Phosphorylation of 3-*O*-methyl-D-glucose by yeast and beef hexokinase

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Received 31 January 1986

Beef heart hexokinase and yeast hexokinase both catalyzed the phosphorylation of 3-*O*-[^{14}C]methyl-D-glucose. The maximal velocity was 3 orders of magnitude lower and the K_m for the glucose analogue 40–120-times higher than those observed with D-[U-^{14}C]glucose. Hence, 3-*O*-methyl-D-glucose should not be considered as a truly nonmetabolized analogue of D-glucose.

Hexokinase *3-O-Methyl-D-glucose* *Phosphorylation*

1. INTRODUCTION

The analogue of D-glucose 3-*O*-methyl-D-glucose is widely used in studies of D-glucose transport [1–7]. Indeed, it is usually assumed that 3-*O*-methyl-D-glucose is not phosphorylated. Recent observations raise doubt about the validity of such an assumption. In 1984 Gatley et al. [8] observed, in rat hearts perfused with a medium containing ^{14}C-labelled 3-*O*-methyl-D-glucose, the presence in the tissue homogenate of a labelled compound which behaved on anion-exchange HPLC as a sugar-phosphate and was eliminated after treatment of the heart extract with alkaline phosphatase. In this study, however, the position at which the glucose analogue was labelled and its purity were not defined. More recently, Gancedo and Gancedo [9] observed, over 18 h incubation, the formation from 3-*O*-methyl-D-[1-^{3}H]glucose of a phosphorylated compound in either intact yeast cells or yeast extracts incubated in the presence of ATP. None of these observations unambiguously demonstrates, although they both suggest, that 3-*O*-methyl-D-glucose is indeed phosphorylated at the intervention of yeast or mammalian hexokinase. Indeed, in these two studies, the kinetics of 3-*O*-methyl-D-glucose phosphorylation were not established and, hence, the results could have been conceivably affected by either the presence of contaminating labelled D-glucose or the conversion of 3-*O*-methyl-D-glucose to D-glucose prior to its phosphorylation. In this study, therefore, we have investigated the kinetics of 3-*O*-[^{14}C]methyl-D-glucose phosphorylation by both yeast and beef heart hexokinase.

2. MATERIALS AND METHODS

Lyophilized beef heart hexokinase (Sigma, St. Louis, MO), solubilized yeast hexokinase (Boehringer, Mannheim), 3-*O*-[^{14}C]methyl-D-glucose (59 mCi/mmol) and 3-*O*-methyl-D-[U-^{14}C]glucose (295 mCi/mmol) both from Amersham (England), D-[U-^{14}C]glucose (257–341 mCi/mmol; New England Nuclear, Boston, MA) and unlabelled 3-*O*-methyl-D-glucose (Aldrich, Beerse, Belgium) were purchased from the cited companies. The beef heart hexokinase was solubilized in H_2O, divided in aliquots of 1.0 unit each, and again lyophilized prior to being used in each experiment.

The reaction mixture (0.1 ml) consisted of a Hepes-NaOH buffer (50 mM, pH 7.4) containing 150 mM KCl, 7 mM $MgCl_2$, 1.0 mM EDTA, 5.0 mM ATP, the required hexose(s), and either 0.25 U mammalian or 0.28 U yeast hexokinase. After 30 min incubation at 30°C, the reaction was

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00145793/86/$3.50

halted by addition of 2.0 ml iced water, the diluted reaction mixture being then passed through a column (0.5 ml) of Dowex 1-X8 (formate form) for separation of the hexose phosphates by anion-exchange chromatography [10].

The results were corrected for the blank value (0.1–0.2% of total radioactivity) obtained in the absence of enzyme. All reaction velocities given below are expressed per unit enzyme (by reference to the nominal activity, as indicated by the supplying company). In the case of yeast hexokinase, the maximal velocity obtained with D-[U-^{14}C]glucose was in fair agreement with such a nominal activity, taking into account the fact that our measurements were performed at 30°C instead of 25°C. With mammalian hexokinase, however, the maximal velocity obtained with D-[U-^{14}C]glucose was somewhat lower than the nominal value, this apparent decrease in activity being conceivably attributable to repeated lyophilizations.

The results presented in this report are restricted to the data obtained with 3-*O*-[^{14}C]methyl-D-glucose. Indeed, in the case of 3-*O*-methyl-D-[U-^{14}C]glucose, the significance of experimental data was considerably obscured by the presence of contaminating D-[U-^{14}C]glucose (representing about 1% of total radioactivity; unpublished). This resulted both in a lack of proportionality between the extent of phosphorylation and either the amount of enzyme or length of incubation and in much higher apparent reaction velocities than those obtained with 3-*O*-[^{14}C]methyl-D-glucose.

All results are expressed as the mean values together, if required, with the SE and number of individual observations (n), each of which was usually derived from triplicate measurements.

3. RESULTS

In the presence of 3-*O*-[^{14}C]methyl-D-glucose (0.1 mM), the rate of phosphorylation of the hexose averaged 7.20 ± 1.04 pmol/min (n = 6) with mammalian hexokinase, and 24.4 ± 1.8 pmol/min (n = 9) with yeast hexokinase. The amount of 3-*O*-[^{14}C]methyl-D-glucose phosphorylated was proportional to the amount of enzyme (3 mU–0.3 U) and length of incubation (15–30 min), with a coefficient of variation (SD/mean) for the reaction velocity of 8.8% (n = 9).

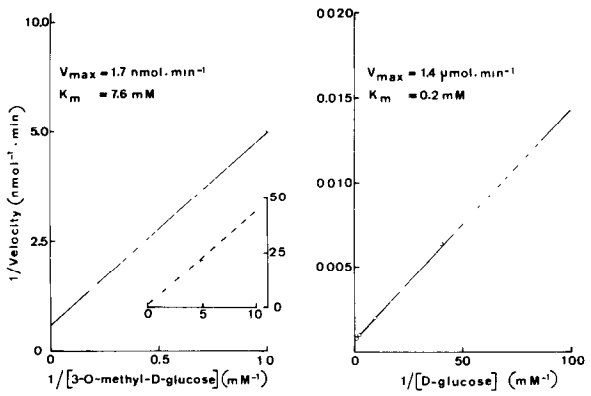

Fig.1. Reciprocal plot for the phosphorylation of 3-*O*-[^{14}C]methyl-D-glucose (left) and D-[U-^{14}C]glucose (right) by yeast hexokinase. Inset: data obtained at low concentrations of the hexose. The scale for the abscissa and ordinate axis in the inset were reduced in the same proportion relative to the corresponding scale in the main graph, to illustrate the parallelism of the two experimental lines.

At increasing concentrations of 3-*O*-[^{14}C]methyl-D-glucose (0.1–100.0 mM), reciprocal plots yielded linear relationships for both yeast (fig.1) and mammalian (fig.2) hexokinase. When compared to the results obtained with D-[U-^{14}C]glucose, the maximal velocity was 3 orders of magnitude lower and the K_m 38–118-times higher for 3-*O*-methyl-D-glucose than for D-glucose. The K_m for D-glucose was higher in the case of yeast hexokinase than mammalian hexokinase (cf. figs 1 and 2).

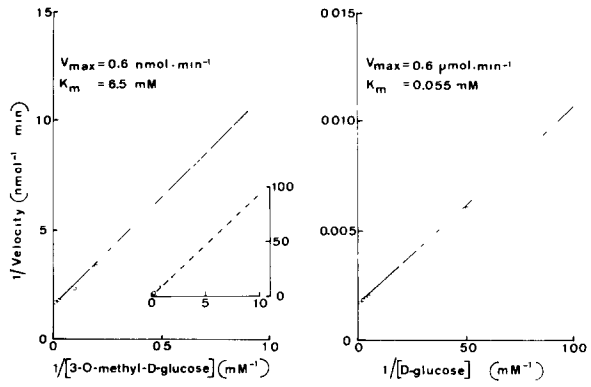

Fig.2. Reciprocal plot for the phosphorylation of 3-*O*-[^{14}C]methyl-D-glucose (left) and D-[U-^{14}C]glucose (right) by beef heart hexokinase. Same presentation as in fig.1.

Increasing concentrations of unlabelled D-glucose (0.01–10.0 mM) caused a dose-related decrease in the phosphorylation of 3-*O*-[^{14}C]methyl-D-glucose (0.1 mM) as catalyzed by either yeast or mammalian hexokinase (not shown). Virtual suppression of 3-*O*-[^{14}C]methyl-D-glucose phosphorylation (94.7 ± 3.6% inhibition; $n = 5$) was observed at the highest concentration of D-glucose (10.0 mM). At lower concentrations of D-glucose, the experimental values (y) for the reaction velocity, relative to the paired control reading (no D-glucose present), and the corresponding theoretical values (x) calculated from the respective K_m values for D-glucose and 3-*O*-methyl-D-glucose (see figs 1 and 2) yielded, by regression analysis ($y = 0.05 \pm 0.97x$), a coefficient of correlation amounting to 0.922 ($n = 14$; $P < 0.001$).

4. DISCUSSION

This study was performed with 3-*O*-methyl-D-glucose labelled with ^{14}C in the methyl moiety of the molecule. Our results unambiguously indicate that both yeast hexokinase and beef heart hexokinase indeed catalyze the phosphorylation of the D-glucose analogue.

It should be duly underlined that both the affinity of the enzyme for 3-*O*-[^{14}C]methyl-D-glucose and the maximal rate of phosphorylation of the glucose analogue were vastly different from the corresponding values found with D-[U-^{14}C]glucose. Our results do not necessarily invalidate, therefore, the use of 3-*O*-methyl-D-glucose as a tool in the study of D-glucose transport. For instance, the data obtained here with beef heart hexokinase would yield a ratio between 3-*O*-[^{14}C]methyl-D-glucose and D-[U-^{14}C]glucose phosphorylation amounting to 10^{-5} when the hexoses are tested at a tracer concentration (e.g. 5.0 μM) and 10^{-4} when they are tested in the concomitant presence of the homologous unlabelled hexose (e.g. 1.0 mM). Obviously, much higher ratios would be reached, especially in the presence of tracer concentrations, if labelled 3-*O*-methyl-D-glucose were contaminated with labelled D-glucose. The fact that commercially available 3-*O*-methyl-D-[U-^{14}C]glucose is indeed on occasions contaminated, may thus result in severe overestimation of 3-*O*-methyl-D-glucose phosphorylation.

The findings here collected in the concomitant presence of 3-*O*-[^{14}C]methyl-D-glucose and unlabelled D-glucose suggest that both sugars compete for the same catalytic site of the enzyme.

In conclusion, the present data demonstrate that 3-*O*-methyl-D-glucose should no more be considered as a strictly nonmetabolized analogue of D-glucose.

ACKNOWLEDGEMENTS

This study was supported in part by grants from the Belgian Foundation for Scientific Medical Research and NATO Scientific Affairs Division. We thank C. Demesmaeker for secretarial help.

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