concentration of 30–300mg wet wt./ml of buffer. After 5min centrifugation at 1000*g*, the supernatant was sonicated (3 × 5s) and added in portions of either 15μl (radioisotopic procedure) or 300μl (non-radioisotopic procedure) to the assay medium. The islets (approx. 1700 islets/ml) and tumoral cells (approx. 3×10^6 cells/ml) were sonicated, and the homogenates added in 15μl portions each to the assay medium.

The radioisotopic (Giroix *et al.*, 1984) and non-isotopic (Malaisse *et al.*, 1985*a*) procedures used to measure glucose phosphorylation were identical with those described in the cited references. The incubation time amounted to 20min at 30°C and 60min at 6–7°C. The method used to calculate K_m values from data obtained in the simultaneous presence of unlabelled α- or β-D-glucose and D-[U-^{14}C]glucose in anomeric equilibrium was also previously described (Giroix *et al.*, 1985*b*).

All results are expressed as the means ± S.E.M., together with the numbers of individual observations. The statistical significance of difference between mean values was assessed by the use of Student's *t* test.

Results

Liver hexokinase

At 30°C the phosphorylation of D-[U-^{14}C]glucose in anomeric equilibrium over a range of concentrations between 25μM and 2.5mM yielded in a double-reciprocal plot a single straight line with a $V_{max.}$ close to 17.1pmol/60min per μg wet wt. and a K_m close to 0.42mM (Fig. 1*a*). The activity of this hexokinase-like enzyme was inhibited by glucose 6-phosphate. For instance, in the presence of 1.0mM-glucose 6-phosphate, the reaction velocity averaged 28.9 ± 2.2 and 24.9 ± 1.6% ($n = 6$ in both cases) of the corresponding mean control value measured at D-[U-^{14}C]glucose concentrations of 0.25 and 0.5mM respectively. Incidentally, in the presence of 0.25mM-D-[U-^{14}C]-glucose and 1.0mM-glucose 6-phosphate, the phosphorylation rate of the hexose averaged no more than 0.073 ± 0.003pmol/60min per μg wet wt. ($n = 3$) in the absence of ATP, as distinct from 1.782 ± 0.177pmol/60min per μg wet wt. ($n = 8$) in the presence of ATP (5.0mM).

At 6–7°C, unlabelled α-D-glucose, used at concentrations of 30 and 80μM, inhibited more than did unlabelled β-D-glucose ($P < 0.005$ and 0.02 respectively) the phosphorylation of D-[U-^{14}C]glucose (20μM) in anomeric equilibrium (Table 1). From the results obtained in the presence of 80μM unlabelled α- or β-D-glucose, it was calculated that the α/β ratio in K_m values for D-glucose averaged 0.382 ± 0.139 ($P < 0.005$ as compared with unity), with K_m values close to 0.38mM and 1.0mM for α-and β-D-glucose respectively. At the low temperature and at a concentration of 0.5mM, the phosphorylation of α-D-[U-^{14}C]glucose averaged 87.4 ± 3.0% ($n = 9$) of the corresponding mean value found with β-D-[U-^{14}C]glucose (100.0 ± 1.8%; $n = 9$), i.e. 0.41 ± 0.07pmol/60min per μg wet wt. Such an anomeric difference ($P < 0.005$), taken together with the anomeric difference in K_m values (see above), indicates that the maximal velocity of liver hexokinase was higher for β- than for α-D-glucose. Thus, on the basis of the two sets of data, the α/β ratio in $V_{max.}$ was calculated to be 0.512 ± 0.027.

Liver glucokinase

At 30°C and at glucose concentrations in excess of 2.5mM, the phosphorylation of D-[U-^{14}C]glucose in anomeric equilibrium indicated the presence of a high-K_m glucokinase-like enzymic activity (Fig. 1*b*). After subtraction of the values attributable to hexokinase, the high-K_m enzymic activity yielded, in a double-reciprocal plot, a curvilinear pattern comparable with that obtained with purified glucokinase (Niemeyer *et al.*, 1975) and characterized by an apparent K_m for glucose close to 24mM. At high glucose concentrations (10–20mM) the rate of D-[U-^{14}C]glucose phosphorylation was little affected by glucose 6-phosphate. For instance, in the presence of 1.0mM- and 3.0mM-glucose 6-phosphate respectively, the reaction velocity averaged 103.7 ± 4.5 and 82.9 ± 4.6% ($n = 4$ in each case) of the paired control value (no glucose 6-phosphate), all measurements being made at 10.0mM-D-[U-^{14}C]glucose. The values just mentioned for D-[U-^{14}C]glucose phosphorylation in the presence of glucose 6-phosphate were corrected for the readings obtained in the presence of glucose 6-phosphate but in the absence of ATP. Such readings averaged, in the presence of glucose 6-phosphate at 1.0 and 3.0mM respectively, 7.6 ± 0.2 and 15.5 ± 0.5% ($n = 3$ in both cases) of the paired value found in the presence of ATP (5.0mM), at the same concentration of both D-[U-^{14}C]glucose (10.0mM) and unlabelled glucose 6-phosphate.

Further experiments designed to investigate the anomeric specificity of liver glucokinase were performed over 60min incubation at 6–7°C. At

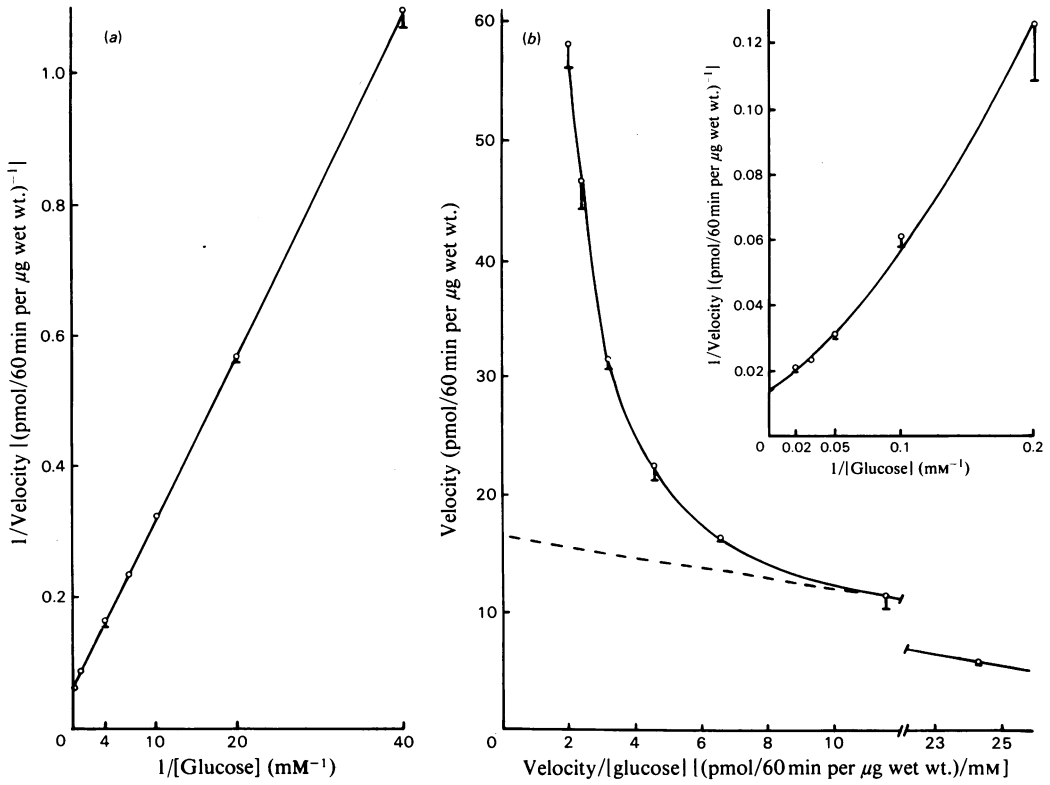

Fig. 1. D-[*U*-^{14}C]*Glucose phosphorylation by liver homogenates incubated at 30°C*

(*a*) Double-reciprocal plot at glucose concentrations ranging from 25μM to 2.5mM; mean values (±S.E.M., whenever it exceeds the size of the mean symbol) refer to three separate experiments. (*b*) Hofstee plot at glucose concentrations ranging from 0.25 to 30.0mM; mean values (±S.E.M.) refer to three to six separate experiments. The inset illustrates a Lineweaver–Burk plot of the high-K_m enzymic activity, as calculated after subtraction of the contribution attributable to the low-K_m enzymic activity (broken line), in the 5.0–50.0mM range of glucose concentrations.

Table 1. *Effects of unlabelled α- and β-*D*-glucose on the phosphorylation of* D-[*U*-^{14}C]*glucose in anomeric equilibrium by liver homogenates incubated at 6–7°C*

For experimental details see the text.

D-[U-^{14}C]Glucose in anomeric equilibrium (μM)	Unlabelled D-glucose (μM)		D-[U-^{14}C]Glucose phosphorylation rate (pmol/60min per μg wet wt.)
	α	β	
20.0	—	—	0.120±0.001 (4)
20.0	30.0	—	0.107±0.002 (4)
20.0	—	30.0	0.121±0.002 (4)
20.0	80.0	—	0.099±0.003 (4)
20.0	—	80.0	0.111±0.002 (4)

30.0mM, the phosphorylation rate of α-D-glucose, as measured by the non-isotopic procedure, averaged 6.48±1.34pmol/60min per μg wet wt. ($n=6$). At the same concentration (30mM), the paired α/β ratio in reaction velocity averaged 0.716±0.063 ($n=5$). The velocity was thus higher ($P<0.05$) in the presence of β- than of α-D-glucose.

Relative to the value collected with each anomer at this high concentration (30mM), the results obtained at lower concentrations were not significantly different with α- and with β-glucose (Table 2), suggesting that glucokinase does not display any obvious difference in affinity for these two anomers. At the most, there was a trend for the

Table 2. *Relative values for α- and β-D-glucose phosphorylation by liver homogenates at 6–7°C*
Results obtained by a non-isotopic (left) or a radioisotopic procedure (right) are expressed as percentages of the corresponding values found at 30.0mM of the same anomer. Mean values (±S.E.M.) are derived from three to six separate experiments.

Concn. (mM)	D-[U-^{12}C]Glucose		D-[U-^{14}C]Glucose	
	α	β	α	β
0.5	10.1±4.1	11.2± 7.3	5.4±0.2	4.5±0.2
2.5	19.0±7.9	24.5±10.1	26.4±1.5	20.4±0.9
5.0	46.7±4.7	41.4± 9.2	42.7±3.5	35.1±1.8
10.0	70.1±5.4	62.1± 7.7	61.6±2.4	50.4±2.7
20.0	81.9±4.8	81.5± 4.9	84.5±6.3	72.7±6.4
30.0	100.0	100.0	100.0	100.0

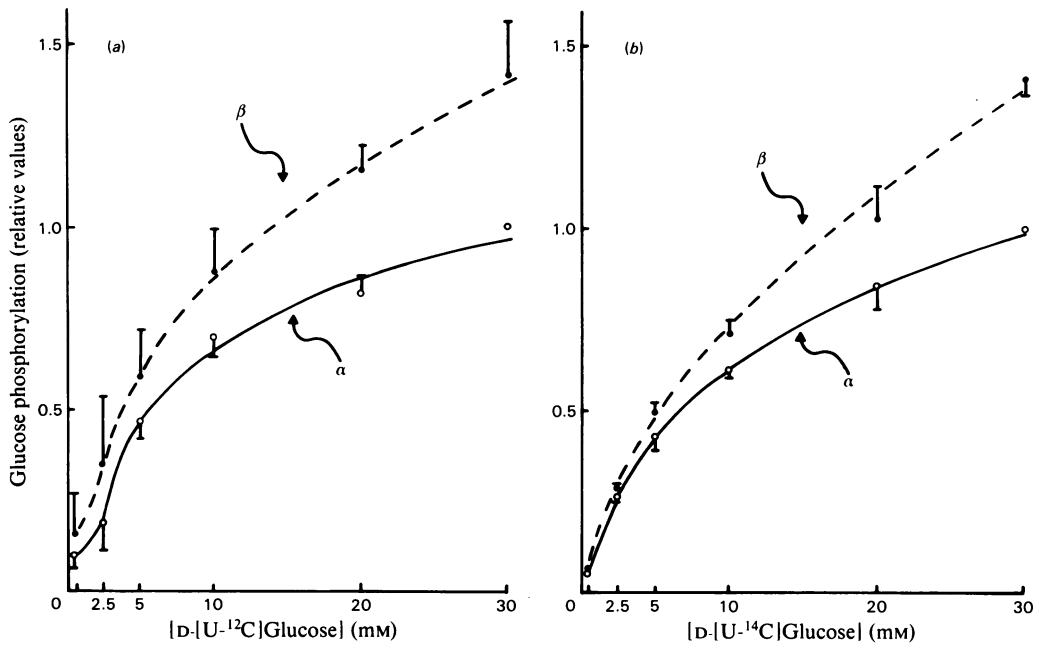

Fig. 2. *Comparison of α- and β-D-glucose phosphorylation by liver homogenates incubated at 6–7°C*
For the sake of comparison, the results obtained by a non-isotopic (*a*) or a radioisotopic procedure (*b*) are expressed relative to the mean values found within the same experiments in the presence of 30mM-α-D-glucose. Absolute rates for such a reference value are quoted in the text. Mean values (±S.E.M.) are derived from three to six separate experiments.

relative values in reaction velocities to be somewhat higher with α-D-glucose at intermediate concentrations (5.0–10.0mM). Nevertheless, the mean absolute rate of phosphorylation was always higher with β- than with α-D-glucose (Fig. 2*a*).

Comparable results were obtained when the rate of α-D-[U-^{14}C]glucose was compared with that of β-D-[U-^{14}C]glucose. Thus, at 30.0mM, the phosphorylation rate of α-D-glucose averaged 4.67±0.52pmol/60min per μg wet wt. ($n=4$). At the same concentration (30mM), the paired α/β ratio in reaction velocity averaged 0.707±0.050 ($n=3$; $P<0.05$ as compared with unity). Relative to the value obtained with each anomer at this high concentration (30mM), the results obtained at lower concentrations tended to be greater with α-D-glucose, such a difference achieving statistical significance ($P<0.05$) only at 10.0mM-hexose concentration. This finding suggests that glucokinase may display a slightly greater affinity for α- than for β-D-glucose. Nevertheless, the mean absolute rate of phosphorylation was always higher with β- than with α-D-[U-^{14}C]glucose (Fig. 2*b*). Even in the presence of glucose 6-phosphate

Table 3. *Effects of unlabelled α- and β-D-glucose on the phosphorylation of D-[U-^{14}C]glucose in anomeric equilibrium by liver homogenates incubated at 6–7°C in the presence of glucose 6-phosphate (3.0mM)*

The rate of D-[U-^{14}C]glucose phosphorylation is expressed in absolute terms (first column), or relative to the mean paired value found in the presence of the unlabelled anomers (second column, pooled data obtained with α- and β-D-[U-^{12}C]glucose), or relative to the mean corresponding value found in the presence of unlabelled β-D-glucose (third and fourth columns).

[U-^{14}C]Glucose in anomeric equilibrium (mM)	α- or β-D-[U-^{12}C]Glucose (mM)	D-[U-^{14}C]Glucose phosphorylation rate (pmol/60min per μg wet wt.)	(%)	α (% of β)	β (% of β)
2.0	—	0.70±0.06 (8)	100.0±3.4 (8)		
2.0	3.0		108.4±4.3 (16)	99.8±6.4 (8)	100.0±3.6 (8)
2.0	8.0		96.2±2.5 (16)	95.0±3.4 (8)	100.0±3.7 (8)
4.0	—	1.72±0.28 (7)	100.0±3.1 (7)		
4.0	6.0		107.5±7.7 (14)	95.9±5.5 (7)	100.0±6.2 (7)
4.0	16.0		101.7±6.6 (14)	95.0±3.5 (7)	100.0±5.4 (7)
10.0	—	3.44±0.06 (4)	100.0±1.7 (4)		
10.0	20.0		83.0±1.4 (8)	93.9±1.6 (4)	100.0±1.8 (4)
10.0	40.0		71.7±0.8 (8)	99.1±1.4 (4)	100.0±1.9 (4)

(3.0mM), used to inhibit liver hexokinase, the mean phosphorylation rate of α-D-[U-^{14}C]glucose remained lower than that of β-D-[U-^{14}C]glucose. Thus the α/β ratio in reaction velocity averaged 0.811±0.080 and 0.728±0.071 (degrees of freedom: 14 and 9) in the 2.5–3.3mM and 5.0–10.0mM ranges of glucose concentrations respectively ($P<0.05$ and 0.005).

In order to explore further the affinity of liver glucokinase for the α- and the β-anomer of D-glucose, a last series of experiments was performed in the presence of D-[U-^{14}C]glucose, in anomeric equilibrium, mixed with unlabelled α- or β-D-glucose (Table 3). Glucose 6-phosphate (3.0mM) was always present in the reaction mixture in order to inhibit liver hexokinase. In the absence of unlabelled anomers, the reaction velocity increased as the concentration of D-[U-^{14}C]glucose was raised from 2.0mM to 4.0 and 10.0mM. The unlabelled anomers failed to affect significantly the phosphorylation of D-[U-^{14}C]glucose, except at high concentrations of both D-[U-^{14}C]glucose and the unlabelled anomers. This finding is consistent with both the sigmoidicity and elevated apparent K_m for glucose of liver glucokinase (Niemeyer *et al.*, 1975). The phosphorylation of D-[U-^{14}C]glucose tended to be lower in the presence of unlabelled α- than of unlabelled β-D-glucose (Table 3). However, such a difference was quite modest and only achieved statistical significance ($P<0.05$) under one experimental condition (i.e. in the presence of 10mM-D-[U-^{14}C]glucose and 20mM of the unlabelled anomers). The results confirm that glucokinase may display a slightly greater affinity for α- than for β-D-glucose.

Insulin-producing cells

We have previously characterized the anomeric specificity of hexokinase in both normal pancreatic islets (Malaisse *et al.*, 1985*b*) and insulin-producing tumoral cells (Malaisse-Lagae *et al.*, 1985). The α/β ratio in $V_{max.}$ averaged 0.765±0.022 and 0.690±0.021 and the α/β ratio in K_m values averaged 0.389±0.041 and 0.505±0.060 in normal islets and tumoral cells respectively. Therefore the present experiments were restricted to study the anomeric specificity of the high-K_m glucokinase-like enzymic activity present in these two preparations of insulin-producing cells (Ashcroft & Randle, 1970; Malaisse *et al.*, 1976*b*; Meglasson *et al.*, 1983; Meglasson & Matschinsky, 1983).

At a low glucose concentration (1.0mM), the α/β ratio in reaction velocity averaged 0.624±0.015 and 0.751±0.013 in normal islets and tumoral cells respectively, in fair agreement with our prior study in which the anomeric specificity of hexokinase was assessed by a different procedure. At higher glucose concentrations (5.0–30.0mM), the rate of α-D-[U-^{14}C]glucose phosphorylation was also lower than that of the corresponding β-anomer (Fig. 3). Even when the rate of phosphorylation observed at low glucose concentrations (1.0 or 5.0mM) was subtracted from that found at the highest glucose concentrations (20.0 and 30.0mM), the increment in reaction velocity evoked by α-D-glucose remained lower than that attributable to β-D-glucose. The α/β ratio for such increments averaged 0.623±0.040 and 0.614±0.012 ($P<0.005$, as compared with unity) in islets and tumoral cells respectively. Thus the high-K_m enzyme in insulin-producing cells, like that in liver, yielded a higher

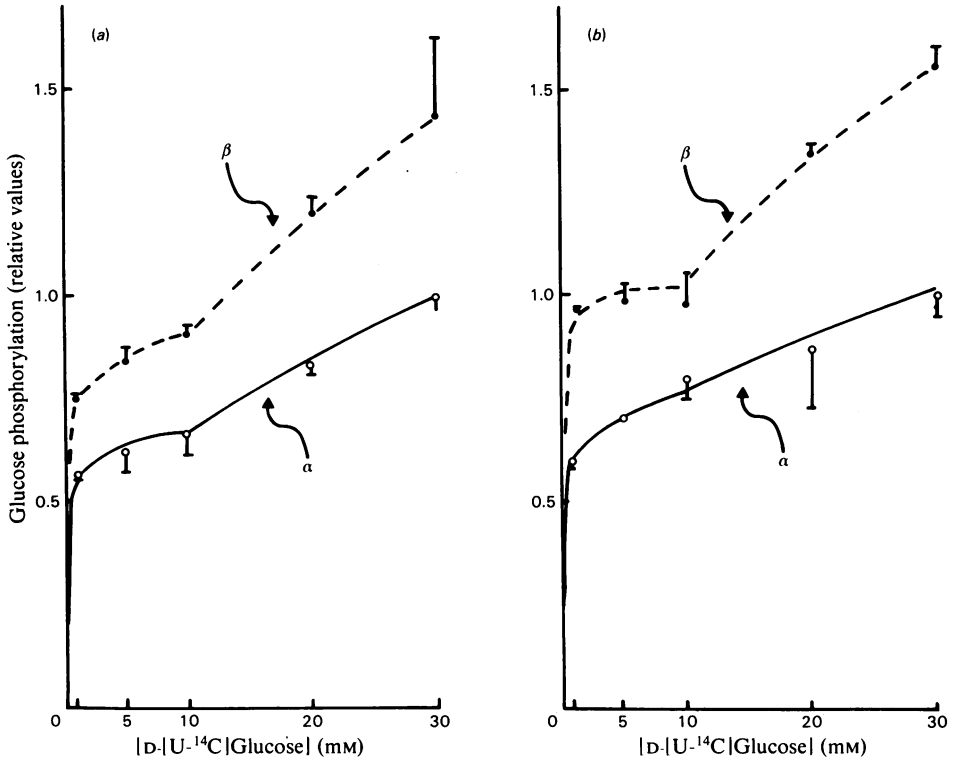

Fig. 3. *Comparison of α- and β-D-[U-^{14}C]glucose phosphorylation by (a) insulin-producing-tumoral-cell and (b) pancreatic-islet homogenates incubated at 6–7°C*

Mean values (±S.E.M.) refer to three individual measurements. For the sake of comparison, all results are expressed relative to the mean values found in the presence of 30mM-α-D-glucose. Such reference values amounted to 14.8±0.5pmol/60min per 10^3 tumoral cells and 49.4±2.1pmol/60min per islet.

velocity with β- than with α-D-glucose. The affinity of the high-K_m enzyme for the anomers of D-glucose could not be unambiguously assessed because of the much larger contribution of hexokinase, relative to that of glucokinase, in insulin-producing cells as distinct from liver. Nevertheless, when the increments in reaction velocity above the value obtained at the lowest glucose concentration (1.0mM) were expressed relative to the mean increment attributable to the same anomer at the highest glucose concentration (30.0mM), no significant difference could be found between α- and β-D-glucose. For instance, in tumoral cells, the values for such relative increments averaged, in the presence of α- and β-D-glucose respectively, 14.0 and 13.0% at 5.0mM-glucose, 24.1 and 22.7% at 10.0mM-glucose, and 61.6 and 65.5% at 20.0mM-glucose. This suggests that the apparent K_m values for D-glucose of the glucokinase-like enzymic activity in insulin-producing cells are not vastly different for α- and β-D-glucose.

Discussion

The present results reveal that liver hexokinase, although displaying a higher K_m for glucose than did the enzyme characterized by the same procedure in erythrocytes (Malaisse *et al.*, 1985*a*), parotid (Malaisse *et al.*, 1985*b*) or pancreatic islets (Giroix *et al.*, 1984), demonstrates the same anomeric specificity as in the latter tissues, namely a lower K_m for and lower $V_{max.}$ with α- than with β-D-glucose.

Our results indicate that the reaction velocity catalysed by glucokinase is higher with β- than with α-D-glucose, whatever the glucose concentration (5–30mM) or tissue examined (liver, pancreatic islets, insulin-producing tumoral cells). The affinity of glucokinase tended to be higher for α- than for β-D-glucose. Such a difference, however, was often at or below the limit of statistical significance. It should be underlined that the anomeric affinity of glucokinase was assessed from

both the concentration-dependency of glucose phosphorylation (by radioisotopic and non-isotopic procedures) and the inhibitory action of unlabelled anomers on the phosphorylation of D-[U-^{14}C]glucose in anomeric equilibrium. In the latter case, glucose 6-phosphate was used to inhibit the activity of hexokinase. Our results confirm that glucose 6-phosphate, even in the absence of ATP, may allow for a limited rate of glucose phosphorylation by liver homogenates, e.g. as catalysed by D-glucose 6-phosphate phosphohydrolase (Alvarez & Nordlie, 1977; Singh & Nordlie, 1982). Therefore the effect of the unlabelled anomers on the phosphorylation of D-[U-^{14}C]glucose in anomeric equilibrium may, in the presence of glucose 6-phosphate, not depend solely on the anomeric affinity of glucokinase. Along the same line of thought, it could be objected that the present work was conducted with crude homogenates rather than purified enzymes. However, we were eager to explore the activity of hepatic enzymes under the same conditions as those used for the study of enzymic activities in normal pancreatic islets. In the latter case, the purification of enzymes is not easy in view of the limited amount of tissue readily available (Meglasson & Matschinsky, 1984).

An important difference between the present results and those previously reported (Meglasson & Matschinsky, 1983; Miwa *et al.*, 1983) consists in the fact that the rate of α-D-glucose phosphorylation, as catalysed by glucokinase, was never higher than that of β-D-glucose. We have no satisfactory explanation for this discrepancy. In the light of the present results, however, we consider it unjustified to ascribe to glucokinase a key role in the higher rate of glycolysis found in intact islets exposed to α- as distinct from β-D-glucose (Malaisse *et al.*, 1976*a*). There are further objections to the latter proposal. First, the glucose 6-phosphate concentration is higher in islets exposed to β- than to α-D-glucose (Idahl *et al.*, 1975; Malaisse *et al.*, 1976*a*). Secondly, in erythrocytes, the rate of glycolysis is also higher in the presence of α- than of β-D-glucose, although the phosphorylation of glucose is catalysed in these cells solely by hexokinase (Malaisse *et al.*, 1985*a*). Lastly, we have recently observed that, even when glucose is used at a concentration (3.3mM) low enough to avoid any significant participation of glucokinase in the phosphorylation of this hexose, the rates of phosphorylation and insulin release remain higher in islets exposed to α- as distinct from β-D-glucose (Malaisse *et al.*, 1985*c*). These converging observations support the view (Malaisse *et al.*, 1983) that the critical reaction(s) responsible for the anomeric specificity of glucose metabolism in intact cells take(s) place at a site distal to the phosphorylation of this hexose.

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