INSOLUBLE HEXOKINASE IN THE BRUSH BORDER REGION OF RAT INTESTINAL EPITHELIAL CELLS

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In several reports it has been shown that a considerable amount of the total hexokinase activity in rat intestinal epithelial cells is particle-bound [1–4]. Srivastava et al. [4] determined the intracellular distribution of hexokinase activity in the mucosa of both rat and guinea pig small intestine. They found that in rat 60% of the hexokinase activity was associated with the particulate fraction of homogenates. Further subfractionation and characterization of the subfractions by means of marker enzymes, led the authors to conclude that the particle-bound hexokinase was predominantly recovered in the mitochondrial fraction [4].

During our studies on energy metabolism of intestinal epithelium it was found that ATP contributed less than 15% to the total amount of adenine nucleotides [5]. This low phosphorylation potential led us to investigate the energy metabolism in a part of the cell where mitochondria are absent, the brush border region.

Preparations of brush borders were made essentially according to Harrison and Webster [6]. After removal of viscous material contaminating the everted intestine by 1 min vibration (100 Hz) in cold saline, brush borders were released by further vibration for 20 min in 2.5 mM EDTA, containing 1 mM dithiothreitol (pH 7.4). As described by Harrison and Webster [6], the brush borders were harvested by centrifugation (2 min at 700 *g*) after treatment with glass fibers to remove nuclear aggregates. The precipitates were washed in cold isolation medium 3–5 times. The material obtained did not contain mitochondrial material, as judged by the absence of cytochrome *c* oxidase. However, the washed brush border did contain hexokinase activity. The Michaelis constants for this hexokinase are 10^{-4} M for glucose (compare also

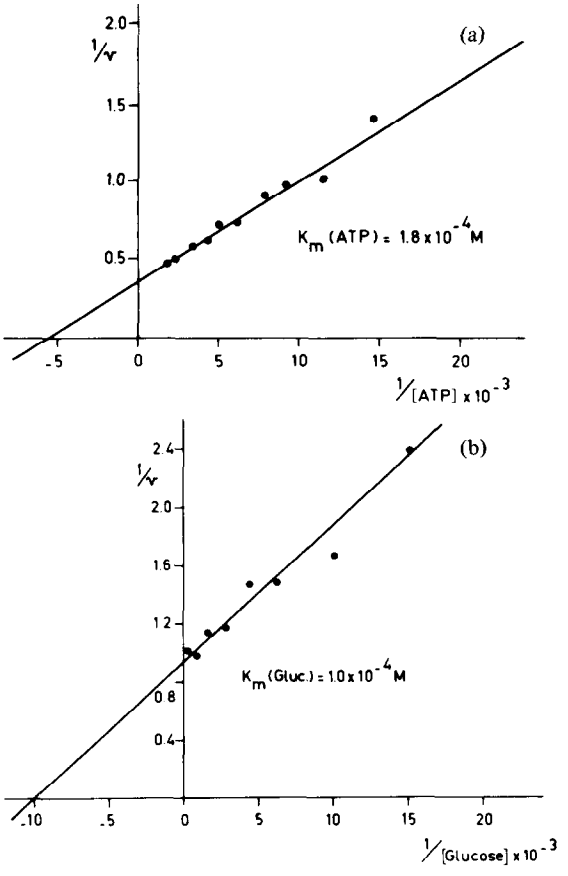

Fig. 1. Determination of the Michaelis constants of brush border-bound hexokinase. The reaction mixture (3 ml) contained: 0.4 mM $NADP^+$, 3 U glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 10 mM $MgCl_2$, 46 mM tris-HCl (pH 7.4), 1 mM dithiothreitol and about 1 mg of brush border protein. a) the ATP concentration was varied, while the glucose concentration was 3.3 mM; b) the glucose concentration was varied, while the ATP concentration was 5 mM. The reaction was followed at 340 and 380 nm in an Amino-Chance dual Wavelength spectrophotometer at 20°.

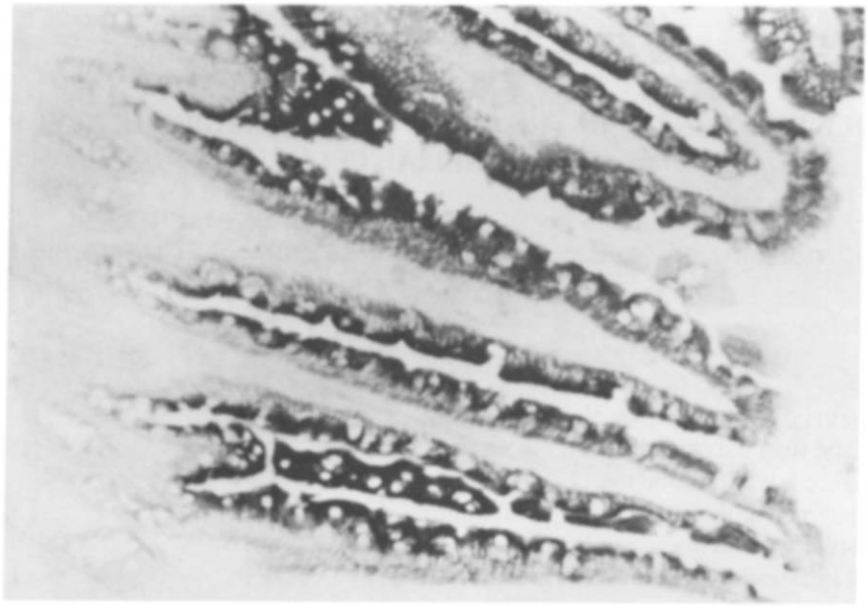

Fig. 2. Histochemical demonstration of hexokinase activity in rat jejunal mucosa. The reaction medium contained: 24 mM glucose, 0.5 mM $NADP^+$, 1.2 mM ATP, 1.4 mM $MgCl_2$, 0.44 mM nitroblue tetrazolium, 12 mM imidazole buffer, 8.5 mM KCN, 0.7 μg/ml glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 33 mg/ml gelatin. The cryostat sections (10 μm thick) were incubated without fixation for 30 min at 20° and pH 7.4.

Table 1.
Distribution of hexokinase and alkaline phosphatase activities in fractions of isolated brush borders.

Treatment of brush border preparation	*Hexokinase activity* nmoles/min/fraction	%	*Alkaline phosphatase act.* nmoles/min/fraction	%
Tris-disruption[a]				
supernatant	0.9	7	0.1	0.2
pellet	11.2	93	48.0	99.8
Ultrasonic disruption[b]				
supernatant	1.3	8	0.4	0.7
pellet	15.8	92	55.0	99.3
No treatment[c]				
supernatant	1.3	14	0.2	0.4
pellet	8.0	86	55.1	99.6

The recovery of hexokinase activity was 90% for the untreated preparation whereas the disrupted preparations showed a recovery of 120–150% compared with the intact brush border preparation. The alkaline phosphatase recovery was 90% for the untreated and 120% for both disrupted preparations, also compared with intact brush borders.

[a] According to [9], in 0.6 mM EDTA, 0.25 mM dithiothreitol and 1 M tris-HCl (pH 7.0) at 0° for 5–10 min, followed by the addition of bovine serum albumin (final concentration 0.5%) prior to centrifugattion for 60 min at 151,000 *g*.

[b] The brush border preparation was suspended in 0.6 mM EDTA, 0.25 mM dithiothreitol and 0.5% bovine serum albumin (pH 7.0) and sonicated (20 kHz at 0–5° for 2 × 30 sec), prior to centrifugation for 60 min at 151,000 *g*.

[c] Intact brush border preparation, contained in the same solutions as above. Hexokinase activity was measured as described in fig. 1 with 3.3 mM glucose and 5 mM ATP. Alkaline phosphatase was measured with *p*-nitrophenylphosphate as the substrate as described in [10].

[4]) and 1.8×10^{-4} M for ATP (fig. 1). The V_{max} (20°) for glucose phosphorylation was calculated to be 9.2 mU/mg protein.

Mayer et al. [7] made the interesting observation that perfusion of the intestinal lumen with glucose, prior to harvesting the epithelium, caused a rapid increase of soluble hexokinase activity, whereas the sum of particle-bound and soluble hexokinase activities did not change significantly. Earlier observations of this group [4] mentioned that the mitochondrial fractions of intestinal mucosa homogenates contained the bulk of the insoluble hexokinase. In the present study, however, employing isolated and washed brush borders, the activity cannot be due to contamination with mitochondria. Moreover, the preferential localization of hexokinase activity in the border region of the cell was confirmed by histochemical studies, carried out in principle as described by Meyer [8] for cryostat sections of liver, as shown in fig. 2. By this method isolation artifacts are circumvented, ruling out any possible redistribution of enzyme activity between subcellular fractions. This experiment also shows that the hexokinase activity of the brush border region is extremely low in crypt cells, when compared with villous cells in which glucose absorption occurs.

In order to obtain further information on the localization of this hexokinase within the brush border region, use was made of the observation of Eichholz and Crane [9] that brush borders disintegrate in 1.0 M tris-HCl (pH 7.0). The completeness of disintegration was verified by microscopic observation. An alternative method of disintegration, sonication at 0°, was also employed. It can be seen from table 1 that after both types of disintegration, centrifugation at 151,000 *g* for 60 min yields a pellet with about 90% of the total activity present in the brush border preparation. This suggests that the hexokinase studied is mainly structurally-bound and that about 10% is soluble or solubilized. No solubilization occurred with alkaline phosphatase (table 1 and [11]). The particulate hexokinase and alkaline phosphatase activites were subsequently found to be present in different structures of the brush border. Subfractionation of disrupted brush borders, followed by density gradient centrifugation on glycerol, exactly as described by Eichholz and Crane [9] yielded a fraction rich in alkaline phosphatase and poor in hexokinase activity (not shown). No further information on the exact localization of insoluble, brush border-linked, hexokinase is available at present. The latter activity, which in our hands represents 65% of the total homogenate activity, may well provide soluble hexokinase on glucose-feeding. Mayer et al. [7] previously suggested than insoluble hexokinase may provide soluble hexokinase on glucose-feeding. The store of hexokinase in the brush border region seems therefore of considerable interest, since hexokinase limits glycolytic activity [12].

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References

[1] C. Long, Biochem. J. 50 (1951) 407.
[2] R.K. Crane and A. Sols, J. Biol. Chem. 203 (1953) 273.
[3] C.F. Lange and P. Kohn, J. Biol. Chem. 236 (1961) 1.
[4] L.M. Srivastava, P. Shakespeare and G. Hübscher, Biochem. J. 109 (1968) 35.
[5] W.G.J. Iemhoff, J.W.O. van den Berg, A.M. de Pijper and W.C. Hülsmann, Biochim. Biophys. Acta 215 (1970) 229.
[6] D.D. Harrison and H.L. Webster, Biochim. Biophys. Acta 93 (1964) 662.
[7] R.J. Mayer, P. Shakespeare and G. Hübscher, Biochem. J. 116 (1970) 43.
[8] A.E.F.H. Meyer, Acta Histochem. 28 (1967) 286.
[9] A. Eichholz and R.K. Crane, J. Cell Biol. 26 (1965) 687.
[10] K. Linhardt and K. Walter in: Methoden der enzymatischen Analyse, ed. H.U. Bergmeyer (Verlag Chemie, Weinheim, 1962) p. 779.
[11] S. Clark, Am. J. Anat. 109 (1961) 57.
[12] L.M. Srivastava and G. Hübscher, Biochem. J. 100 (1966) 458.