Evidence for the induction and release of pancreatic folylpolyglutamate hydrolase by the ingestion of folyl polyglutamates in the rat

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Abstract. Pteroyl polyglutamate hydrolase (folyl conjugase), which hydrolyses the dietary polyglutamyl folates into simple forms prior to absorption, has been shown to be induced in rat pancreas in response to dietary polyglutamyl folates but not by ingestion of synthetic unconjugated folates. Folate absorption, as measured by the rise in serum folate levels after ingestion, of dietary conjugated folates is impaired in pancreatectomised animals whereas absorption of synthetic simple folate is not. A severe build-up of folyl conjugase is observed in the lumen of control but not in pancreatectomised animals after dietary folate ingestion. These results taken together would suggest that dietary folates are hydrolysed to monoglutamyl forms suitable for absorption in the lumen; the hydrolysis is catalysed by a luminal folyl conjugase of pancreatic origin induced by dietary conjugated folates.
Key words. Folyl conjugase; polyglutamyl folate; intestinal absorption; pancreatic induction; acid microenvironment.

Naturally occurring dietary folates are mainly conjugated forms containing a mixture of polyglutamyl folates with 3–7 γ-linked glutamate residues[1]. These polyglutamyl folates have to be hydrolysed to simple monoglutamyl forms prior to intestinal absorption[2]. The enzyme folyl polyglutamate hydrolase (EC 3.4.12.10), commonly referred to as folyl conjugase, breaks down the dietary polyglutamyl folates to simple monoglutamyl forms suitable for absorption. Though folyl conjugase has been isolated and characterised from various sources such as liver[3], plasma[4], intestine[5,6] and other tissues[7,8] in different species, the origin of the intestinal folyl conjugase that hydrolyses dietary folates prior to absorption, and the site of hydrolysis, have been controversial. Various reports supported the concept that the digestion of dietary folates is an intestinal function and that the hydrolytic process occurred in the enterocyte[9–11]. We have reported earlier the possible involvement of a folyl conjugase of pancreatic origin in the hydrolysis of dietary folates prior to absorption in the rat[12]. This report provides evidence for the role of dietary folyl polyglutamates in the pancreatic induction of the folyl conjugase which is responsible for their hydrolysis before absorption.

Materials and methods

Male Wistar albino rats weighing 150–170 g were used throughout the study. The pancreas was removed at various time intervals after ingestion of yeast-conjugated folates (40 μg/kg b.wt), a 10% (w/v) homogate was prepared in 0.25 M sucrose and folyl conjugase activity was determined (ng folate released/mg protein/15 min). The reaction system, optimized as described earlier[12], contained 100 ng of *Lactobacillus casei* active conjugated folates (extracted from Torula yeast) as substrate in 0.1 M acetate buffer, pH 4.2, and 0.2–0.3 mg enzyme protein in a total volume of 1.0 ml. The reaction was initiated by adding the enzyme and was carried out at 37 °C for 15 min. Then, 0.2 ml of 10% ascorbate pH 6.0 was added and the reaction was terminated by heating the incubation mixture for 5 min in a boiling water bath. The final volume was made to 2.0 ml. The amount of unconjugated folates released was taken as a measure of the enzyme activity.

In other experiments, the pancreas was removed through a small opening made in the abdominal region on the ventral side of the animal under mild anaesthesia and the incision was sutured under aseptic conditions. The bile duct was left undisturbed. The intestines from control and 24 h post-pancreatectomised rats were removed at different time intervals after ingestion of yeast conjugated folates, and divided into 4 equal segments beginning from the duodenojejunal end. The luminal contents of each segment were washed out with 10 ml of ice cold 0.25 M sucrose and their conjugase activities determined[12].

Absorption of folates was determined as described earlier[13] from the rise in serum folate levels at various time intervals after ingestion of either simple folic acid (Sigma) or dietary (yeast) conjugated folates. Absorption of folates was similarly determined in rats 24 h after pancreatectomy.

Folate activity was determined by microbiological assay using *L. casei* ATCC 7469 as the test organism[14].

Results

Folyl conjugase levels in pancreas were observed to be induced about 2-fold following conjugated folate ingestion (fig. 1). The enzyme activity was raised from 50 ng simple folate released/mg protein in control (0 min) rats to 110 ng/mg protein, 30 min after conjugated folate ingestion. These levels returned to the base level values 120 min after folate ingestion. However, there was no conspicuous rise observed in pancreatic folyl conjugase levels after ingestion of unconjugated synthetic folic acid, indicating no induction of the enzyme (fig. 1).

Concomitant with the induction of pancreatic folyl conjugase, a rise in serum folate levels was also observed 30

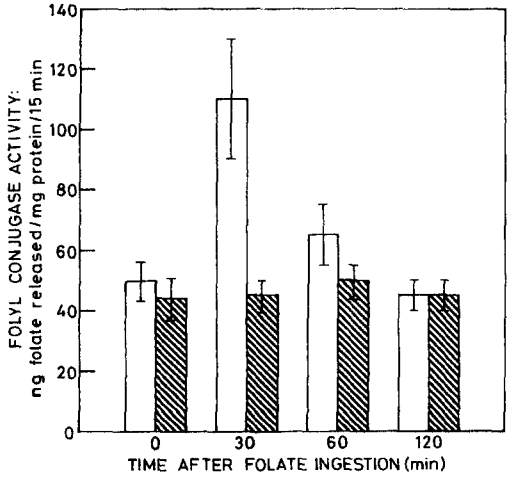

Figure 1. Rise in pancreatic folyl conjugase activity following ingestion of dietary polyglutamyl (□) and synthetic unconjugated (▨) folates in normal rats. The values represent the mean ± SEM (n = 5).

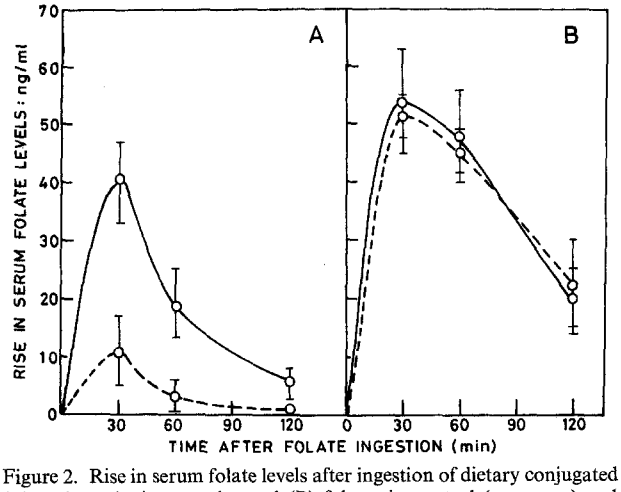

Figure 2. Rise in serum folate levels after ingestion of dietary conjugated (A) and synthetic unconjugated (B) folates in control (o———o) and pancreatectomised (o- - - - -o) rats. The values represent the mean ± SEM (n = 6).

min after folate ingestion, indicating a normal absorption pattern of dietary folyl polyglutamates. The absorption of dietary conjugated folates and synthetic simple folates as determined by the rise in serum folate levels after ingestion in normal and pancreatectomised animals is shown in figure 2. The serum folate levels are elevated to 40 ng/ml above the baseline (0 min) in control animals 30 min following ingestion of conjugated folates (fig. 2 A). The rise in serum folate levels after ingestion is impaired by 75–80% in pancreatectomised rats, indicating a defect in the absorption of dietary conjugated folates. The absorption of simple folates is, however, not impaired in pancreatectomised animals (fig. 2 B), indicating normal physiological folate absorption in the pancreatectomised rats.

A determination of intestinal luminal folyl conjugase showed (fig. 3) that the enzyme activity was progressively elevated beginning from the proximal region, showing higher activity at the jejunal and mid-gut regions 30–60 min after dietary folate ingestion. Total enzyme levels in the entire small intestine showed (fig. 3) very little enzyme activity in the fasting animals at 0 min. The enzyme levels are elevated almost 8–9-fold; an initial level of 200 ng of folates released (which is a measure of conjugase activity) increased to 1660 ng 60 min after conjugated folate ingestion. No such increase in the luminal folyl conjugase activity was observed in pancreatectomised animals. The total luminal folyl conjugase activity released after conjugated folate ingestion in pancreatectomised rats is only 20% that of the control animals, the values being 760 ng and 3580 ng released folate activity, respectively. Similarly, very low enzyme activity was observed in control animals after simple unconjugated folate ingestion (fig. 3).

Discussion

The role of pancreatic folyl conjugase in the breakdown of dietary polyglutamyl folates prior to absorption is not well established. We have reported earlier the possible

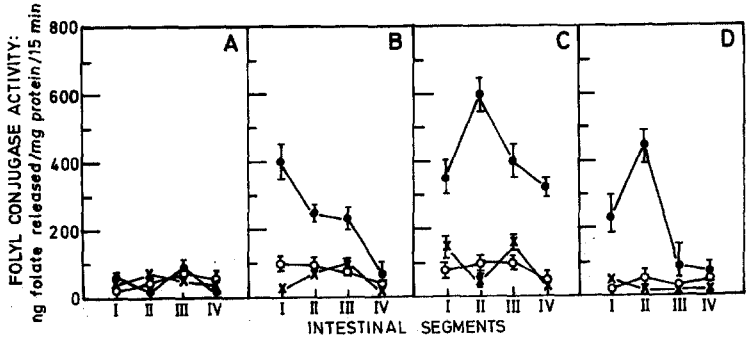

Figure 3. Luminal folyl conjugase activity in 4 sequential equally divided small intestinal segments (I, II, III & IV) after ingestion of dietary conjugated folates in control (●———●) and pancreatectomised (x———x) rats and also after synthetic unconjugated folate ingestion (o———o) in control rats at 0 (A), and after 30 (B), 60 (C) and 120 (D) min. The values represent the mean ± SEM (n = 5).

involvement of pancreatic folyl conjugase in the hydrolysis of dietary folates at the intestinal site of absorption [12] in the rat. A high build-up of folyl conjugase activity at the intestinal lumen of normal rats and not in pancreatectomised rats (fig. 3) clearly indicates the pancreatic origin of luminal folyl conjugase. This is supported by the observation of the pancreatic induction of folyl conjugase activity following ingestion of dietary polyglutamyl folate (fig. 1). Intestinal mucosal folyl conjugase was not, however, induced or elevated following dietary folate ingestion (untabulated results). The possibility that the low luminal folyl conjugase levels in the pancreatectomised animals are the result of a diabetic state was ruled out since luminal folyl conjugase activity is observed after dietary polyglutamyl folate ingestion in streptozotocin diabetic rats. The total folyl conjugase levels in the streptozotocin diabetic animals were 3650 ng/mg protein which was quite comparable with control values (see results). Thus the induction of folyl conjugase is not related to the diabetic status of the rat but to the presence of the intact pancreas per se.

We have shown earlier that in the rat, folate absorption is not a carrier-mediated process as no precursor/product relationship existed between folates bound to the intestinal mucosal surface and folates appearing in the blood stream after folate ingestion [15]. Further, folate absorption was shown to be a concentration-dependent passive uptake, requiring an acidic microenvironment (around pH 4.0) generated and regulated by an intestinal mucosal brush border Mg^{2+} ATPase [16]. The pancreatic folyl conjugase with a pH optimum of 4.2 would be ideally suited to function in the acidic microenvironment generated to facilitate folate uptake [16]. Our data on the absorption of dietary folates are indicative of the induction of folyl conjugase (fig. 2). The absorption of dietary conjugated folates was impaired 75–80% in the pancreatectomised animals where that of synthetic simple folates was not. Similar impairment in the absorption processes of dietary folates was observed by us earlier [12] in aged animals, where pancreatic secretion of folyl conjugase in response to dietary folate intake was found to be 4–5-fold lower. These observations taken together would clearly suggest that in the rat dietary polyglutamyl folates are hydrolysed to suitable simple forms (possibly at the mucosal surface) by a luminal folyl conjugase of pancreatic origin which requires ingestion of conjugated folates for induction. It is noteworthy that ingestion of unconjugated folates did not induce the release of the conjugase from the pancreas.

Intestinal mucosal folyl conjugase did not seem to be involved in the digestion of dietary folates. It could instead have a role in controlling folate coenzyme functions by altering τ-glutamyl folate chain lengths [17], thereby regulating the mucosal cell metabolism.

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