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Kinetic Properties of Phosphofructokinase in the Mucosa of Mouse and Rat Small Intestine

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ABSTRACT. The regulatory properties of mouse jejunal mucosa phosphofructokinase have been studied in crude extracts freed from low molecular weight effectors on Sephadex G-100, and compared to those of the rat. Both isoenzymes displayed cooperativity with respect to fructose 6-phosphate at pH 7.0 in the presence of inhibitory concentrations of ATP. The rat isoenzyme was activated to a greater extent by AMP and P_i and inhibited to a greater extent by creatine phosphate than the mouse isoenzyme. Both isoenzymes were strongly activated by glucose 1,6-bisphosphate and strongly inhibited by citrate. The general similarity in properties of the two isoenzymes reflects the fact that both rat and mouse intestinal mucosa are characterised by high rates of aerobic glycolysis.

A recent investigation (Khoja and Kellett 1983) of the isoenzyme type of phosphofructokinase from the epithelial cells of rat small intestine showed that the enzyme from this tissue is different from those of other tissues, muscle, liver and brain; therefore, the mucosal isoenzyme was named phosphofructokinase D. This name followed the nomenclature of Tsai and Kemp (1973) who reported that animal tissues contain three principal isoenzymes of phosphofructokinase, type A found as the sole isoenzyme in skeletal muscle, type B found as the major isoenzyme in liver, and type C found as a significant isoenzyme in brain.

Some other investigations of phosphofructokinase isoenzymes have been carried out in rat tissues (Tanaka *et al.* 1971) and mouse tissues (Ommenn and Cohen 1971). These investigations have also shown multiple forms of phosphofructokinase.

The regulatory properties of phosphofructokinase have been used to distinguish the types of isoenzymes present in animal tissues (Tsai and Kemp 1974). The A, B and C isoenzymes of rabbit displayed different regulatory properties. The A isoenzyme is inhibited by phosphocreatine, while isoenzymes B and C are not, and isoenzyme B is the most readily inhibited by 2,3-diphosphoglycerate. All isoenzymes are inhibited by ATP and citrate. Recently, Khoja (1986) has reported that the regulatory properties of rat mucosal phosphofructokinase are different and distinct from those of other isoenzymes in the rat. The mucosal isoenzyme was less inhibited by ATP and was found to be more activated than the other isoenzymes as the mucosa is characterized by an exceptionally high rate of aerobic glycolysis (Hanson and Parsons 1976, Porteous 1978). In intestinal mucosa, Jamal *et al.* (1984) have indicated that phosphofructokinase is the rate-limiting enzyme of glycolysis.

The purpose of the present study is the comparison of the kinetic properties of phosphofructokinase from jejunal mucosa in the rat and the mouse.

Material and Methods

All biochemicals were obtained from either Sigma or Boehringer-Mannheim and were used without further purification. All chemicals were AnalaR grade from BDH. Sephadex G-100 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

Animals were male Wistar rats (200-250g) and male MFI mice (50-60g) fed *ad libitum* on standard laboratory diet (Oxoid modified 41B) with free access to water, obtained from King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia.

Rats or mice were anaesthetized with Sagatal (0.1 ml/100 g body wt). The jejunum was quickly removed, flushed with ice-cold extraction buffer (50 mM-Tris-chloride, pH 8.0) containing 100 mM-ammonium sulphate, 30 mM-potassium fluoride, 5 mM-2-mercaptoethanol, 1 mM-EDTA and proteinase inhibitors as 1 mM-phenylmethylsulphonyl fluoride, 1 mM-6-amino-n-hexanoic acid and 0.5 mg of Soya-bean trypsin inhibitor/ml, opened by a lengthwise incision and gently blotted with tissue. The mucosa was then collected by gentle scraping of the luminal surface with a microscope slide and homogenized directly in a Potter-Elvehjem homogenizer with 3 volumes (v/w) of extraction buffer. The homogenates were then centrifuged at 75000 g for 30 min at 4°C. The pellets were discarded and the particle-free supernatants were chromatographed directly on a column (2.5 × 50 cm) of Sephadex G-100 equilibrated with the same buffer.

Phosphofructokinase activity under optimal conditions at pH 8.0 was measured at 27°C as described by Ling *et al.* (1965). The regulatory properties were expressed as the activity ratio, v/V, where v is the activity at pH 7.0 determined under the conditions defined by Jamal and Kellett (1983) and V is the maximal activity at pH 8.0. One unit of activity is defined as the formation of 1 μmol of fructose 1,6-bisphosphate/min.

Results and Discussion

All studies of phosphofructokinase from the small intestinal mucosa were performed in the presence of proteinase inhibitors as described above to prevent proteolysis of the mucosal isoenzyme by endogenous proteinases (Khoja *et al.* 1983).

The regulatory properties of phosphofructokinase were determined at pH 7.0 because assays of this enzyme at pH 7.8 or higher showed a hyperbolic curve with respect to fructose 6-phosphate (Srivastava and Hubscher 1966), and hence the regulatory properties cannot be determined.

The fructose 6-phosphate saturation curve of the intestinal mucosa phosphofructokinase at 2.5 mM-ATP of rat and mouse is shown in Fig. 1. The enzyme from both animals showed sigmoidal velocity curves with different apparent K_m values. The apparent K_m for the rat isoenzyme was 0.4 mM while that of the mouse isoenzyme was 0.5 mM. The activity ratio at 0.5 mM-fructose 6-phosphate or less was higher in the rat isoenzyme than that in the mouse, but the activity ratio at concentrations more than 0.5 mM-fructose 6-phosphate was higher in the mouse isoenzyme with a V_{max} of 90% of the activity at pH 8.0, whereas the V_{max} of the rat isoenzyme was 80%.

Figure 2 shows the inhibition by ATP of rat and mouse jejunal mucosa phosphofructokinase at pH 7.0, in the presence of 0.5 mM-fructose 6-phosphate. The rat isoenzyme was slightly more inhibited by ATP than the mouse isoenzyme, where both showed maximum velocity at 0.5 mM ATP. However, this increase in susceptibility to inhibition by ATP is reflected in an increase in apparent K_m for fructose 6-phosphate.

Activitors

As previously described (Khoja 1986), the effect of activators and inhibitors on the activity of phosphofructokinase from jejunal mucosa were determined at the apparent K_m values for fructose 6-phosphate. Therefore, the effects of activators were studied at pH 7.0 in the presence of 2.5 mM-ATP and with fructose 6-phosphate at 0.4 mM for rat mucosa, and 0.5 mM for mouse mucosa.

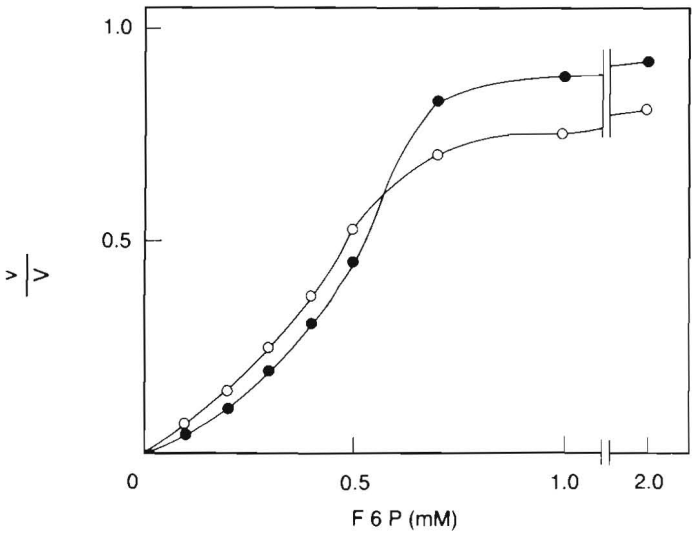


Fig. 1. Fructose 6-phosphate saturation curve of mucosal phosphofructokinase partially purified by chromatography on Sephadex G-100. Enzyme activity was assayed at pH 7.0
(○) rat (●) mouse

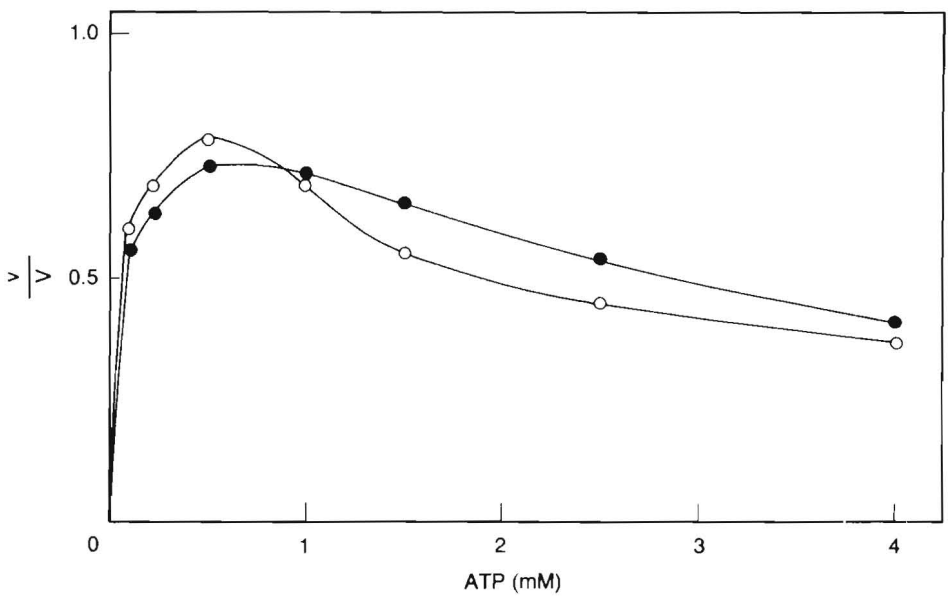


Fig. 2. Dependence of mucosal phosphofructokinase activity on ATP concentration in the presence of 0.5 mM-fructose 6-phosphate
(○) rat (●) mouse

Inorganic phosphate proved to be a strong activator for both isoenzymes, but the rat isoenzyme was more activated than the mouse isoenzyme (Fig. 3). The extent of activation of these isoenzymes were 100% and 85% for rat and mouse respectively at 10 mM inorganic phosphate. The concentrations of P_i required to give half-maximal activation were 0.06 mM and 1.25 mM for the rat and the mouse isoenzymes respectively.

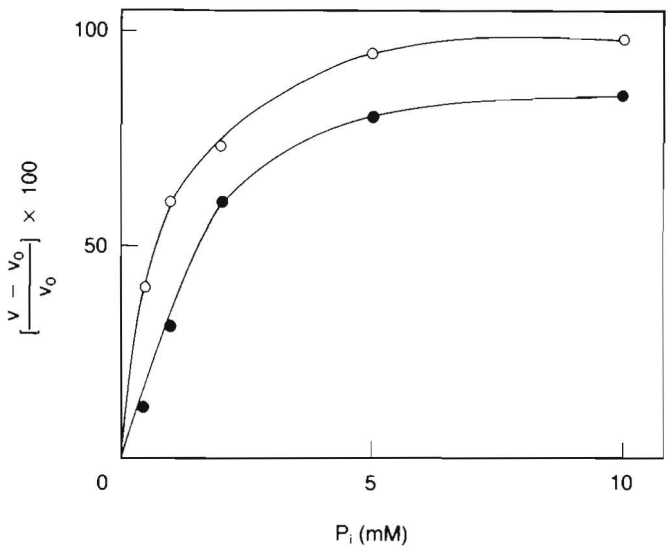
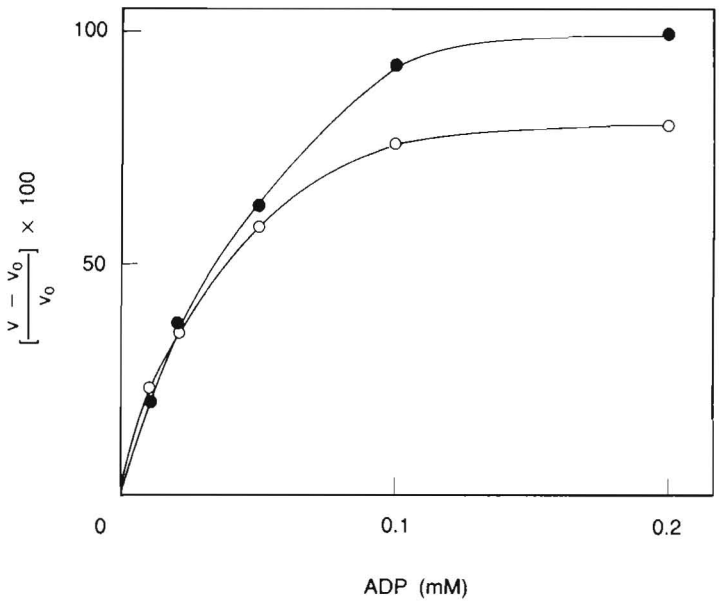


Fig. 3. The effect of inorganic phosphate on the activity of mucosal phosphofructokinase in the presence of 2.5 mM-ATP
(○) rat (●) mouse

The effect of adenine nucleotides activators is shown in Fig. 4. ADP and AMP were both powerful activators for the rat and the mouse isoenzymes, but AMP was stronger than ADP in full agreement with an earlier report from our laboratory (Khoja 1986) that muscle, liver, brain and mucosal isoenzymes were more activated with AMP. However, the concentrations of ADP required to give half-maximal activation were 0.025 mM and 0.035 mM for the rat and the mouse isoenzymes respectively. The concentrations of AMP required to give half-maximal activation were 0.01 mM and 0.035 mM for the rat and the mouse isoenzymes respectively.

Figure 5 shows the activation curve of phosphofructokinase from the rat and the mouse jejunal mucosa by glucose 1,6-*bis*phosphate. It can be seen that both



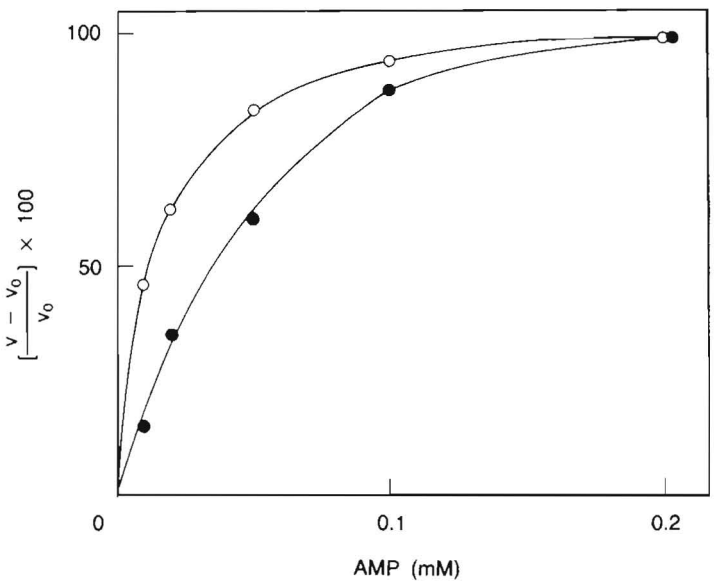


Fig. 4. The effect of adenine nucleotides on the activity of mucosal phosphofructokinase in the presence of 2.5 mM-ATP

(a) ADP (b) AMP

(○) rat (●) mouse

isoenzymes were highly activated with this metabolite, but the rat isoenzyme was only slightly more activated, whereas the extent of activation was nearly the same (95%) at 0.2 mM-glucose-1,6-bisphosphate.

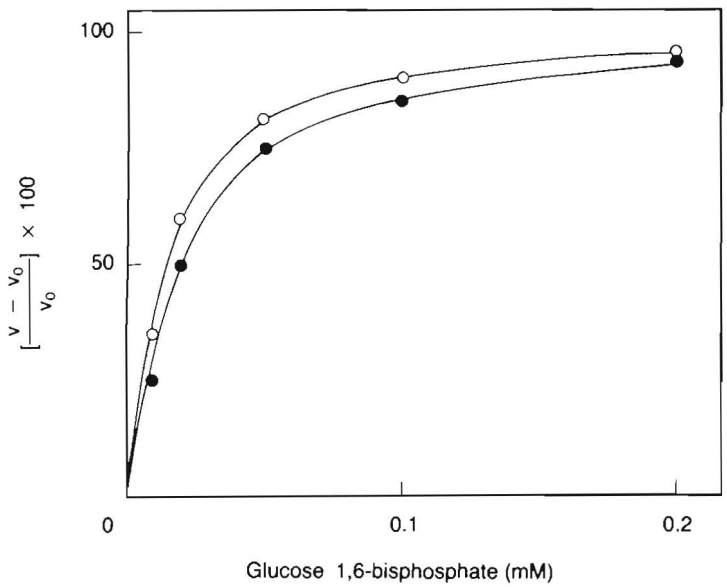


Fig. 5. The effect of glucose 1,6-bisphosphate on the activity of mucosal phosphofructokinase in the presence of 2.5 mM-ATP
(○) rat (●) mouse

Inhibitors

The inhibitors of phosphofructokinase were compared under the same conditions as for the influence of activators, that is, at 2.5 mM-ATP, 0.4 mM, and 0.5 mM fructose 6-phosphate for the rat and the mouse isoenzymes at pH 7.0 respectively. Figure 6 shows the effect of citrate and creatine phosphate on the activity of rat and mouse mucosal phosphofructokinase. The enzymes from both animals were inhibited by citrate and creatine phosphate, but the inhibition by citrate was stronger than that of creatine phosphate. However, there was not much difference in the extent of the inhibition of the enzyme activities between the rat and the mouse, so that 50% inhibition was observed at 1.1 mM and 1.3 mM-citrate respectively. It has been reported by several workers that citrate is a potent inhibitor for skeletal muscle and mucosal phosphofructokinase (Kemp 1971, Tsai

& Kemp 1974 and Khoja 1986) which is also in agreement with these results. Creatine phosphate inhibited the rat isoenzyme more than the mouse isoenzyme, so that the extent of inhibition at 2 mM-creatine phosphate was 38% and 12% for the rat and mouse isoenzymes respectively.

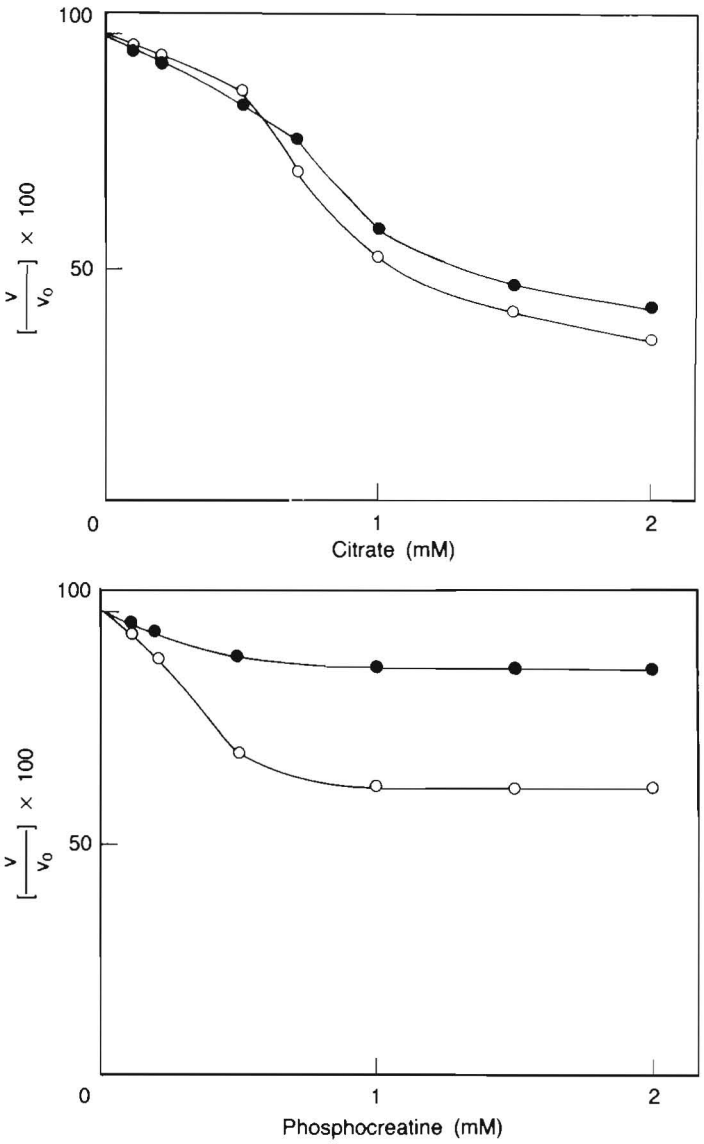


Fig. 6. The effect of (a) citrate and (b) phosphocreatine on the activity of mucosal phosphofructokinase in the presence of 2.5 mM-ATP
(○) rat (●) mouse

In general, these results show that rat and mouse mucosal isoenzymes are not greatly dissimilar in their regulatory properties. Both animals have a single isoenzyme species in intestinal mucosa (Khoja *et al.* 1983). In the rat, it has been proposed that the specific isoenzymes play significant roles in the different tissues as shown with the kinetic properties (Khoja 1986). The rat mucosal isoenzyme is more affected by inorganic phosphate and AMP as activators and was more inhibited by creatine phosphate than the mouse isoenzyme. Both isoenzymes are sensitive to inhibition by ATP and citrate. However, the occurrence of some similarities among the species in the kinetic properties as reported here is not surprising since both tissues display a high rate of aerobic glycolysis.

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الخواص الحركية لإنزيم فوسفو فركتوكاينار في غشاء الأمعاء الدقيقة المخاطي للفأر الكبير والصغير

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درست الخواص الحركية لإنزيم فوسفو فركتوكاينار من الأمعاء الدقيقة للفأر الصغير mouse، وذلك بعد تمرير الإنزيم الخاص على عمود تنقية يحتوي على مادة Sephadex G-100 وذلك لغرض فصل المواد ذات الوزن الجزيئي الصغير، ثم مقارنة هذه الخواص بمثيلاتها في الأمعاء الدقيقة للفأر الكبير rat.

أوضحت النتائج بأن الإنزيم في الحالتين يتجاوب للتفاعلات مع فركتوز ٦ ـ فوسفات عند الأس الهدروجيني ٧، وكلاهما أيضاً يثبط عند تركيزات عالية من ادينوسين ثلاثي الفوسفات. فبالرغم من وجود الجلايكوليسيس في خلايا الأمعاء الدقيقة بنسبة عالية، فإن إنزيم فوسفو فركتوكاينار في هذين الحيوانين قد تأثر بدرجة كبيرة بوساطة بعض مواد نواتج الأيض مثل، سترات، فوسفو كارياتين، ادينوسين ثنائي الفوسفات، ادينوسين أحادي الفوسفات، جلوكوز ـ ١، ٦ ـ ثنائي الفوسفات.