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Phosphofructokinase D from the epithelial cells of rat small intestine

Samir M. KHOJA and George L. KELLETT*
Department of Biology, University of York, Heslington, York YO1 5DD, U.K.

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Phosphofructokinase from the epithelial cells of rat small intestine was characterized with respect to isoenzyme type in a comparison of its properties with those of the skeletal-muscle, brain and major liver isoenzymes by using five different techniques, namely electrophoresis on cellulose acetate and in polyacrylamide gels, chromatography on DEAE-cellulose, $(NH_4)_2SO_4$ precipitation and immunotitration. When precautions were taken to inhibit the formation of active proteolytic artifacts by the action of endogenous proteinases, each technique revealed that rat intestinal mucosa contains only a single form of phosphofructokinase. The mucosal isoenzyme was found to be very similar to, although not identical with, the major liver isoenzyme and to be quite distinct from the skeletal-muscle isoenzyme when studied by the techniques of cellulose acetate electrophoresis, chromatography on DEAE-cellulose and immunotitration, whereas the converse was true when studied by the techniques of $(NH_4)_2SO_4$ precipitation and polyacrylamide-gel electrophoresis. The mucosal isoenzyme was distinct from the brain isoenzyme when studied by each of the five techniques. Tsai & Kemp [(1973) *J. Biol. Chem.* **248**, 785–792] 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. Phosphofructokinase from mucosa is distinct from each of these isoenzymes. Following the nomenclature of Tsai & Kemp (1973), the isoenzyme from the mucosa of rat intestinal epithelial cells is designated phosphofructokinase D. The mucosal and liver isoenzymes behave so similarly with respect to their charge and immunological characteristics, on which the typing of isoenzymes is conventionally based, that it is likely that some tissues reported to contain the liver isoenzyme contain instead the mucosal isoenzyme.

The existence of different molecular forms of phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) was first suggested by the observation that congenital deficiency of muscle phosphofructokinase in man is associated with partial deficiency of the enzyme activity in erythrocytes (Tarui *et al.*, 1965; Layzer *et al.*, 1967). Since then it has been firmly established that phosphofructokinase exists in isoenzymic forms in the tissues of both man and animals (Layzer *et al.*, 1969; Layzer & Conway, 1970; Taylor & Bew, 1970; Tanaka *et al.*, 1971; Kurata *et al.*, 1972; Tsai & Kemp, 1973, 1974; Layzer & Rasmussen, 1974; Kirby & Taylor, 1974; Dunaway *et al.*, 1974; Gonzalez *et al.*, 1975; Kahn *et al.*, 1975, 1979).

In animals, the different isoenzymes have been distinguished on the basis of charge, by DEAE-cellulose chromatography and cellulose acetate electrophoresis, and on the basis of immunological cross-reactivity. In this way, it has been shown that there are at least three major isoenzymes of phosphofructokinase in rabbit tissues: type A found as the sole isoenzyme in muscle, type B found as the major isoenzyme in liver, and type C found as a significant isoenzyme in brain (Tsai & Kemp, 1973, 1974). Gonzalez *et al.* (1975) have further reported that these three isoenzymes are sufficient to explain the observed distributions in the tissues of the mouse, guinea pig and rat and have ascribed bands of intermediate mobilities observed in cellulose acetate electrophoresis to the formation of hybrids between these isoenzymes.

Tanaka *et al.* (1971) have reported that, in addition to the major isoenzymes of muscle and liver, which they designate as type I (instead of A)

* To whom reprint requests should be addressed.

and type IV (instead of B), rat tissues contain two other isoenzymes, namely types II and III. Gonzalez *et al.* (1975) reconciled their observations with those of Tanaka *et al.* (1971) by suggesting that one of types II and III would prove to be identical with type C and the other to be a hybrid of type C with A or B. This suggestion remains to be established. As well as the major liver isoenzyme (B), rat liver also contains a minor isoenzyme accounting for about 15% of the total phosphofructokinase activity in liver (Dunaway *et al.*, 1974, 1978).

The A, B and C isoenzymes of rabbit display distinct regulatory properties. Thus the A isoenzyme is inhibited by phosphocreatine, whereas isoenzymes B and C are not, and B is the isoenzyme most readily inhibited by 2,3-bisphosphoglycerate. All three isoenzymes are inhibited by ATP and citrate, although C is rather less so than the others. Tsai & Kemp (1974) have proposed that these regulatory differences confer on phosphofructokinase tissue-specific diversity in its role as the principal rate-limiting enzyme of glycolysis.

We have previously studied the regulation of phosphofructokinase in the epithelial cells of the rat small intestine (Jamal & Kellett, 1983). This tissue is characterized by an exceptionally high rate of aerobic glycolysis (Hanson & Parsons, 1976; Porteous, 1978), and we have shown that purified phosphofructokinase from mucosal cells is very active at pH7.0 and not readily inhibited by ATP (Khoja *et al.*, 1983). These observations suggested that mucosal phosphofructokinase might be different from other isoenzymes. We therefore decided to characterize it further. In so doing, as well as the usual criteria of molecular charge and immunological cross-reactivity, we used two further criteria, namely solubility in $(NH_4)_2SO_4$ and electrophoresis in polyacrylamide gels. These proved to be essential in establishing that mucosal phosphofructokinase is distinct from the previously documented A, B and C isoenzymes.

The term isoenzyme is now properly reserved for forms that have been shown to be genetically distinct, as, for example, in the case of human muscle, platelet and liver isoenzymes, which are encoded by genes in chromosomes 1, 10 and 21 respectively (Weil *et al.*, 1980; Vora & Francke, 1981; Vora *et al.*, 1982). Such multigenic control does not appear to have been established for the rat. Nevertheless, it is strongly suggested by the existence of different molecular forms of phosphofructokinase in rat tissues, similar in general character to those of the human isoenzymes (Taylor & Bew, 1970; Tanaka *et al.*, 1971; Kurata *et al.*, 1972; Kirby & Taylor, 1974; Dunaway *et al.*, 1974; Gonzalez *et al.*, 1975). With this reservation in mind, the term isoenzyme is used to describe the multiple forms reported here.

Materials and methods

All biochemicals were obtained from either Sigma or Boehringer–Mannheim, and used without further purification. All chemicals were AnalaR grade from BDH.

Animals were female Wistar rats (250–300g) fed *ad lib.* on standard laboratory diet (Oxoid, modified 41B) with free access to water. Rats were killed by cervical dislocation; tissues were excised and mucosa was collected and homogenized in extraction buffer (100mM-K_2HPO_4, pH8.0) containing 30mM-KF, 1mM-EDTA, 5mM-2-mercaptoethanol, 1mM-phenylmethanesulphonyl fluoride, 1mM-6-amino-n-hexanoic acid and 0.5mg of soyabean trypsin inhibitor/ml. Tissue samples for electrophoresis were homogenized directly in electrophoresis buffer containing proteinase inhibitors. Tissue homogenates were then centrifuged for 30min at 75000 *g* and the supernatants, referred to subsequently as crude extract, used for experiments.

Cellulose acetate electrophoresis was carried out at 4°C on strips (5.7cm × 14.0cm) of Whatman Cellogel in buffer (5mM-Tris/phosphate, pH8.0) containing 5mM-$(NH_4)_2SO_4$, 0.1mM-ATP, 5mM-2-mercaptoethanol and 0.1mM-EDTA. Electrophoresis of enzyme samples (5–10μl, 1–16units/ml) was performed at 300V (2mA/strip) for 2h, after which enzyme activity was detected by use of the agar-gel staining technique described by Kemp (1971). Bands of stain appeared both in the agar gel and on the surface of the Cellogel.

Polyacrylamide-gel electrophoresis was carried out in vertical slabs (170mm × 150mm × 1mm) of 5% acrylamide in the same buffer system as for cellulose acetate electrophoresis. Gels were first pre-equilibrated against buffers for 2h and then electrophoresis of the enzyme samples (5–15μl, 1–5units/ml) was performed at 4°C for 5h at 30mA per gel. The gels were stained for phosphofructokinase activity by the method of Kemp (1971) as modified by Kahn *et al.* (1979).

$(NH_4)_2SO_4$ fractionation was performed by the addition of the appropriate weight of solid $(NH_4)_2SO_4$ to the crude extract, and the protein precipitates were collected by centrifugation for 30min at 75000*g* after the solutions had been left for 2h. For the determination of $(NH_4)_2SO_4$-precipitation curves, protein fractions were solubilized in extraction buffer. The crude extract, the supernatant and the solubilized precipitate were then assayed, and the recovery of phosphofructokinase activity was 100% within experimental error at each $(NH_4)_2SO_4$ saturation used. When the $(NH_4)_2SO_4$ fraction was to be used for chromatography, the precipitate was solubilized in 25mM-Tris/phosphate buffer, pH8, containing 5mM-$(NH_4)_2SO_4$, 1mM-EDTA, 5mM-2-mercaptoethanol and 1mM-phenylmethanesulphonyl fluoride, dialysed overnight, and

the solution was then applied to a column (25 cm × 1.5 cm) of Whatman DE52 DEAE-cellulose equilibrated with the same buffer. Phosphofructokinase activity was eluted with a gradient of 5–200 mM-$(NH_4)_2SO_4$ established by two reservoirs each of 3 column volumes.

Rat skeletal-muscle phosphofructokinase was purified to homogeneity as follows. Frozen muscle was extracted in 50 mM-Tris/phosphate buffer, pH 8.0, containing 30 mM-KF, 1 mM-EDTA, 5 mM-2-mercaptoethanol and 100 mM-$(NH_4)_2SO_4$, and the homogenate was centrifuged at 75000 *g* for 30 min. The pellet obtained by fractionation of the resulting supernatant with $(NH_4)_2SO_4$ from 40 to 60% saturation was resuspended in 100 mM-Tris/phosphate buffer, pH 8.0, containing 0.2 mM-fructose 1,6-bisphosphate, 30 mM-KF, 1 mM-EDTA and 2 mM-dithiothreitol (buffer A) and heated to 59°C for 5 min. After centrifugation, solid $(NH_4)_2SO_4$ was added to the supernatant to 60% saturation to precipitate phosphofructokinase. The resulting pellet was resuspended in buffer A and chromatographed on a column (58 cm × 2.2 cm) of Bio-Gel A-1.5 m equilibrated with the same buffer. Fractions containing phosphofructokinase activity were pooled and applied directly to a column (8 cm × 3.5 cm) of DEAE-cellulose pre-equilibrated with buffer A. The column was washed with this starting buffer until the A_{280} of the washings had returned to zero. Phosphofructokinase was then eluted as homogeneous enzyme with buffer A containing instead 300 mM-Tris/phosphate. The preparation proceeded with 60–70% yield to produce a product of specific activity about 220 units/mg, that was stable for several weeks as a suspension in 60%-satd. $(NH_4)_2SO_4$ in the presence of 2 mM-ATP at 4°C. The major isoenzyme of rat liver phosphofructokinase was partially purified to give a product of specific activity about 50 units/mg of protein as described by Kemp (1971).

Antibody to purified enzyme was prepared as follows. Enzyme (0.5 mg) was resuspended in 1 ml of 50 mM-Tris/phosphate buffer, pH 8.0, containing 0.2 mM-fructose 1,6-bisphosphate, 1 mM-dithiothreitol and 1 mM-EDTA. The enzyme solution was mixed with an equal volume of Freund's complete adjuvant and then injected subcutaneously into a rabbit at four separate sites in the neck and flank. The dose was repeated with Freund's incomplete adjuvant at 7, 14 and 28 days. Serum prepared from blood collected at 31 days was stored in a deep-freeze at −80°C and used as antibody without further purification when required.

Immunotitration of enzyme activity was done by incubating 0.10 ml of tissue extract (activity 0.15 unit/ml) with antiserum and extraction buffer in a total volume of 0.20 ml contained in polycarbonate centrifuge tubes for 30 min at room temperature (approx. 20°C). The samples were then centrifuged at 75000 *g* for 30 min, and the phosphofructokinase activity remaining in solution was assayed at pH 8.0 and 27°C as described by Ling *et al.* (1965). One unit of activity is defined as the formation of 1 μmol of fructose 1,6-bisphosphate/min.

Results

We have previously shown that the mucosa of the rat small intestine contains only a single form of phosphofructokinase (Khoja *et al.*, 1983). However, the mucosal form is subject to proteolysis by endogenous proteinases, giving rise to two active products that can be readily distinguished from native enzyme and from each other by cellulose acetate electrophoresis or chromatography on DEAE-cellulose. All studies of mucosal phosphofructokinase were therefore performed reasonably quickly on crude extracts derived from homogenates of mucosa prepared in the presence of proteinase inhibitors as described above. In these circumstances, proteolysis was inhibited and the results were not obfuscated by the presence of active proteolytic artifacts. Because of the use of crude extract, experiments were performed not only with single extracts, but also with mixtures of the mucosal extract with each of the others; each mixture was prepared on a 1:1 basis so it contained the same number of units of phosphofructokinase activity/ml from each of the pair of extracts. No significant difference was observed between the behaviour of any of the isoenzymes in a single extract or in a mixture of extracts. The differences in physical properties were therefore not caused by secondary factors such as protein concentration or interactions with contaminating proteins.

Fig. 1 compares the behaviour on cellulose acetate electrophoresis of phosphofructokinase in a crude mucosal extract (*b*) with that in crude extracts of liver (*a*), brain (*d*) and skeletal muscle (*e*). The single band obtained with the mucosal extract confirms

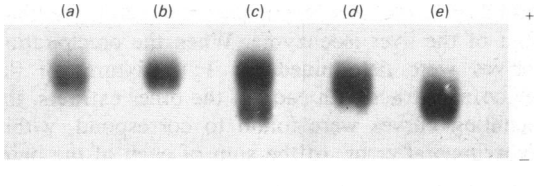

Fig. 1. *Cellulose acetate electrophoresis of phosphofructokinase isoenzymes in crude tissue extracts*
(*a*) Liver, 0.9 unit/ml; (*b*) mucosa, 2.0 units/ml; (*c*) 1:1 mixture of mucosa and muscle, total activity 1.4 units/ml; (*d*) brain, 8 units/ml; (*e*) muscle, 16 units/ml. For experimental details see the text. One-fifth of the strip is shown.

that proteolysis was inhibited under the selected extraction conditions and that mucosa contains only a single isoenzyme. Gonzalez *et al.* (1975) have studied the distribution of phosphofructokinase isoenzymes in the tissues of different species. They reported that in each of the species the liver isoenzyme is the most anodic and the skeletal-muscle isoenzyme is the least anodic isoenzyme and that the difference in their mobilities is less for the rat than for any other species. Fig. 1 confirms that the difference is indeed small. Surprisingly, however, the mucosal form proved to be slightly more anodic than the liver isoenzyme, so that the order of the relative mobilities of the different isoenzymes were mucosa (1.0) > liver > brain > muscle (0.9). This order was consistent, although the difference between individual isoenzymes was sometimes hard to measure. When a 1:1 mixture of the mucosal and muscle extracts was subjected to electrophoresis, no change in the relative mobility of the isoenzymes was observed (Fig. 1, lane *c*). The same was true for other combinations of the mucosal with each of the other two isoenzymes (results not shown).

The $(NH_4)_2SO_4$-precipitation curves of phosphofructokinase activity from crude extracts of liver, mucosa, brain and skeletal muscle are presented in Fig. 2. The precipitation curves of the mucosal and muscle isoenzymes are monophasic and identical within experimental error, both isoenzymes being almost completely precipitated between 45 and 55% saturation with $(NH_4)_2SO_4$. The precipitation curve for the brain isoenzyme is also monophasic, with precipitation occurring at a slightly lower saturation, between 40 and 50%. In contrast, the precipitation curve of phosphofructokinase activity from liver extracts is biphasic, in agreement with the report by Dunaway *et al.* (1974). About 75% of the total activity is precipitated between 30 and 40% saturation and the remainder between 45 and 55% saturation. The former phase corresponds to the major isoenzyme of liver (used in all other experiments described here) and the latter to the minor isoenzyme. Interestingly, Dunaway *et al.* (1981) have shown that the minor isoenzyme migrates on electrophoresis in agarose gels with the same mobility as the muscle isoenzyme, which is less than that of the liver isoenzyme. When the precipitation curves were determined for 1:1 mixtures of the mucosal extract with each of the other extracts, the resulting curves were found to correspond, within experimental error, to the sum of each of the pairs determined individually (Fig. 2*b*).

The elution profiles of the major liver, mucosal, brain and skeletal muscle forms of phosphofructokinase and DEAE-cellulose chromatography are compared in Fig. 3(*a*). The major liver isoenzyme was only partially purified by heat treatment of crude extract (Kemp, 1971) and $(NH_4)_2SO_4$ fractionation between 30 and 40% saturation (Fig. 2), whereas the muscle isoenzyme was obtained by $(NH_4)_2SO_4$ fractionation between 45 and 55% saturation; as before, crude extract was used for mucosal and brain phosphofructokinase. Samples were eluted from the DEAE-cellulose column with a gradient of 5–200 mM-$(NH_4)_2SO_4$ and, for the mucosal enzyme, chromatography was performed in the presence of 1 mM-phenylmethanesulphonyl fluoride. As expected on the basis of the previous results, each isoenzyme was eluted as a single peak of activity and the muscle isoenzyme, which was the least anodic enzyme on cellulose acetate electro-

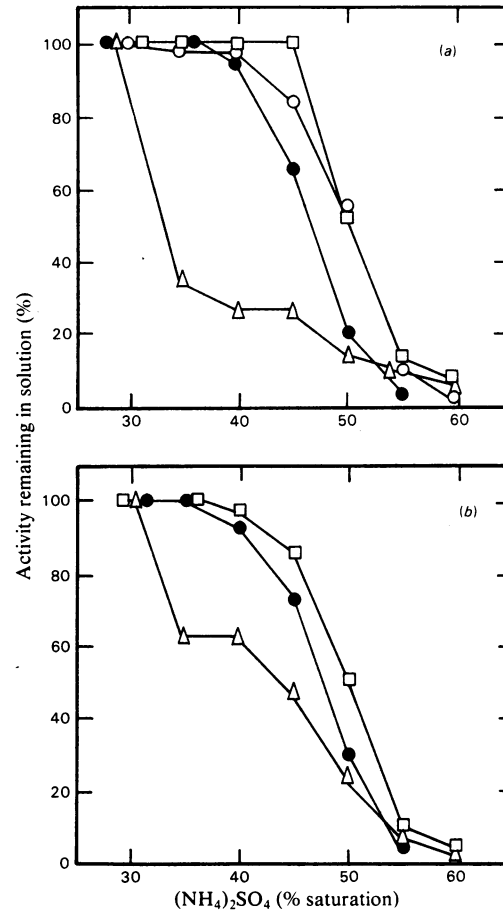

Fig. 2. *$(NH_4)_2SO_4$-precipitation curves of phosphofructokinase activity from crude tissue extracts*
(*a*) ○, Mucosa (2.5 units/ml); □, skeletal muscle (19.2 units/ml); ●, brain (3.5 units/ml); △, liver (0.8 unit/ml). (*b*) 1:1 mixtures of extracts of: □, mucosa and muscle (total activity 3.7 units/ml); ●, mucosa and brain (total activity 2.8 units/ml); △, mucosa and liver (total activity 1.1 units/ml). For experimental details see the text.

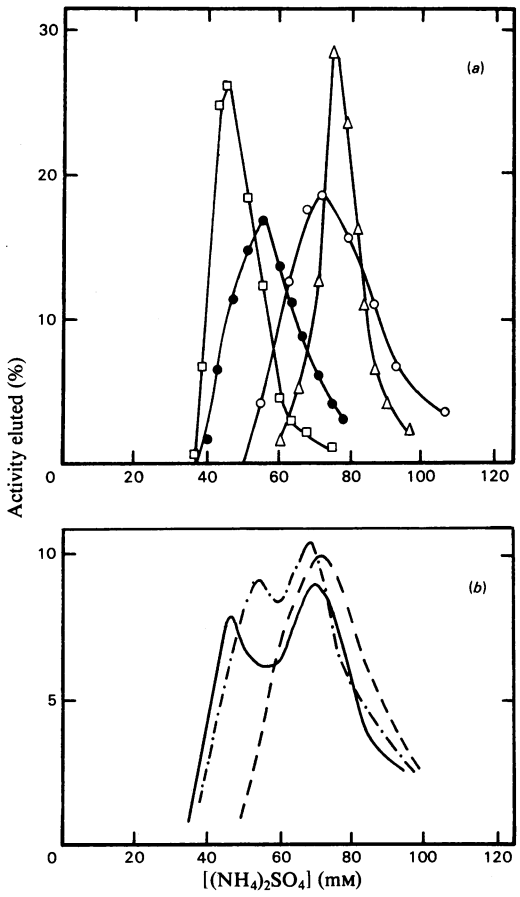

Fig. 3. *Chromatography of phosphofructokinase isoenzymes on DEAE-cellulose*

(*a*) O, Mucosa, crude extract (3.1 units/ml); □, skeletal muscle, $(NH_4)_2SO_4$ fraction (50 units/ml); ●, brain, crude extract (8 units/ml); △, liver, $(NH_4)_2SO_4$ fraction (20 units/ml). (*b*) 1:1 mixtures of crude extracts of: ——, mucosa and muscle (total activity 5.5 units/ml); –·–·–, mucosa and brain (total activity 4.5 units/ml); – – – –, mucosa and liver (total activity 1.5 units/ml). The experimental points have been omitted for the sake of clarity. For experimental details see the text.

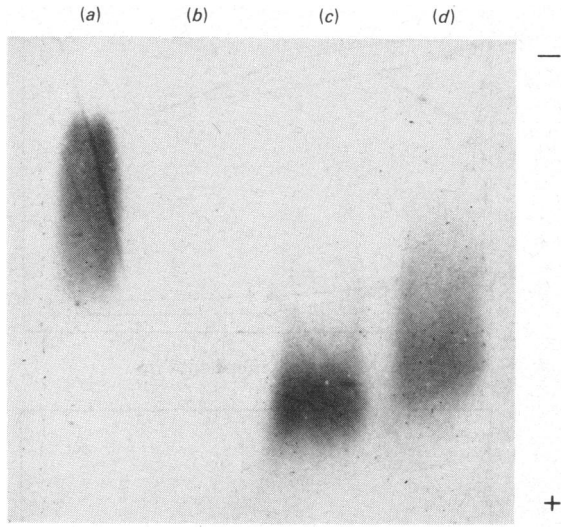

Fig. 4. *Electrophoresis of the mucosal, skeletal-muscle and major liver isoenzymes of phosphofructokinase in 5%-polyacrylamide gels*

(*a*) Skeletal muscle, purified isoenzyme; (*b*) liver, purified isoenzyme (the enzyme does not penetrate the gel); (*c*) mucosa, crude extract; (*d*) brain, crude extract. For experimental details see the text. One-half of the strip is shown.

phoresis, was eluted first at a concentration of 46 mM-$(NH_4)_2SO_4$, followed in turn by phosphofructokinase from brain, mucosa and liver at 55 mM, 72 mM and 76 mM respectively (Fig. 3*a*). Elution profiles were also determined for 1:1 mixtures of crude mucosal extract with crude extracts of skeletal muscle, brain and liver (Fig. 3*b*). In each instance the elution profile of a given mixture corresponded to the sum of the profiles for each component separately. (In comparing the elution profiles of the partially purified major liver isoenzyme with the mixture of liver and mucosal extracts, allowance should be made for the fact that the mixture contains 8–10% of activity as the minor liver isoenzyme. The latter is eluted before the muscle isoenzyme and is not shown.)

Gel electrophoresis of the phosphofructokinase isoenzymes was performed in 5% polyacrylamide in a buffer containing 0.1 mM-ATP. In the presence of ATP, the muscle enzyme is stabilized as a tetramer rather than as an aggregate (Lad *et al.*, 1973; Liddle *et al.*, 1977). In contrast, the major liver phosphofructokinase remains aggregated. Hence, Fig. 4 shows that the liver isoenzyme does not penetrate the gel (lane *b*), whereas the muscle isoenzyme migrates into the gel easily (lane *a*), so confirming the observations by Brand & Söling (1974) and also by Kahn *et al.* (1979). The major liver isoenzyme was found not to enter the gel, irrespective of whether partially purified enzyme or crude liver extract was used (results not shown).

The relative mobilities of the mucosal and brain enzymes, which also penetrate the gel, and that of the muscle isoenzyme were determined to be 1.0, 0.85 and 0.55 respectively. Their order is similar to that observed in cellulose acetate electrophoresis, implying that mucosal and brain enzymes are also tetramers under the conditions of electrophoresis.

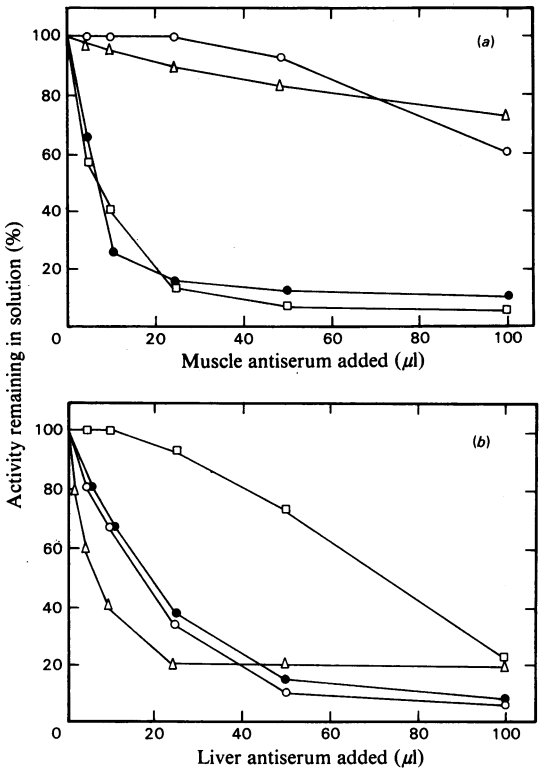

Fig. 5. *Immunotitration of the mucosal, skeletal-muscle and major liver isoenzymes of phosphofructokinase in crude tissue extracts with antisera raised in rabbit to (a) purified rat skeletal-muscle phosphofructokinase and (b) purified major liver phosphofructokinase*

Isoenzyme: O, mucosa; □, skeletal muscle; ●, brain; △, liver. For experimental details see the text.

The behaviour of the mucosal enzyme is in agreement with our previous observation that, in polyacrylamide gradient gels under identical conditions, the mucosal enzyme is indeed a stable tetramer (Khoja *et al.*, 1983).

Immunotitration studies of phosphofructokinase activity present in crude rat tissue extracts were performed with antisera raised in rabbits against either the purified major liver isoenzyme or the purified skeletal-muscle isoenzyme of rat. Although the antiserum to the muscle isoenzyme was, as expected, strongly cross-reactive with the muscle isoenzyme, it was almost as cross-reactive with the brain isoenzyme. However, it displayed very little cross-reactivity with either the liver or mucosal forms of phosphofructokinase (Fig. 5*a*). Antiserum to the major liver isoenzyme precipitated about 80% of the total phosphofructokinase activity from the crude liver extract (Fig. 5*b*), leaving about 20% of the activity corresponding to the minor liver isoenzyme in solution, as observed by Dunaway *et al.* (1974). The liver antiserum also strongly cross-reacted with the mucosal enzyme; indeed liver antiserum was able to precipitate some 95% of the total mucosal phosphofructokinase activity and was almost, though not quite, as effective as against the major liver isoenzyme. The same was true for the brain isoenzyme. In contrast, cross-reactivity of antiserum to the liver isoenzyme with the muscle isoenzyme was relatively weak. We have not raised antibody to the rat mucosal enzyme because the latter is relatively susceptible to proteolysis when purified.

Discussion

We have previously reported that rat intestinal mucosa contains only a single isoenzyme of phosphofructokinase (Khoja *et al.*, 1983). This conclusion is supported here by the results obtained with five different techniques. Thus the phosphofructokinase activity of a crude mucosal extract prepared in the presence of proteinase inhibitors migrated as a single band on cellulose acetate and in polyacrylamide gels, was eluted as a single peak from DEAE-cellulose, displayed by a sharp $(NH_4)_2SO_4$-precipitation curve and was completely precipitated by antiserum raised against the major liver isoenzyme in a single phase.

It has been conventional practice to characterize isoenzymes on the basis of their charge properties, by cellulose acetate electrophoresis and DEAE-cellulose chromatography, and immunological properties. On the basis of these criteria, mucosal phosphofructokinase appears to be very similar to, though not completely identical with, the major phosphofructokinase isoenzyme of liver. Thus in cellulose acetate electrophoresis the mucosal isoenzyme migrates very slightly ahead of the liver isoenzyme (Fig. 1), whereas on DEAE-cellulose chromatography the mucosal isoenzyme is eluted at a slightly lower $(NH_4)_2SO_4$ concentration than the liver isoenzyme (Fig. 3). Furthermore, the muscle and brain isoenzymes are clearly distinct from the other two by either technique. Similarly, in immunotitration studies, the mucosal isoenzyme cross-reacts only weakly with antiserum to the muscle isoenzyme, but cross-reacts almost, though not quite, as strongly with antiserum to the major liver isoenzyme as the antiserum does to the liver isoenzyme itself, whereas the brain isoenzyme cross-reacts strongly with both antisera. It would therefore be easy to conclude that mucosa contains the major liver isoenzyme.

That this is not the case is demonstrated by the use of two further criteria, namely solubility in $(NH_4)_2SO_4$ and polyacrylamide-gel electrophoresis. The former was originally used by Dunaway *et al.*

(1974) to distinguish between the liver and muscle isoenzymes. We have confirmed their observations and have shown further that by this criterion the mucosal isoenzyme is indistinguishable from the muscle isoenzyme, is similar to, though distinguishable from, the brain isoenzyme and is completely different from the liver isoenzyme (Fig. 2).

Similarly, polyacrylamide-gel electrophoresis is able to distinguish between the mucosal and major liver isoenzymes immediately, for the latter cannot penetrate 5%-polyacrylamide gels, whereas the former can (Fig. 4). In addition, polyacrylamide-gel electrophoresis also readily distinguishes the mucosal from the brain and muscle isoenzymes. The relative mobilities of the bands of phosphofructokinase activity from mucosa, brain and skeletal muscle were 1.0, 0.85 and 0.55 respectively. The mobility difference between the mucosal and muscle isoenzymes in gels is much greater than on cellulose acetate (1.0 and 0.90 respectively) in the same buffer system. Thus their relative mobility in polyacrylamide-gel electrophoresis depends not only on the difference in their individual charges but also on the difference in steric hindrance experienced by each isoenzyme when migrating in the gel. The great resolving power of polyacrylamide-gel electrophoresis makes it a valuable system for distinguishing between phosphofructokinase isoenzymes, and has been exploited by Kahn *et al.* (1979) to identify the F_4 isoenzyme present in human tissues.

Tsai & Kemp (1973) have previously reported that animal tissues contain three major isoenzymes of phosphofructokinase, those of skeletal muscle (A), liver (B) and brain (C). From the data presented above it is clear that the mucosal isoenzyme represents a further isoenzyme type that has not been previously recognized. Following the nomenclature adopted for phosphofructokinase by Tsai & Kemp (1973), the mucosal isoenzyme will be termed D. As noted earlier, the typing of isoenzymes is often performed solely on the basis of charge and immunological characteristics, in which respects the mucosal and major liver isoenzymes are very similar. It is therefore likely that the presence of the liver isoenzyme has been incorrectly assigned in the literature to some tissues which contain instead the mucosal isoenzyme.

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