SPECIFIC INACTIVATION OF ANIMAL HEXOKINASES BY XYLOSE *IN VITRO, IN SITU* AND *IN VIVO*

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1. Introduction

A highly specific inactivation of yeast hexokinase induced by xylose was found [1], and identified as involving a potentially reversible paracatalytic phosphorylation of the enzyme [2] at a serine residue [3]. Xylose is a non-phosphorylatable glucose analogue that competitively inhibits the hexokinase reaction and activates the marginal adenosine triphosphatase activity of the enzyme [4]. There was no information on whether this peculiar inactivation could be restricted to a particular type of hexokinase. As we report here, animal hexokinases are similarly susceptible to specific inactivation induced by xylose in the presence of MgATP in vitro, in situ and in vivo.

2. Materials and methods

2.1. *Biological material*

A strain of Ehrlich-Lettré, ascites carcinoma was grown on month 2 Swiss mice and harvested about the 7th day after inoculation. The cells were washed twice with 115 mM NaCl, 5 mM sodium phosphate (pH 7.4).

Human erythrocytes from hospital surpluses were washed in the same buffer.

Calf-brain hexokinase was prepared as in [5] and used for in vitro studies.

2.2. *In vitro studies*

The in vitro studies were carried out with brain hexokinase. Enzyme, 0.1 U, was incubated in 0.5 ml 125 mM KCl_2, 5 mM sodium phosphate, 10 mM TES buffer (pH 7.4) with 5 mM phosphoenolpyruvate and 1 U pyruvate kinase as ATP-regenerating system, plus additions when indicated of 0.1 M D-xylose, 5 mM MgATP and 0.1 M *N*-acetyl-D-glucosamine, and incubated at 37°C. At the indicated times residual hexokinase activity was estimated with 50 μl aliquots in 1 ml 50 mM TES buffer (pH 7.4), 6% Ficoll, 5 mM glucose, 2.5 mM MgATP, 0.5 mM $NADP^+$ and 0.5 U glucose-6-phosphate dehydrogenase, following the increase in $A_{340\ nm}$.

2.3. *In situ studies*

The in situ studies were carried out with ascites tumor cells and erythrocytes crosslinked with dimethylsuberimidate and permeabilized with digitonin as in [6]. Permeabilized cells were suspended in 20 vol. inhibition mixture as above and incubated at 37°C with constant shaking. With ascites cells, aliquots were taken at the indicated times, centrifuged at 1500 × *g* for 5 min, and assayed as above. In erythrocytes, hexokinase was assayed by a 2 step isotopic method with 1.25 mCi/mmol D-[^{14}C]glucose; the reaction was stopped by adding 50 μl aliquots to 50 μl 2 M glucose–150 mM EDTA, the solution was filtered through Whatman DE-81 paper and washed with 200 ml water, after which the filters were dried and radioactivity counted.

2.4. *In vivo studies*

The in vivo studies were carried out with intact ascites tumor cells suspended in 10 vol. 115 mM NaCl, 5 mM sodium phosphate (pH 7.4), plus 10 mM pyruvate as oxidizable substrate, and 0.1 M xylose, with or without *N*-acetylglucosamine, and incubated

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at 37°C, shaking in air. At the indicated times 1 ml aliquots were centrifuged at 1500 × *g* for 5 min, the pellet was suspended in 1.8 ml 125 mM KCl, 5 mM $MgCl_2$, 5 mM sodium phosphate (pH 7.4) and was lysed by adding 0.1 ml 2% digitonin, and allowed to stand for 15 min at room temperature, after which the residual hexokinase activity was assayed by spectrophotometry as indicated above.

3. Results and discussion

While the studies on the D-xylose-induced inactivation of yeast hexokinase [1,3] were carried out either in vitro or in vivo, we had the additional possibility of observing the two major isoenzymes of animal hexokinase [7] in the intermediate state of permeabilized cells in which enzymes can be studied in their normal macromolecular environment but in otherwise controllable conditions [6].

As can be seen in fig.1, incubation of brain hexokinase (isoenzyme I), permeabilized erythrocytes (mostly isoenzyme I) or permeabilized ascites tumor cells (mostly isoenzyme II) with a saturating concentration of xylose [8] in the presence of near saturating MgATP and in otherwise physiological conditions (pH 7.4, 0.1 M K^+, 37°C) leads within 1–2 h to an inactivation of ~40%, which can be prevented by the simultaneous presence of another competitive inhibitor of hexokinase, namely *N*-acetylglucosamine [8] equimolar with xylose. This behaviour in vitro and in situ closely parallels that of the yeast enzyme in vitro, although to a lesser extent [1,3]. In the case of ascites tumor cells, which in contrast with the erythrocytes can respire pyruvate, it was also observed that aerobic incubation of intact cells with xylose and pyruvate leads to progressive inactivation of the hexokinase measurable in homogenates, up to a loss of > 60%. In vivo, *N*-acetylglucosamine protected only partly, presumably because of poor permeability [9]. It is not known why the inactivation does not approach completion; appropriate controls rule out exhaustion of ATP in the experimental control used. In the residual activity of the xylose-treated brain enzyme, no changes in the affinities for the substrates or the allosteric inhibition by glucose 6-phosphate have been detected.

The generalization to hexokinases of this unique inactivation initially observed with the yeast enzyme

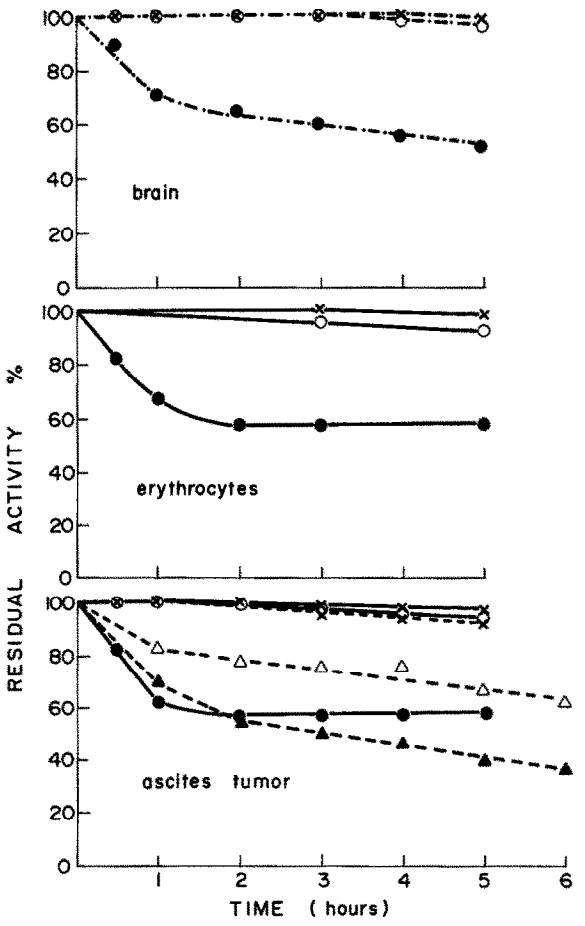

Fig.1. Time course of the xylose-induced inactivation of animal hexokinases. In vitro (- · -), in situ (—), and in vivo (- - -). Control without sugar, with and without mgATP or pyruvate in the in vivo experiment (×), xylose and MgATP (●), xylose, *N*-acetylglucosamine and MgATP (○); in vivo with xylose (▲) or xylose and N-acetylglucosamine (△).

[1] has an intrinsic value for studies on the mechanism of the hexokinase reaction and for comparative studies of common features of this enzyme in widely separated eucaryotes. The present observation obviously opens a way to experimental approaches involving highly selective partial inactivation of hexokinase in a variety of normal and tumoral animal tissues. Finally the possibility of a previously unsuspectable side reaction in the absorption test involving a xylose load [10] should be taken into account, since with it there could be a significant inactivation of the hexokinase of the intestinal mucosa [11].

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