On the primary site of control in the spontaneous development of small-intestinal sucrase-isomaltase after birth

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We have compared the appearance in the small intestine of baby rabbits, of sucrase and isomaltase activities and of the sucrase-isomaltase mRNA. For the latter we used a cDNA probe encompassing ~4.1 kb from the 5'-end of pro-sucrase-isomaltase cDNA [(1986) Cell 46, 227–234]. Sucrase-isomaltase mRNA and the enzyme activities developed simultaneously from the 15th day after birth onwards. Over two orders of magnitude the enzymatic activities and sucrase-isomaltase mRNA matched one another closely, thus ruling out translation as the main site of biosynthetic control during spontaneous development, while rendering very probable transcription as the primary site of control. However, we cannot rule out the possibility that, prior to day 15, sucrase-isomaltase mRNA might be degraded so rapidly that it is not translated.

(Small intestine) *Development* *Sucrase-isomaltase*

1. INTRODUCTION

In most mammals small-intestinal lactase (e.g. [1,2]) (and β-glucosidase [3]) activities are fully developed at birth, whereas sucrase and isomaltase activities develop at the time of weaning, e.g. in the rat [1] and the rabbit [4] from day 15–18 to approx. day 25–30 of extrauterine life. An exception is man, who is fully equipped with small-intestinal α- and β-glycosidases at birth [5,6].

In the 'average mammal' the small-intestinal glycosidase activities are due to a maltase with sucrase activity, a second maltase with 1,6-α-glucosidase (isomaltase) activity; two more maltases, each with glucoamylase activity; a trehalase; a lactase and a glycosylceramidase. Six of these glycosidases occur as heterodimers (save for further dimerization [7,8]); i.e. the sucrase-isomaltase complex, the glucoamylase complex and the β-glycosidase complex. The subunits of each heterodimer are synthesized as a single, very long polypeptide chain ('pro' form) (review [9]). Thus, the two subunits of each heterodimer belong to the same translational unit and are subjected to the same biological control. As originally suggested [10,11] and then amply demonstrated (e.g.[12–16]; see review [9]) for sucrase-isomaltase, these heterodimers are synthesized as a single, long (~200 kDa) polypeptide chain (e.g. as a pro-sucrase-isomaltase), which is split posttranslationally into the 'final' subunits. This mode of synthesis explains also the species distribution (review [17]) (and thus the phylogeny) [9,16,18] and the mode of anchoring to the membrane of sucrase-isomaltase [16,19,20].

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Abbreviations: S, sucrase; I, isomaltase; proSI, pro-sucrase-isomaltase; SSC, 0.15 M NaCl, 0.015 M Na_3citrate, pH 7.0

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Since the sucrase-isomaltase complex accounts for all the sucrase, nearly all the isomaltase (a little isomaltase activity is accounted for by the glucoamylase complex) [21–25] and much of the maltase activity of the small intestine, it is not surprising that sucrase, isomaltase and much of the maltase activities appear simultaneously during development (e.g. [1,4]). Little is known of the location of the primary site of control of 'spontaneous' development (i.e. not precociously triggered by, say, hormonal administration) of the brush border glycosidases. For another type of brush border enzymes, i.e. for aminopeptidase N, evidence has been presented that the major site of control is at the level of translocation [26].

Recently, the cDNA of rabbit pro-sucrase-isomaltase (proSI) has been sequenced and become available [16]. We have now used this probe to measure the appearance of SI mRNA and compare it with that of sucrase and isomaltase activities. The highly satisfactory matching between mRNA levels and the enzyme activities over two orders of magnitude effectively rules out that the control of biosynthesis of sucrase-isomaltase is located at the translational level. Most likely it is located at the level of transcription, although we cannot rule out the possibility that SI mRNA, prior to day 15 after birth, may be degraded so fast that it cannot enter the translation machinery.

2. MATERIALS AND METHODS

New Zealand White rabbits were killed at 1, 5, 11, 15, 21, 26 and 35 days after birth. An adult rabbit was killed and used as a control. Mucosa was scraped from the proximal half of the small intestine of each rabbit and immediately processed or kept frozen at −80°C. In rabbits aged 1, 5 and 11 days the whole intestinal wall of the proximal half of the intestine was used instead of the scraped mucosa.

The samples were homogenized at a concentration of 2.5% in 2 mM Tris-50 mM mannitol (pH 7.1). Sucrase and isomaltase enzymatic activities were assayed according to Auricchio et al. [27]. For all samples before day 26 the method was modified according to Ciccimarra et al. [28], thus increasing the sensitivity and reliability of the determination of liberated glucose: 200 μl of tissue were incubated with 100 μl of 3% substrate in 0.15 M maleate bufer (pH 5.8) for 30 and 60 min at 37°C. After incubation the samples were boiled for 3 min and deproteinized with 50 μl of 10% $ZnSO_4 \cdot 7H_2O$ and 50 μl of 0.6 N NaOH. After centrifugation the glucose concentration was determined, using 200 μl of the supernatant. The $Zn(OH)_2$ deproteinization eliminates any interference with the glucose assay due to the high concentration of the intestinal homogenates used when the enzyme activity is low (see also [29,30]. Both assay methods gave the same results from day 26 onwards.

Total RNA extraction was carried out as described by Frazier et al. [31] using buffer-saturated phenol and proteinase K digestion. Dot blots were set up using Schleicher-Schuell nitrocellulose membranes and a Bio-Dot Apparatus (Bio-Rad). Before binding to filters, RNA was denatured in 1 M NaCl, 40 mM phosphate buffer (pH 7.0) and 6% formaldehyde, at 55°C for 15 min. Three different dilutions of RNA of each sample (10, 3, 1 μg) were applied to the filter. Nitrocellulose filters were baked at 80°C for 2 h. Hybridization was performed in the presence of 50% formamide at 42°C. Two different probes were prepared by nick-translation: the sucrase-isomaltase probe is a fragment covering more than the 5′-half of the rabbit cDNA; a cDNA actin probe was chosen as a control since the levels of actin mRNA are expected to remain constant. The two probes were used at a specific activity of at least 10^8 cpm/μg DNA. Filters were washed at high stringency, 0.2×SSC (SSC: 0.15 M NaCl, 0.015 M Na_3 citrate, pH 7.0) at 65°C. Autoradiography was carried out using Kodak X-Omat films and intensifying screens at −80°C. The quantitation of the specific sucrase-isomaltase mRNA was carried out using a Loebl-Joyce densitometer.

3. RESULTS

Fig.1 compares the sucrase and isomaltase activities with the levels of SI mRNA in the small intestines of rabbits of different ages. The observed correspondence extends over two orders of magnitude. The SI mRNA is first detectable at 15 days of age; it reaches adult level at 35 days (fig.2A). The appearance and increase of SI mRNA levels (figs 1,2A) are not artifactual, since a control probe (actin cDNA) did not show any change with age (fig.2B).

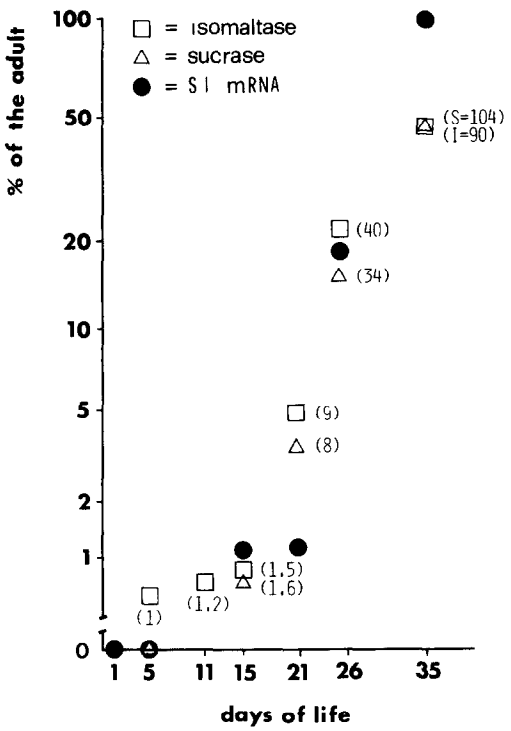

Fig.1. Development of sucrase-isomaltase activities and of SI mRNA after birth. Homogenates of small intestine were assayed for sucrase (△) and isomaltase (□) activities at the various time points after birth. The enzyme activity is expressed as % of the adult value and reported on a semilogarithmic scale. In parentheses the sucrase and isomaltase activities at different ages, expressed in mU/g protein, are shown. The corresponding adult values were 220 for sucrase and 190 for isomaltase. SI mRNA levels (●), detected by dot blot and quantitated by a densitometer, are also reported as % of the adult levels.

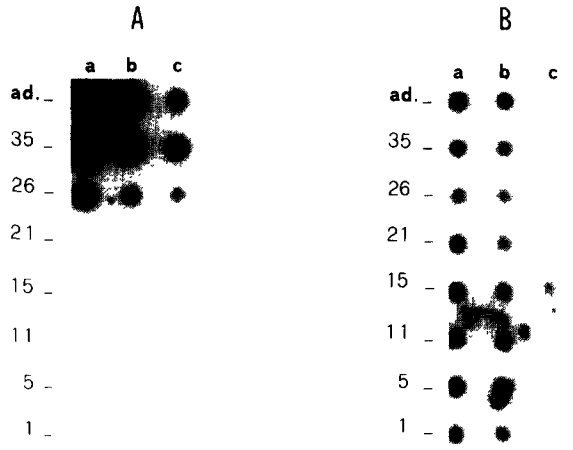

Fig.2. Dot-blot analysis of sucrase-isomaltase mRNA during intestinal development. RNAs were extracted from rabbit intestine at different ages (days after birth indicated on the left; ad, adult). RNA samples were applied, at different dilutions (a,10 μg; b,3 μg; c,1 μg), and blotted to two replicate nitrocellulose filters, hybridized to SI cDNA (A) or actin cDNA (B), respectively.

Sucrase activity appears at the same day as SI mRNA, i.e. at day 15, whereas traces of isomaltase activity are already detectable at day 5 (fig.1). This requires some comment.

Most previous investigators (e.g. [1,4]) either failed to detect, or barely detected this 'early' isomaltase. This activity is attributable either to a lysosomal enzyme [32] or perhaps to the glucoamylase complex, which does develop slightly ahead of sucrase-isomaltase [33] and has, at least in some species, some isomaltase activity [21–25]. Be as it may, the minor isomaltase activity which begins to appear at day 5 (fig.1) is certainly not related to the sucrase-isomaltase complex (in particular, it is not its isomaltase subunit appearing earlier and independently), because the SI cDNA probe which we used was approx. 4.1 kb long, starting from the 5′-end of SI cDNA, i.e. it corresponded to the whole anchoring segment, the 'stalk', the whole of the isomaltase region and a part of the sucrase region. (In proSI, amino acids 1 – ~931 encompass the anchor, the stalk and the isomaltase region [16]. See also [20].) Thus, if the isomaltase activity present at day 5–11 were identical with (or of it had extensive homology with) the isomaltase subunit of the SI complex, it would have hybridized with the probe which we have used. This interpretation is most likely to be correct, because this early isomaltase appears prior to SI mRNA, and because the SI mRNA levels parallel sucrase activity, although the cDNA probe used, while coding for the whole isomaltase region, did so for only a small portion of the sucrase region in proSI (as mentioned above).

4. DISCUSSION

Danielsen et al. [26] have reported that another intestinal brush border enzyme, aminopeptidase N, is apparently regulated at the translational

level, since total mRNAs from fetal or adult pig intestine are equally effective in stimulating aminopeptidase synthesis in cell-free translation systems. This enzyme (unlike sucrase-isomaltase) is present in traces in embryonic life but appears mainly perinatally (reviews [34–36]). Also, it does not respond to dexamethasone [37], whereas sucrase-isomaltase does.

As to sucrase-isomaltase, it has been indicated that the site of control of development (in organ cultures, from the inhibition by actinomycin D of dexamethasone induction) is likely to be at the transcriptional level [38].

Our data (fig.1) show a close matching of the appearance of SI mRNA with that of the enzyme, which extends over two orders of magnitude. This observation clearly rules out translation as the primary site of biosynthetic control in the spontaneous development of SI. The most straight forward explanation of our data is that the control is at the level of transcription; however, we cannot rule out at present the possibility that in preweaning baby rabbits SI mRNA may be degraded too fast to enter the translation machinery or to be detected in the dot-blot test. The mRNA of another small-intestinal, cytosolic protein (a fatty acid-binding protein) appears shortly before birth (in rat [39]).

The minor isomaltase activity present at day 5–11, i.e. before the appearance of SI mRNA, is, as mentioned in section 3, unrelated to the sucrase-isomaltase complex and does not indicate an early, isolated synthesis of the isomaltase subunit alone (the cDNA probe used encompassed the whole of the isomaltase portion also). This early isomaltase activity is either due to a kindred enzyme, the glucoamylase complex (which is known to appear slightly ahead of sucrase-isomaltase [33] and may have some isomaltase activity [21–25]), or, more probably, to a lysosomal enzyme [32].

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