SPECIFICITY OF HEXOKINASES TOWARDS SOME UNCOMMON SUBSTRATES AND INHIBITORS

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1. Introduction

The specificities of animal hexokinase towards analogues of glucose as substrate and of glucose 6-phosphate as allosteric inhibitor have been extensively studied [1–3]. Of other hexokinases, that of yeast [4–6] is of particular interest on theoretical [7-10] and practical grounds. These two enzymes have related but also significantly different substrate specificities.

During the last few years a number of sugar derivatives of potential interest as substrates or inhibitors of hexokinases have become available, and some of them could be useful for metabolic studies and even for chemotherapy. Among those already tested with yeast hexokinase, 5-thioglucose seems to offer a definite potential medical interest [11,12]. Most of them have apparently never been tested with any hexokinase.

We report here a systematic study of 9 uncommon analogues of glucose with yeast and in some cases with brain hexokinase, as well as of some of their phosphorylated products with the latter enzyme.

2. Materials and methods

2.1. *Chemicals*

All sugars were of the D-series: 1,5-anhydroglucitol, mannosamine hydrochloride, 5-thioglucose and 6-deoxyglucose were obtained from Sigma; 1-thioglucose, 2-deoxy-2-fluoroglucose and 3-deoxy-3-aminoglucose from Calbiochem; *N*-acetylmannosamine from P-L Biochemicals; 6-deoxy-6-aminoglucose from US Biochemicals Corp. An ~0.1% glucose impurity in 1-thioglucose was detected with glucose 6-phosphate dehydrogenase and eliminated with glucose oxidase. No significant phosphoryl at able impurities were detected enzymatically in either 2-deoxy-2-fluoroglucose or mannosamine. Coenzymes were obtained from Sigma. TES was from Calbiochem.

2.2. *Enzymes*

Isozymes I and II of yeast hexokinase [9] were obtained from Sigma (type C-301 and C-302, respectively). A partially purified preparation of bovine brain hexokinase (preparation CD [13]) was prepared by P. A. Lazo in this laboratory. The auxiliary enzymes were obtained from Sigma.

2.3. *Enzymatic assays*

Hexokinase activity was assayed, at pH 7.4 (50 mM TES buffer) in the presence of 0.1 M KCl and 5 mM $MgCl_2$ and at 37°C, by:

(A) Coupling with pyruvate kinase and lactate dehydrogenase (1 U each) to follow the production of ADP, with 2 mM MgATP, 3 mM phosphoenolpyruvate, and 0.2 mM NADH, in a volume of 0.5 ml, with enzyme and substrate as appropiate;

(B) As in (A) but in cuvettes of 2 mm optical path to increase the practical range of concentrations of accumulated hexose 6-phosphate; concentrations as in (A) except for NADH which was 1 mM and MgATP as indicated;

(C) Coupling to glucose 6-phosphate dehydrogenase (1 U for glucose and 5 U for 5-thioglucose and 0.5 mM $NADP^+$. The reaction was started by the addition of the hexokinase, following the ΔA_{340}.

Abbreviation: TES, *N*-tris(hydroxymethyl)methyl-2-amino ethane sulfonic acid

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3. Results

The glucose analogues studied have been tested with the isozymes I and II of yeast hexokinase. Results were essentially similar with both isozymes within a factor of ~2. The more interesting ones were also tested with brain hexokinases. The results with these compounds as substrates or competitive inhibitors are summarized in table 1.

Among the compounds tested the best substrate for both yeast and brain hexokinase, and for both maximal phosphorylation rate and apparent affinity ($1/K_m$) is 2-fluoroglucose. The former observation confirms the finding in [6]. Mannosamine is a fairly good substrate for the yeast and brain enzymes. 5-Thioglucose is very slowly phosphorylated by both enzymes, with fairly good apparent affinity, particularly for the brain enzyme; accordingly if administered to an animal it could both inhibit glucose phosphorylation and be slowly phosphorylated. 1,5-Anhydroglucitol is a weak substrate for yeast hexokinase, both in rate and in affinity; the latter result does not confirm the report [4] that it had a rather low K_m for this enzyme. 3-Deoxy-3-aminoglucose (tested only with the isozyme II) and 1-thioglucose are only marginal substrates, with phosphorylation coefficients [1] of 10^{-5}. *N*-Acetylmannosamine and 6-deoxy-6-aminoglucose are not appreciably phosphorylated by the yeast enzyme; it is to be noted that *N*-acetylmannosamine has considerably less affinity for the enzyme as competitive inhibitor than *N*-acetylglucosamine, in contrast with the relative apparent affinities of the parent sugars mannose and glucose [4].

2-Deoxy-2-fluoroglucose 6-phosphate and 5-thioglucose 6-phosphate were tested as analogues of glucose 6-phosphate for allosteric inhibition of brain hexokinase. 2-Deoxy-2-fluoroglucose 6-phosphate did not appreciably inhibit its own formation with brain hexokinase, as shown in fig.1 in contrast with the autoinhibition in the time course of glucose phosphorylation in the same conditions. This fact validates the potential usefulness of 2-deoxy-[2-^{18}F]fluoroglucose as an indicator of the intensity of energy metabolism in different areas of the brain in humans [14], since the accumulation of its 6-phosphate would not interfere with the continuation of normal hexokinase activity. Because of the poor phosphorylation rate of 5-thioglucose, its 6-phosphate was prepared with yeast hexokinase [12] and tested for inhibition of fructose phosphorylation by method (A), with results indicating strong inhibition. Later it has been found (F. Bedoya, A. S., unpublished) that 5-thioglucose 6-phosphate (Sigma) has a K_i as low as 7 μM and that this inhibition is not decreased by increase in [ATP], in contrast with the inhibition by glucose 6-phosphate.

We observed that glucose 6-phosphate dehydrogenase from yeast can use 5-thioglucose 6-phosphate as substrate, with an oxidation coefficient [1] of ~0.005 that of glucose 6-phosphate. It seems that both a

Table 1
Kinetic parameters for yeast and animal hexokinases

D-Sugars	Yeast		Animal	
	K_m (mM)	Rel. max. rate[a]	K_m (mM)	Rel. max. rate[a]
1,5-Anhydroglucitol	20	0.02	–	–
1-Thioglucose	5	4×10^{-4}	–	–
2-Deoxy-2-fluoroglucose	0.2	0.5	0.2	0.9
Mannosamine	5	0.2	–	0.2[b]
N-Acetylmannosamine	50[c]	$<2 \times 10^{-4}$	–	–
3-Deoxy-3-aminoglucose	30	0.003	–	–
5-Thioglucose	4	0.01	0.1[d]	0.003
6-Deoxy-6-aminoglucose	–	$<5 \times 10^{-4}$[e]	–	–
6-Deoxyglucose	50[c]	–	–	–

[a] Relative to glucose as 1; [b] at 50 mM; [c] K_i, as inhibitor of glucose phosphorylation; [d] K_i, as inhibitor of fructose phosphorylation; [e] at 25 mM

All assays were done at pH 7.4 in the presence of 0.1 M KCl as in the text. A dash indicates that no test was done. When appropiate, controls were done to rule out the possibility of interference of the glucose analogue with the auxiliary system

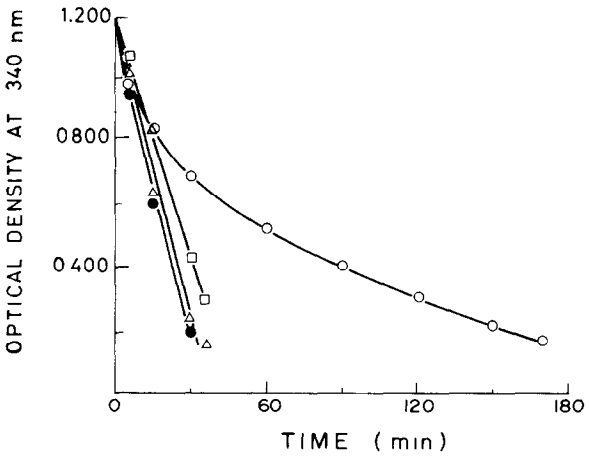

Fig.1. Lack of allosteric inhibition of brain hexokinase by 2-deoxy-2-fluoroglucose 6-phosphate. The time course of hexokinase activity was followed with method (B) in section 2 with: 5 mM glucose with 0.5 mM ATP (○——○) and 5 mM ATP (●——●); 5 mM 2- fluoroglucose (□——□) and 5 mM mannose (△——△) with 0.5 mM ATP

smaller maximal rate and a smaller affinity contribute to this markedly lessened efficiency, thus confirming in part the observations in [12].

4. Discussion

The substitution of an oxygen atom by a sulphur atom in the ring of glucopyranose leads to a moderate loss of apparent affinity for hexokinases and a large loss in maximal phosphorylation rate. In contrast, it seems that the same substitution in the 6-phosphate derivative maintains the requirements for allosteric inhibition.

The fact that the hexokinases of brain and yeast are so similar in their sensitivity and ability to phosphorylate 5-thioglucose makes it unlikely that testis or tumors could have an isozyme much more sensitive to inhibition by this compound. Obviously, interference with glycolysis in a peripheral tissue would be intolerable if accompanied by parallel damage to the central nervous system. Nevertheless, differential facility of access to the hexokinase in different tissues in vivo, or differential stability of the phosphorylated 5-thioglucose could allow for a significant degree of differential toxicity.

The known high specificity with respect to C-2 in glucose 6-phosphate of the allosteric site of animal hexokinase [2,3] is reinforced by the observation that it does not tolerate even the substitution of the hydroxyl group by a fluorine atom, a change that makes little difference at the sugar substrate site.

The fact that 6-deoxyglucose has an affinity for yeast hexokinase not much different from that of xylose [4] indicates that the inability of 6-deoxyglucose to induce the inactivation of yeast hexokinase as xylose does [15] depends probably on a high specificity for the induction of the conformational change required for this inactivation.

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