Glucose-6-phosphatase in normal adult human intestinal mucosa

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1. The existence of specific glucose-6-phosphatase activity in human intestinal mucosa has been somewhat controversial.
2. We have demonstrated the presence of low levels of specific glucose-6-phosphatase activity in normal human adult intestinal mucosa. Activity was found in oesophagus, stomach, duodenum and colon.
3. Immunoblot analysis using antibodies monospecific for the 36.5 kDa liver glucose-6-phosphatase catalytic subunit demonstrated that intestinal mucosa contains low levels of the glucose-6-phosphatase enzyme protein.
4. The low levels of activity together with problems of proteolysis make human intestinal biopsies unsuitable for use in the diagnosis of type 1 glycogen-storage disease.

INTRODUCTION

The liver plays an important role in the regulation of blood glucose homeostasis [1, 2]. Whenever blood glucose levels fall, the liver releases glucose into the bloodstream for use by other tissues which cannot make significant levels of glucose (e.g. brain) (Fig. 1). The two pathways by which glucose is made in the liver are glycogenolysis and gluconeogenesis (Fig. 1). The enzyme glucose-6-phosphatase (EC 3.1.3.9) catalyses the terminal step of both pathways (Fig. 1) [1, 2]. The importance of hepatic glucose-6-phosphatase in the maintenance of blood glucose levels first became obvious from the studies of patients with type 1 glycogen-storage disease (glucose-6-phosphatase deficiency or Von Gierke’s disease) [3–5]. The microsomal glucose-6-phosphatase system has been unequivocally demonstrated to be present also in kidney [6], pancreatic islets [7] and gall bladder [8], although its role in these tissues is not so clear.

Glucose-6-phosphatase is multicomponent and consists of a catalytic subunit with its active site inside the lumen of the endoplasmic reticulum, a regulatory protein and three transport systems which allow the substrates and products of the enzyme (glucose 6-phosphate, phosphate and glucose) to cross the endoplasmic reticulum membrane [3–5] (Fig. 1). A deficiency of any of the protein components of hepatic glucose-6-phosphatase will impair both hepatic glucose output and glucose-6-phosphatase activity and result in type 1a, 1b, 1c or 1d glycogen-storage disease [3–5]. Recently, it has been realized that there is an association between type 1b glycogen-storage disease and Crohn’s-like colitis [9, 10], which suggests the possibility that there might also be a specific glucose-6-phosphatase in intestinal mucosa.

This possibility is further supported by a number of reports of histochemical detection of glucose-6-phosphatase activity in the intestine of mouse and rat [11–14] and reports of biochemical detection of low levels of glucose-6-phosphatase activity in the...
intestinal of rat, mouse, rabbit, guinea pig, chicken and man [15–20]. Unfortunately, however, these reports all failed to unequivocally establish whether the activity measured was due to the presence of the microsomal glucose-6-phosphatase system or merely due to the non-specific hydrolysis of glucose 6-phosphate by other non-specific phosphatases. In addition, there have also been contrasting reports that glucose-6-phosphatase is not present in rat intestine [19, 21, 22] or human intestine [22].

We have therefore used microassay techniques, together with monospecific antibodies to the catalytic subunit of liver microsomal glucose-6-phosphatase [6], to determine unequivocally whether specific microsomal glucose-6-phosphatase is present in normal human intestinal mucosa.

MATERIALS AND METHODS

Materials

The monosodium salt of glucose 6-phosphate was obtained from BDH Chemicals, Poole, Dorset, U.K. Phenylmethylsulphonyl fluoride (PMSF), trypsin–chymotrypsin inhibitor, 1,10-o-phenanthroline, pepstatin A, streptavidin/horseradish peroxidase complex, histone 2A and 4-chloro-1-naphthol were purchased from Sigma Chemicals, Poole, Dorset, U.K. The cocktail of protease inhibitors [containing 50 µg of antipain dihydrochloride/ml, 40 µg of PMSF/ml, 10 µg of aprotinin/ml, 40 µg of bestatin/ml, 100 µg of chymostatin/ml, 0.5 mg of E-64/ml, 0.5 mg of EDTA (di-sodium salt)/ml, 0.5 µg of leupeptin/ml, 0.7 µg of pepstatin/ml and 330 µg of phosphoramidon/ml] used to thaw the fast-frozen mucosal samples was obtained from Boehringer Mannheim, London, U.K. Nitrocellulose was purchased from Schleicher and Schuell, Dassel, Germany, and biotin-labelled secondary antibody was obtained from Amersham International, Amersham, Bucks, U.K. All other reagents were of Analar grade or better.

Patients

Patients attending the Department of Clinical Measurement, Ninewells Hospital and Medical School, Dundee, for routine endoscopy were asked to give informed consent for four pinch biopsies of endoscopically normal mucosa to be taken from a single area of gut. No individual was sampled from more than one site or on more than one occasion. No patient with malignant disease of contra-indication to biopsy was approached. No complications other than mild surface bleeding were observed. The study was approved by the Tayside Health Board Committee on Medical Ethics.

Samples

Pinch mucosal biopsies (wet weight 30–40 mg) were treated in one of two ways. Biopsies used for measuring specific glucose-6-phosphatase activity were placed immediately into ice-cold 0.25 mol/l sucrose/5 mmol/l 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (Hepes), pH 7.4 (SH buffer) containing 10 mmol/l 1,10-o-phenanthroline, 0.02% PMSF, 0.01 mg of trypsin/chymotrypsin inhibitor/ml and 0.1 mmol/l pepstatin A. The samples were quickly homogenized by hand in a Jencons glass/teflon homogenizer in SH buffer containing protease inhibitors to a final volume of 10 times the original wet weight of tissue before being centrifuged for 1 min at 10,000g. The supernatant was separated immediately for rapid assay (see below). Biopsies for Western immunoblot analysis were wrapped in aluminium foil and frozen in liquid nitrogen immediately on being taken and were stored at −70°C. The samples were thawed in SH buffer containing the commercially available ‘cocktail’ of protease inhibitors described above and were homogenized rapidly by hand in a Jencons glass/teflon homogenizer to a final volume of 10 times the original frozen weight. Aliquots of homogenate were immediately boiled for 5 min in sample buffer containing 2% (w/v) SDS and 1 mol/l mercaptoethanol.

Assays

Glucose-6-phosphatase activity was measured as previously described [23] using 30 mmol/l glucose 6-phosphate as substrate. Specific glucose-6-phosphatase activity was calculated as nmol of P_i produced min⁻¹ mg⁻¹ of protein.

Non-specific phosphatase activity was measured by pretreatment of the sample of supernatant at pH 5 and 37°C for 15 min to inactivate the glucose-6-phosphatase. The pH was returned to 6.5 with 0.4 mol/l Hepes before assay with 30 mmol/l glucose 6-phosphate as substrate as described above. The non-specific phosphatase assay is based on the observation that incubation under the conditions used completely destroys the ability of the specific glucose-6-phosphatase enzyme to hydrolyze glucose 6-phosphate without any loss of non-specific phosphatase activities (for a recent review, see [4]).

Protein concentrations were measured by the method of Lowry et al. as modified by Peterson [24] using BSA as standard.

SDS/PAGE and immunoblot analysis

Mucosal homogenates were subjected to electrophoresis on 7–16% SDS/polyacrylamide gels as described by Laemmli [25] to separate proteins. The separated proteins were then transferred onto nitrocellulose by the method of Towbin et al. [26] using an LKB semi-dry blot apparatus and were detected with sheep antibodies previously shown to be monospecific for the catalytic subunit of glucose-6-phosphatase [6, 7]. A biotin–streptavidin/horseradish peroxidase detection system was used with 4-chloro-1-naphthol/H_2O_2 as substrate.
RESULTS

The specific glucose-6-phosphatase activities measured in mucosal samples from 20 individuals are shown in Fig. 2. The non-specific phosphatase activity measurements which were subtracted from the total phosphatase activities (see the Methods section) for each site were in the ranges: oesophagus, 0–30 nmol min\(^{-1}\) mg\(^{-1}\) of protein; stomach 0–130 nmol min\(^{-1}\) mg\(^{-1}\) of protein; duodenum, 5–10 nmol min\(^{-1}\) mg\(^{-1}\) of protein; ascending colon, 0–6.2 nmol min\(^{-1}\) mg\(^{-1}\) of protein; descending colon, 0–100 nmol min\(^{-1}\) mg\(^{-1}\) of protein. Median values were 0, 2.5, 6.9, 2.8 and 2.0 nmol min\(^{-1}\) mg\(^{-1}\) of protein, respectively.

The level of specific glucose-6-phosphatase activity found in the mucosal samples was variable. In all cases the level of activity in the gut mucosa was much lower than the average specific activity of 490 nmol min\(^{-1}\) mg\(^{-1}\) of microsomal protein previously reported for the glucose-6-phosphatase enzyme in control adult human liver [27], but it was in the same range as that previously found in human gall bladder mucosa [8].

Microsomes isolated from rat liver and human gut mucosa samples were subjected to immunoblot analysis (see Figs. 3 and 4) using a sheep antihepatic microsomal glucose-6-phosphatase antiserum previously shown to be monospecific for the 36,500 Da polypeptide which contains the active site of glucose-6-phosphatase [6, 7]. Fig. 3 (lanes 1–8) and Fig. 4 (lane 4) show that oesophagus, stomach, duodenum and colon mucosa all contain the liver-specific glucose-6-phosphatase protein. However, the most common result with gut mucosa was the...
appearance of smaller-molecular-mass immuno-reactive proteolytic breakdown products of the glucose-6-phosphatase protein (Fig. 4, lanes 2, 3, 6, 7 and 8) or occasionally no immuno-reactive peptides in badly degraded samples (e.g. Fig. 4, lanes 1 and 5) even in the presence of 'cocktails' of protease inhibitors.

**DISCUSSION**

The role of the liver glucose-6-phosphatase enzyme in hepatic glucose production has been understood since the 1950s (for an early review, see [2]). In contrast, the role of glucose-6-phosphatase in other tissues (e.g. pancreatic islet β-cells and intestinal mucosa) is much less clear. The importance of glucose production by the intestinal mucosa has not been well defined nor have differences in glucose metabolism in the various regions of the gut been clearly established. In intestinal mucosa, glutamine has been shown to be a quantitatively more important fuel than glucose [28]. Intestinal mucosa has been shown to contain several of the enzymes of gluconeogenesis [29, 30], and both gluconeogenesis and intestinal glucose production increase during starvation [17, 31]. Glycogen levels in intestinal mucosa have also been shown to vary depending on the metabolic state of the animal studied [32], but the glycolytic enzymes of intestinal mucosa have not been extensively investigated. These studies suggested that glucose-6-phosphatase must be present in intestinal mucosa as glucose-6-phosphatase is the terminal step of gluconeogenesis (Fig. 1).

Enzymic analysis of glucose-6-phosphatase activity in adult human intestinal biopsy samples showed that there are low levels of specific glucose-6-phosphatase activity in all the areas of intestine that were tested (Fig. 2). However, the activities measured were very variable and unstable (in a time-dependent manner), as indeed were the non-specific phosphatase levels that we measured. Immunoblot analysis (Fig. 3) confirmed that the 36.5 kDa specific glucose-6-phosphatase catalytic subunit is present in intestinal mucosa. It also revealed evidence of proteolysis in the majority of biopsies (Fig. 4) even when a variety of 'cocktails' of protease inhibitors were used to inhibit proteolysis. The proteolysis that occurs in human intestinal biopsy samples is the most likely explanation for the variable measurements that we obtained and for the controversy in the literature concerning whether glucose-6-phosphatase is present or absent in human intestinal mucosa, e.g. [17, 20]. The low activity and proteolysis also make it difficult to carry out metabolic studies of glucose-6-phosphatase in intestinal mucosa.

The current methods of unequivocally diagnosing glucose-6-phosphatase deficiencies (glycogen-storage disease types 1a, 1aSP, 1b, 1c and 1d) all rely on enzymic analysis of glucose-6-phosphatase activity in liver biopsy samples (for recent reviews see [3–5]). The presence of the specific glucose-6-phosphatase system in normal human intestinal mucosa would potentially allow diagnosis of the type I glycogen-storage diseases using intestinal biopsies rather than the more invasive liver wedge or needle biopsies, and it has been suggested that it is possible to diagnose type I glycogen-storage disease using intestinal biopsies [19, 20]. However, unfortunately, the high levels of glucose-6-phosphatase proteolysis and low levels of activity in human intestinal mucosa mean that intestinal biopsies cannot be used to unequivocally diagnose genetic deficiencies of the glucose-6-phosphatase system because the most common cause of low or absent activity is proteolysis not glycogen-storage disease.

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**REFERENCES**

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