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Dissociated effects of 2-deoxy-D-glucose on D-[2-^{3}H]glucose and D-[5-^{3}H]glucose conversion into ^{3}HOH in rat erythrocytes

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When rat erythrocytes were preincubated with 2-deoxy-D-glucose, the generation of both ^{3}H-labelled acidic metabolites and ^{3}HOH from D-[5-^{3}H]glucose, the total production of L-lactate, and the generation of $^{14}CO_2$, ^{14}C-labelled acidic metabolites and ^{14}C-labelled lactate from D-[1-^{14}C]glucose or D-[U-^{14}C]glucose were all lower than in erythrocytes preincubated in the absence of a hexose or in the presence of 3-*O*-methyl-D-glucose. However, preincubation with 2-deoxy-D-glucose failed to decrease the generation of ^{3}H-labelled acidic metabolites and L-[3-^{3}H]lactate from D-[2-^{3}H]glucose, while decreasing the production of ^{3}HOH more severely from D-[2-^{3}H]glucose than from D-[5-^{3}H]glucose. This may be attributable not solely to inhibition of D-glucose phosphorylation by 2-deoxy-D-glucose and 2-deoxy-D-glucose 6-phosphate, but also to inhibition by 2-deoxy-D-glucose 6-phosphate of hexose 6-phosphate interconversion in the reaction catalysed by phosphoglucoisomerase, as also observed with the purified enzyme. The generation of ^{3}HOH from D-[2-^{3}H]glucose should therefore be considered as a tool to assess the efficiency of interconversion of hexose 6-phosphates in the reaction catalysed by phosphoglucoisomerase, rather than to estimate D-glucose phosphorylation rate.

INTRODUCTION

The generation of ^{3}HOH from D-[2-^{3}H]glucose is often used to assess the rate of D-glucose phosphorylation in intact cells [1]. Yet, in several cell types, such a generation underestimates the true rate of D-glucose phosphorylation [2], owing to incomplete detritiation of hexose 6-phosphates in the reaction catalysed by phosphoglucoisomerase. This situation is attributable to the conjunction of several factors, including intramolecular transfer of ^{3}H from D-[2-^{3}H]glucose 6-phosphate to the so-called 'iso' diastereomer of D-[1-^{3}H]fructose 6-phosphate [3], marked isotopic discrimination towards these ^{3}H-labelled esters [4,5] and, possibly, enzyme-to-enzyme channelling of hexose 6-phosphates in the early steps of glycolysis [6].

The present study reveals that, in rat erythrocytes preincubated with 2-deoxy-D-glucose, the generation of ^{3}HOH from D-[2-^{3}H]glucose is impaired in preference to other metabolic variables, such as the conversion of D-[5-^{3}H]glucose into ^{3}HOH. Our results do not solely confirm that the generation of ^{3}HOH from D-[2-^{3}H]glucose underestimates D-glucose phosphorylation, but also reveal that the relative extent of such an underestimation may itself be affected, in a given cell type, by selected factors.

MATERIALS AND METHODS

Experiments in rat erythrocytes

The method used to measure D-glucose metabolism in rat erythrocytes was described previously [7]. Briefly, blood was collected in heparinized tubes. The erythrocytes were separated from the plasma and buffy coat and resuspended in a salt-balanced medium prepared in a Hepes/NaOH buffer containing BSA. This suspension was kept for 30 min on ice or preincubated for 30 min at 37 °C either in the absence of any hexose or in the presence of 3-*O*-methyl-D-glucose (16.7 mM) or 2-deoxy-D-glucose (16.7 mM). The erythrocytes were then washed twice and eventually incubated for 90 min at 37 °C in 0.1 ml of medium containing 8.3 mM-D-glucose mixed with a tracer amount of ^{3}H- or ^{14}C-labelled D-glucose. The generation of ^{3}HOH [8], $^{14}CO_2$ [9], radioactive acidic metabolites [10] or L-lactate [11,12] and total production of L-lactate [13] were measured as described in the cited references.

Experiments with purified enzymes

For the study of bovine heart hexokinase (EC 2.7.1.1; Sigma,. St. Louis, MO, U.S.A.), incubations were conducted over 20 min at 37 °C in 0.1 ml of Tris/HCl buffer (50 mM, pH 7.5) containing D-glucose, mixed with a tracer amount of D-[U-^{14}C]glucose, $MgCl_2$ (7.0 mM), EDTA (1.0 mM), ATP (5.0 mM; sodium salt) and, when required, 2-deoxy-D-glucose or 2-deoxy-D-glucose 6-phosphate. The reaction was initiated by addition of hexokinase and stopped by heating for 5 min at 80 °C, D-[U-^{14}C]glucose 6-phosphate then being separated by anion-exchange chromatography [10].

Two methods were used for the study of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (EC 1.1.1.49; Boehringer, Mannheim, Germany). In the first method, a portion (0.1 ml) of Tris/HCl buffer (50 mM, pH 7.5) contained D-glucose 6-phosphate and/or 2-deoxy-D-glucose 6-phosphate, $MgCl_2$ (7.0 mM), EDTA (1.0 mM) and ammonium acetate (25 mM). The reaction was initiated by adding 0.1 ml of the same buffer containing 2-oxoglutarate (10 mM) mixed with a tracer amount of 2-oxo[1-^{14}C]glutarate, ADP (1.0 mM), NAD^+ (2.0 mM), glucose-6-phosphate dehydrogenase (approx. 17 m-units/ml) and bovine liver glutamate dehydrogenase (3.0 units/ml; EC 1.4.1.3; Boehringer). After incubation for 20 min at 37 °C, the reaction was halted by heating for 5 min at 80 °C. The samples were examined for their content of L-[^{14}C]glutamate, which was separated by ion-exchange chromatography [14]. All measurements were read against NADH standards treated in the same manner.

In the second method, the assay medium (0.2 ml) consisted of the same Tris/HCl buffer containing D-glucose 6-phosphate, mixed with a tracer amount of D-[1-^{14}C]glucose 6-phosphate, $NADP^+$ (5.0 mM) and yeast 6-phosphogluconate dehydrogenase

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Table 1. Metabolic flows in rat erythrocytes after preincubation in the absence or presence of either 3-*O*-methyl-D-glucose or 2-deoxy-D-glucose (16.7 mM each)

All results are expressed as pmol of glucose equivalents/90 min for μl of erythrocytes: [a]$P < 0.025$, [b]$P < 0.01$, [c]$P < 0.001$ versus first column.

Tracer	Metabolite	Hexose present during preincubation		
		None	3-*O*-Methyl-D-glucose	2-Deoxy-D-glucose
D-[5-^{3}H]Glucose	^{3}HOH	4746±158 (20)	5163±167 (20)	3219±117 (20)[c]
	Acidic metabolites	337±22 (20)	359±31 (20)	204±17 (20)[c]
D-[2-^{3}H]Glucose	^{3}HOH	3000±95 (20)	3334±119 (20)	1249±50 (20)[c]
	Acidic metabolites	1493±68 (20)	1733±94 (20)	1803±110 (20)[c]
	L-[3-^{3}H]Lactate	315±30 (16)	285±26 (16)	322±35 (16)[a]
None	Total L-lactate	5082±227 (29)	5134±261 (31)	3713±183 (31)[c]
D-[1-^{14}C]Glucose	$^{14}CO_2$	164±6 (12)	176±5 (12)	91±5 (12)[c]
	Acidic metabolites	4511±120 (12)	4303±113 (12)	3094±131 (12)[c]
	L-[3-^{14}C]Lactate	1810±84 (8)	1621±74 (8)	1422±83 (8)[b]
D-[U-^{14}C]Glucose	$^{14}CO_2$	37±2 (12)	35±2 (12)	22±3 (12)[c]
	Acidic metabolites	4583±144 (12)	4232±101 (12)	3258±182 (12)[c]
	L-[U-^{14}C]Lactate	1793±7 (8)	1677±70 (8)	1424±89 (8)[b]

(0.3 unit/ml; EC 1.1.1.44; Boehringer). The reaction was initiated by adding glucose-6-phosphate dehydrogenase and, after incubation for 20 min at 37 °C, halted by injecting 0.1 ml of HCl (0.5 M). The $^{14}CO_2$ formed was trapped in Hyamine hydroxide during a further incubation for 60 min at 20 °C.

For the study of yeast phosphoglucoisomerase (EC 5.3.1.9; Boehringer), the assay medium (0.2 ml) consisted of Tris/HCl buffer (50 mM, pH 8.0) containing D-glucose 6-phosphate, mixed with a tracer amount of D-[1-^{14}C]glucose 6-phosphate, $MgCl_2$ (7.0 mM), EDTA (1.0 mM), ATP (1.0 mM), yeast phosphoglucoisomerase (approx. 5 m-units/ml) and rabbit muscle phosphofructokinase (1 unit/ml; EC 2.7.1.11; Boehringer) as the auxiliary enzyme. After 15 min incubation at 37 °C, the reaction was halted by heating for 5 min at 80 °C. The D-[1-^{14}C]fructose 1,6-bisphosphate formed during incubation was separated from D-[1-^{14}C]glucose 6-phosphate and D-[1-^{14}C]fructose 6-phosphate as described elsewhere [4].

Presentation of results

All results are expressed as means (±S.E.M.) together with the number of individual determinations (n) or degree of freedom (d.f.). The statistical significance of differences between mean values was assessed by use of Student's t test.

RESULTS

Fate of D-[2-^{3}H]glucose and D-[5-^{3}H]glucose

The primary data collected in the experiments with intact rat erythrocytes are summarized in Table 1.

The production of ^{3}HOH from D-[5-^{3}H]glucose was not significantly different in cells preincubated in the absence or presence of 3-*O*-methyl-D-glucose, but was much lower in cells preincubated with 2-deoxy-D-glucose (Table 1). In the latter case, the readings averaged 65.0±2.4% ($n = 20$) of the mean value recorded in the former cases. Likewise, preincubation with 2-deoxy-D-glucose significantly decreased the generation of ^{3}HOH from D-[2-^{3}H]glucose. The ratio of ^{3}HOH production from D-[2-^{3}H]glucose to that from D-[5-^{3}H]glucose was virtually identical in cells preincubated in the absence of any hexose (62.6±2.8%) or in the presence of 3-*O*-methyl-D-glucose (64.5±2.1%), but severely decreased ($P < 0.001$) in erythrocytes first exposed to 2-deoxy-D-glucose (38.6±1.3%). Incidentally, the ratios recorded under the former conditions were themselves lower ($P < 0.03$) than the value of 71.4±2.2% (d.f. = 30) measured, within the same experiments, in erythrocytes preincubated at 0–4 °C rather than 37 °C in the absence of any hexose.

The generation of ^{3}H-labelled acidic metabolites from D-[5-^{3}H]glucose was also inhibited by 2-deoxy-D-glucose. Relative to the total generation of ^{3}H-labelled metabolites, that of acidic metabolites averaged 6.4±0.7, 6.2±0.9 and 5.7±0.7% in cells preincubated in the absence of any hexose, in the presence of 3-*O*-methyl-D-glucose and in the presence of 2-deoxy-D-glucose respectively. With D-[2-^{3}H]glucose, the latter ratios averaged 32.5±1.5, 33.5±1.5 and 57.7±2.4% (d.f. = 35 in all cases). The lesser generation of ^{3}HOH from D-[2-^{3}H]glucose in cells preincubated with 2-deoxy-D-glucose thus coincided with a greater fractional recovery ($P < 0.001$) of ^{3}H-labelled acidic metabolites. Even the absolute value for the generation of ^{3}H-labelled acidic metabolites from D-[2-^{3}H]glucose was not decreased in cells preincubated with 2-deoxy-D-glucose.

The complementary nature of the changes in ^{3}HOH and ^{3}H-labelled acidic metabolites generation from D-[2-^{3}H]glucose was further documented by the fact that the ratio of the total generation of ^{3}H-labelled metabolites from D-[2-^{3}H]glucose to that from D-[5-^{3}H]glucose averaged 95.4±3.2%, 99.1±3.6% and 94.8±3.8% in cells preincubated in the absence of hexose and in the presence of 3-*O*-methyl-D-glucose or 2-deoxy-D-glucose respectively. None of these ratios differed significantly from either unity or one another.

The production of L-lactate was significantly decreased in cells preincubated with 2-deoxy-D-glucose. However, relative to the total generation of ^{3}H-labelled metabolites from D-[5-^{3}H]glucose, the production of L-lactate was not significantly affected by the presence or absence of an hexose in the preincubation medium. Thus the paired ratio between L-lactate production, expressed as glucose equivalents, and total generation of ^{3}H-labelled metabolites from D-[5-^{3}H]glucose averaged 101.3±6.6%, 96.6±7.2% and 107.9±7.9% in erythrocytes preincubated in the absence and presence of 3-*O*-methyl-D-glucose or 2-deoxy-D-glucose respectively (n = 39–40). No significant amount of ^{3}H-labelled L-lactate was generated from cells exposed to D-[5-^{3}H]glucose. However, in cells exposed to D-[2-^{3}H]glucose, a sizeable generation of L-[3-^{3}H]lactate was observed. As already noted for ^{3}H-labelled acidic metabolites generated from D-[2-^{3}H]glucose, the generation of L-[3-^{3}H]lactate from the same tracer was not decreased by 2-deoxy-D-glucose when expressed in absolute terms. Moreover, relative to the total generation of ^{3}HOH and ^{3}H-labelled acidic metabolites from D-[2-^{3}H]glucose,

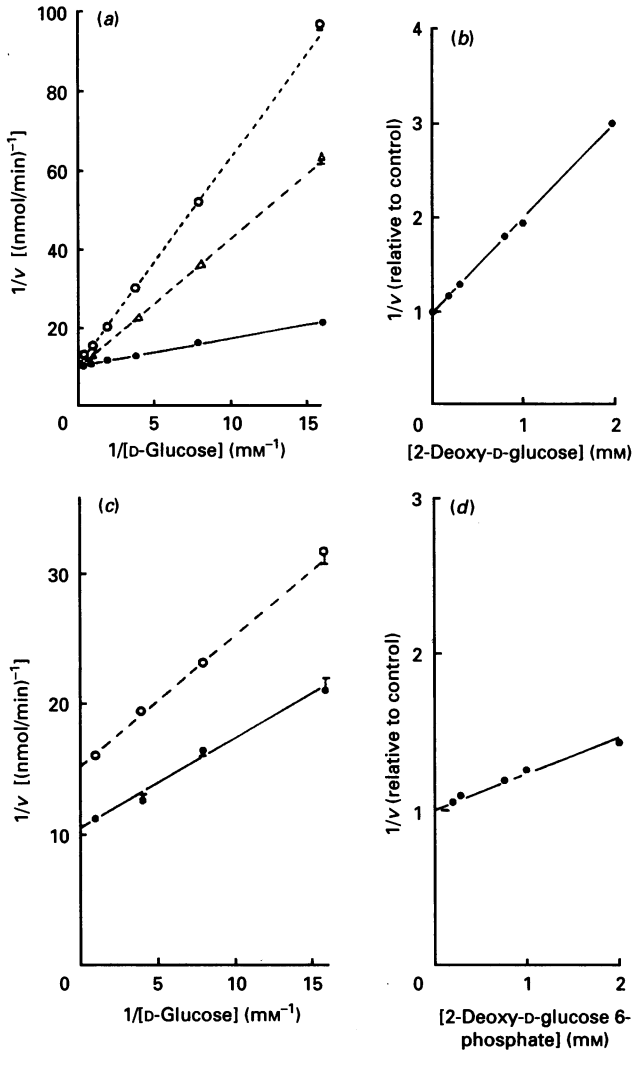

Fig. 1. Effects of 2-deoxy-D-glucose and 2-deoxy-D-glucose 6-phosphate on D-[U-^{14}C]glucose phosphorylation by bovine heart hexokinase

(*a*) Lineweaver–Burk plot at increasing concentrations of D-[U-^{14}C]glucose in the presence of either 2.0 mM- (△----△) or 3.0 mM- (○----○) 2-deoxy-D-glucose and in its absence (●——●). (*b*) Dixon plot for the inhibition of hexokinase activity at increasing concentrations of 2-deoxy-D-glucose in the presence of 62.5 μM-D-[U-^{14}C]glucose. (*c*) Lineweaver–Burk plot at increasing concentrations of D-[U-^{14}C]glucose in the presence of 3.0 mM-2-deoxy-D-glucose 6-phosphate (○----○) or in its absence (●——●). (*d*) Dixon plot for the inhibition of hexokinase activity at increasing concentrations of 2-deoxy-D-glucose 6-phosphate in the presence of 0.1 mM-D-[U-^{14}C]glucose. Mean values (±S.E.M.) are derived from series of three to five individual experiments.

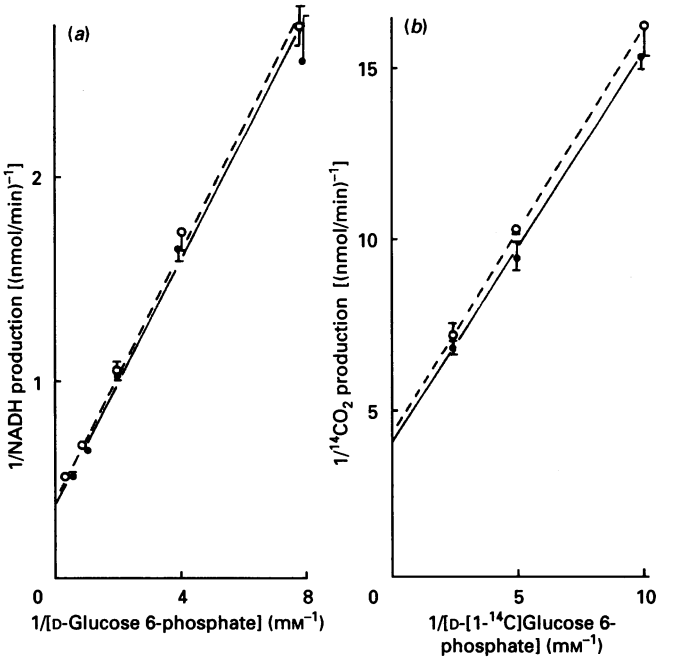

Fig. 2. Lineweaver–Burk plot for the velocity of the reaction catalysed by glucose-6-phosphate dehydrogenase at increasing concentrations of D-glucose 6-phosphate in the presence of 3.0 mM-2-deoxy-D-glucose 6-phosphate (○----○) or in its absence (●——●)

The reaction velocity was judged either from the production of NADH (*a*) or from the conversion of D-[1-^{14}C]glucose 6-phosphate into $^{14}CO_2$ in the presence of 6-phosphogluconate dehydrogenase as the auxiliary enzyme (*b*). Mean values (±S.E.M.) are derived from three individual experiments in each case.

the production of L-[3-^{3}H]lactate was higher ($P < 0.03$ or less) in cells preincubated with 2-deoxy-D-glucose (10.6±0.9%) than in erythrocytes preincubated in either the absence (7.9±0.8%) or the presence (6.1±0.7%) of 3-*O*-methyl-D-glucose.

Fate of D-[1-^{14}C]glucose and D-[U-^{14}C]glucose in rat erythrocytes

Preincubation of erythrocytes with 2-deoxy-D-glucose decreased to a comparable relative extent ($P > 0.3$) the generation of $^{14}CO_2$ from either D-[1-^{14}C]glucose (−46.3±4.1%) or D-[U-^{14}C]glucose (−38.1±8.4%). In the cells preincubated in the absence or presence of 3-*O*-methyl-D-glucose, the generation of $^{14}CO_2$ from D-[U-^{14}C]glucose, when expressed as CO_2 equivalent, averaged 125.6±9.5% (d.f. = 45) of that from D-[U-^{14}C]glucose, indicating a limited recirculation of hexose 6-phosphate in the pentose phosphate pathway.

The mean paired ratio between $^{14}CO_2$ output from D-[1-^{14}C]glucose and the total generation of ^{3}H-labelled metabolites from D-[5-^{3}H]glucose was slightly, but not significantly, lower ($P > 0.1$ or more) in cells preincubated with 2-deoxy-D-glucose (3.6±0.4%) than in erythrocytes preincubated either in the absence of a hexose (3.9±0.5%) or in the presence of 3-*O*-methyl-D-glucose (4.5±0.5%).

The generation of ^{14}C-labelled acidic metabolites from either D-[1-^{14}C]glucose or D-[U-^{14}C]glucose yielded virtually identical results and was decreased by 2-deoxy-D-glucose to 70.2±3.0 and 73.9±4.8% respectively ($n = 12$ in both cases) of the mean control value found in cells preincubated in the absence or presence of 3-*O*-methyl-D-glucose. The total generation of $^{14}CO_2$ and ^{14}C-labelled acidic metabolites from either D-[1-^{14}C]glucose or D-[U-^{14}C]glucose, when expressed as glucose equivalent, was not vastly different from the corresponding total generation of ^{3}H-labelled metabolites from D-[5-^{3}H]glucose, the paired ratio between these two variables ranging in the six experimental conditions between extreme values of 94.5±3.4% and 105.8±3.7% (d.f. = 13 in both cases) and yielding an overall mean value of 99.3±1.6% (d.f. = 78).

In two of the experiments conducted with ^{14}C-labelled D-glucose, the generation of radioactive L-lactate was also measured. The data recorded in cells preincubated with 2-deoxy-D-glucose and then exposed to D-[1-^{14}C]glucose or D-[U-^{14}C]glucose averaged 82.9±4.8% and 83.4±5.2% respectively ($n = 8$ in both cases) of the mean control value found in erythrocytes preincubated in the absence or presence of 3-*O*-

methyl-D-glucose. When measured within the same experiments, the relative decrease in the generation of ^{14}C-labelled L-lactate was not significantly different from that recorded for the generation of radioactive acidic metabolites from either D-[1-^{14}C]glucose or D-[U-^{14}C]glucose. The production of ^{14}C-labelled L-lactate from either D-[1-^{14}C]glucose or D-[U-^{14}C]glucose was much less than the total generation of L-lactate, in fair agreement with prior observations [7].

Enzymic data

The findings so far reported led us to explore the effect of either 2-deoxy-D-glucose or 2-deoxy-D-glucose 6-phosphate on selected enzymic reactions. These experiments were conducted with enzymes purified from biological sources other than rat erythrocytes.

The phosphorylation of D-[U-^{14}C]glucose by bovine heart hexokinase yielded a K_m for D-glucose close to 64 μM (Fig. 1*a*). The phosphorylation of D-[U-^{14}C]glucose was inhibited by 2-deoxy-D-glucose, the relative extent of such an inhibition being inversely related to the concentration of D-glucose. For instance, when 2-deoxy-D-glucose was tested at a concentration of 3.0 mM, the relative extent of its inhibitory action on D-[U-^{14}C]glucose phosphorylation progressively decreased from 84.9 ± 0.2 to 24.9 ± 1.6% as the concentration of the latter hexose was increased from 31 μM to 2.0 mM. As shown in Fig. 1(*a*), the effect of 2-deoxy-D-glucose on D-[U-^{14}C]glucose phosphorylation indeed displayed the typical pattern of a competitive inhibition, with K_i close to 0.4 mM. The concentration-dependency for the inhibitory action of 2-deoxy-D-glucose on the phosphorylation of D-[U-^{14}C]glucose (62.5 μM) is documented, as a Dixon plot, in Fig. 1(*b*). Taking into account the maximal velocity of D-[U-^{14}C]glucose phosphorylation, this Dixon plot also yielded a K_i close to 0.4 mM.

The phosphorylation of D-[U-^{14}C]glucose by bovine heart hexokinase was also inhibited by 2-deoxy-D-glucose 6-phosphate (Figs. 1*c* and 1*d*). However, in this case the relative extent of the inhibition in D-[U-^{14}C]glucose phosphorylation was little affected by the concentration of the latter hexose. A double-reciprocal plot established at increasing concentrations of D-[U-^{14}C]glucose indeed indicated that the data obtained in the absence and presence of 2-deoxy-D-glucose 6-phosphate (3.0 mM) yielded a common intercept with the abscissa, a characteristic feature for non-competitive inhibition (Fig. 1*c*). The K_i for the inhibitory action of 2-deoxy-D-glucose 6-phosphate was 4.3 mM (Fig. 1*d*), well in excess of that characterizing the inhibitory action of D-glucose 6-phosphate on mammalian hexokinase [15,16].

At a concentration of 3.0 mM, 2-deoxy-D-glucose 6-phosphate failed to affect significantly the generation of NADH from NAD^+ and D-glucose 6-phosphate in the reaction catalysed by glucose-6-phosphate dehydrogenase (Fig. 2*a*). Even when tested at 10 mM concentration, 2-deoxy-D-glucose 6-phosphate did not affect the reaction velocity significantly (results not shown). These negative results could not be ascribed to any contribution of 2-deoxy-D-glucose 6-phosphate as a substrate in the latter reaction, at least under the present experimental conditions [17]. Indeed, when 2-deoxy-D-glucose 6-phosphate (0.25–2.0 mM) was tested in the absence of D-glucose 6-phosphate but in the presence of NAD^+, it failed to generate NADH in the reaction catalysed by glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*. Moreover, 2-deoxy-D-glucose 6-phosphate (3.0 mM) also failed to affect the velocity of the reaction catalysed by glucose-6-phosphate dehydrogenase when D-[1-^{14}C]glucose 6-phosphate was used as substrate, with 6-phosphogluconate dehydrogenase present as the auxiliary enzyme and $^{14}CO_2$ measured as the end product (Fig. 2*b*).

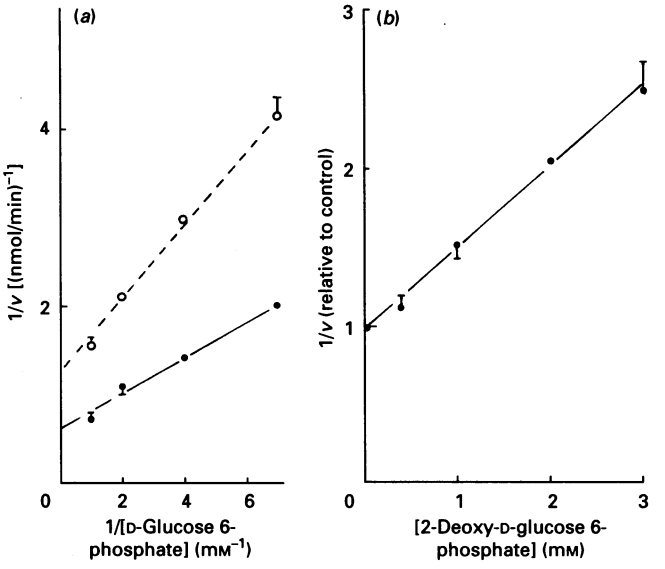

Fig. 3. (*a*) Lineweaver–Burk plot for the velocity of the reaction catalysed by phosphoglucoisomerase at increasing concentrations of D-glucose 6-phosphate either in the presence of 2.0 mM-2-deoxy-D-glucose 6-phosphate (○----○) or in its absence (●——●); (*b*) Dixon plot for the inhibition of phosphoglucoisomerase activity at increasing concentrations of 2-deoxy-D-glucose 6-phosphate in the presence of 0.1 mM-D-glucose 6-phosphate

Mean values (±S.E.M.) are derived from a series of five individual experiments.

At variance with the negative results obtained for glucose-6-phosphate dehydrogenase, 2-deoxy-D-glucose 6-phosphate inhibited the conversion of D-[1-^{14}C]glucose 6-phosphate into D-[1-^{14}C]fructose 6-phosphate in the reaction catalysed by phosphoglucoisomerase (Fig. 3). In these experiments, phosphofructokinase was used as the auxiliary enzyme, the reaction velocity being judged from the production of D-[1-^{14}C]fructose 1,6-bisphosphate. The inhibitory action of 2-deoxy-D-glucose 6-phosphate on the phosphoglucoisomerase reaction appeared of the non-competitive type (Fig. 3*a*). The relative extent of such an inhibitory action was indeed not affected by a rise in D-[1-^{14}C]glucose 6-phosphate concentration in the 0.1–1.0 mM range. The K_i for 2-deoxy-D-glucose 6-phosphate was close to 0.5 mM (Fig. 3*b*). Incidentally, 2-deoxy-D-glucose (0.3–3.0 mM) failed to exert any obvious effect on the activity of phosphoglucoisomerase (results not shown). The decrease in D-[1-^{14}C]fructose 1,6-bisphosphate production caused by 2-deoxy-D-glucose 6-phosphate was not attributable to inhibition of the auxiliary enzyme phosphofructokinase. Indeed, after incubation in the presence of 2-deoxy-D-glucose 6-phosphate, the amount of D-[1-^{14}C]fructose 6-phosphate present in the assay medium was not higher than that found in the absence of 2-deoxy-D-glucose 6-phosphate and did not exceed 0.7 ± 0.5% of the total initial amount of D-[1-^{14}C]glucose 6-phosphate, as compared with 6.5 ± 1.5% for the corresponding generation of D-[1-^{14}C]fructose 1,6-bisphosphate ($n = 10$ in both cases).

A last series of experiments was designed to assess whether 2-deoxy-D-glucose 6-phosphate affects, independently of its inhibitory effect on the velocity of the reaction catalysed by phosphoglucoisomerase, the extent of ^{3}H intramolecular transfer from D-[2-^{3}H]glucose 6-phosphate to D-[1-^{3}H]fructose 6-phosphate on the occasion of a single passage of the aldose ester through the latter reaction. For this purpose, unlabelled D-glucose 6-phosphate (0.25–0.50 mM), mixed with a tracer amount of D-[2-^{3}H]glucose 6-phosphate, was exposed, in the presence of

ATP (1.0 mM), for 90 min at 37 °C to yeast phosphoglucoisomerase (5 m-units/ml) and excess rabbit muscle phosphofructokinase (1 unit/ml). In the control experiments carried out in the absence of 2-deoxy-D-glucose 6-phosphate, the intramolecular ^{3}H transfer, as judged from the production of both D-[1-^{3}H]fructose 1,6-bisphosphate and ^{3}HOH, averaged $54.3 \pm 2.0\%$ ($n = 6$), in fair agreement with a prior observation [4]. When all available data were pooled, the intramolecular ^{3}H transfer was somewhat higher ($P < 0.02$) in the presence of 2-deoxy-D-glucose 6-phosphate (4.0 mM), averaging $60.7 \pm 0.9\%$ ($n = 6$). However, such an increase was not observed in all individual experiments and failed to display any obvious dependency on the concentration of 2-deoxy-D-glucose 6-phosphate in the 1.0–4.0 mM range.

DISCUSSION

The present findings indicate that preincubation of rat erythrocytes with 2-deoxy-D-glucose leads to a significant decrease in the generation of ^{3}HOH from D-[5-^{3}H]glucose or D-[2-^{3}H]glucose, the net production of acidic metabolites from D-[5-^{3}H]glucose, the total production of L-lactate and the generation of $^{14}CO_2$, ^{14}C-labelled acid metabolites and ^{14}C-labelled L-lactate from either D-[1-^{14}C]glucose or D-[U-^{14}C]glucose. As a rule, and when compared within the same experiments, the relative magnitude of the 2-deoxy-D-glucose-induced decrease was commensurate for most of these metabolic variables. There was, however, one outstanding exception to such a rule. In relative terms, the decrease in ^{3}HOH production from D-[2-^{3}H]glucose was much more marked than that from D-[5-^{3}H]glucose, resulting in a severe lowering of the ratio of ^{3}HOH generation from D-[2-^{3}H]glucose to that from D-[5-^{3}H]glucose. The significance of this finding was reinforced by the observation that 2-deoxy-D-glucose, when present in the preincubation medium, failed to decrease the absolute values for the generation of either ^{3}H-labelled acidic metabolites or L-[3-^{3}H]lactate from D-[2-^{3}H]glucose, and actually augmented the relative contribution of such metabolites to the total generation of ^{3}H-labelled metabolites from the same tracer.

Our complementary enzymic data confirm the inhibitory action of 2-deoxy-D-glucose on D-glucose phosphorylation by mammalian hexokinase [18] and further indicate that 2-deoxy-D-glucose 6-phosphate also inhibits both the phosphorylation of D-glucose and the conversion of D-glucose 6-phosphate into D-fructose 6-phosphate as catalysed by phosphoglucoisomerase [19].

In the light of these findings, it seems reasonable to propose that the fall in the ratio of ^{3}HOH production from D-[2-^{3}H]glucose to that from D-[5-^{3}H]glucose, as observed in erythrocytes preincubated with 2-deoxy-D-glucose, may be mainly attributable to the following sequence of events.

First, both residual 2-deoxy-D-glucose not rapidly extruded from the cells during incubation and 2-deoxy-D-glucose 6-phosphate, by inhibiting the rate of D-glucose phosphorylation, decrease the rate of glycolysis as well as the flux through the pentose phosphate pathway and the total generation of L-lactate from either exogenous D-glucose or endogenous 2,3-bisphospho-D-glycerate.

Second, 2-deoxy-D-glucose 6-phosphate, by inhibiting phosphoglucoisomerase activity, may further decrease the frequency of back-and-forth interconversion of D-glucose 6-phosphate and D-fructose 6-phosphate relative to net glycolytic flux. This, in turn, would impair the generation of ^{3}HOH from D-[2-^{3}H]glucose 6-phosphate and D-[1-^{3}H]fructose 6-phosphate.

When the preincubation was conducted in the presence of 2-deoxy-D-glucose, the ratio of ^{3}HOH generation from D-[2-^{3}H]glucose to that from D-[5-^{3}H]glucose was decreased to a mean value of $38.6 \pm 1.3\%$. This is the lowest value so far encountered in rat erythrocytes [2,7]. It is close to the value that might be expected whenever D-[2-^{3}H]glucose 6-phosphate is converted into D-[1-^{3}H]fructose 6-phosphate without significant back-conversion of the ketohexose ester to the aldohexose ester [4].

The present findings indicate not only that the generation of ^{3}HOH from D-[2-^{3}H]glucose underestimates the rate of D-glucose phosphorylation, but also that the relative extent of such an underestimation is variable [20,21]. A comparable, albeit not identical, situation had already been documented either in normal erythrocytes incubated at low temperature or in erythrocytes prepared from diabetic rats [22,23]. In this respect, it should be emphasized that there is no universal relationship between the actual rate of glycolysis in erythrocytes and the ratio of ^{3}HOH production from D-[2-^{3}H]glucose to that from D-[5-^{3}H]glucose. Indeed, a decrease in glycolytic flux may be associated with either an increase in D-[2-^{3}H]glucose detritiation relative to D-[5-^{3}H]glucose utilization, as observed at low temperature, or a decrease in the ratio of ^{3}HOH production from D-[2-^{3}H]glucose to that from D-[5-^{3}H]glucose, as observed in the present study.

It is therefore proposed that the generation of ^{3}HOH from D-[2-^{3}H]glucose should no longer be considered as a reliable indication of D-glucose phosphorylation, but may provide valuable information on the frequency of hexose 6-phosphate interconversion in the reaction catalysed by phosphoglucoisomerase. In this perspective, it may require correction for the generation of ^{3}HOH associated with both the catabolism of D-[1-^{3}H]fructose 1,6-bisphosphate and the circulation of D-[2-^{3}H]-glucose 6-phosphate in the pentose phosphate pathway in cells exposed to D-[2-^{3}H]glucose [7,24].

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