Rat trehalase: cDNA cloning and mRNA expression in adult rat tissues and during intestinal ontogeny

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Oesterreicher, Thomas J., Nanda N. Nanthakumar, John H. Winston, and Susan J. Henning. Rat trehalase: cDNA cloning and mRNA expression in adult rat tissues and during intestinal ontogeny. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1220-R1227, 1998.—A partial rat trehalase cDNA has been cloned and used to examine trehalase mRNA expression. Northern blotting with total RNA from 11 adult rat tissues showed a trehalase transcript only in small intestine, where it was abundant in proximal regions but declined steeply toward the ileum. During development, trehalase mRNA was not detectable in jejunum until postnatal day 19 and then increased markedly through day 25. Modest levels of trehalase mRNA were induced precociously by administration of dexamethasone, with increasing responsiveness evident between the first and second postnatal weeks. In contrast, analysis of sucrase-isomaltase mRNA on the same blots showed maximal induction at both ages. In adrenalectomized animals, the ontogenic increase of trehalase mRNA began as usual but proceeded more slowly than in control animals. Overall, trehalase mRNA expression in the rat displayed both similarities and differences compared with rabbit. Moreover, the differences revealed in glucocorticoid responsiveness of trehalase mRNA and sucrase-isomaltase mRNA suggest that the actions of these hormones on the developing intestine may be more complex than previously recognized.

Trehalase is one of the least studied of a broad group of ectoenzymes found in the apical membrane of mammalian enterocytes (8). The protein was originally suspected of playing a role in glucose transport (24, 30); however, more recent studies (3, 29) have favored its physiological role being the hydrolysis of luminal trehalose. Although this disaccharide is not abundant in modern Western diets, it is widely distributed in both the plant and animal kingdoms, most notably being found in fungi, seaweed, and insects (1). Semenza (33) has pointed out that trehalose is frequently found in diets of some populations of the Far East and may have been more abundant in the diet of early humans. Likewise, trehalose would be expected to be a regular constituent of the diets of omnivorous mammals such as rodents (and in varying proportions depending on the content of fungi, insects, etc.) as well as in herbivores such as lagomorphs (in which fungi would be the main source).

During postnatal development of both rabbits (10) and rats (28), activities of trehalase in the small intestine are low or undetectable during the suckling period and increase during the third postnatal week, coincident with the time course of weaning (12). This ontogenic pattern is shared by several other disaccharidases that are known to be involved in digestion of the carbohydrate components of solid food, namely maltase, glucoamylase, sucrase, and isomaltase (28). Numerous investigators over the past three decades have used sucrase as a prototype enzyme to study the regulation of intestinal maturation (8, 12, 15). Early studies relying on the measurement of enzyme activity and enzyme protein pointed to a dual role of glucocorticoid hormones in its ontogeny. First, there is a surge of circulating corticosterone (the major glucocorticoid of the rodent) at the end of the third postnatal week that controls the rate of the normal developmental increase of sucrase activity. Second, administration of exogenous glucocorticoid during the first two postnatal weeks can elicit a precocious rise of enzyme activity and protein (8, 12). In more recent studies, these patterns of enzyme activity and protein have been shown to be paralleled by changes in the levels of sucrase-isomaltase mRNA (20, 25). Moreover, it has been argued (13) that precocious induction by glucocorticoid has evolutionary significance, specifically, that it represents an adaptive mechanism whereby the intestine of the suckling rodent is elicited to mature in the face of stress associated with precocious loss of milk supply (e.g., due to predation of the dam).

In contrast to the extensive literature on sucrase, there have been relatively few studies on the regulation of trehalase expression in the developing intestine. Galand and colleagues (7, 9) showed a marked increase of trehalase enzyme activity in suckling rats receiving glucocorticoid injections, and similar findings were subsequently reported for both suckling mice (21) and rabbits (6). Recently, the cloning of rabbit trehalase cDNA (29) led to the demonstration that developmental and glucocorticoid-induced changes of trehalase activity in the rabbit intestine are paralleled by changes in steady-state levels of trehalase mRNA (10). To date, there have been no comparable studies at the mRNA level in the developing rat intestine. Moreover, with respect to the effect of exogenous glucocorticoid on trehalase expression, all of the prior studies have been limited to a single age of administration. Thus it is not known whether the increasing responsiveness previously reported for other aspects of intestinal maturation (14, 17, 22, 23) also apply to trehalase. Finally, although adrenalectomy (ADX) has been widely used to assess the role of the endogenous surge of corticosterone in enzymic changes occurring in the third postnatal week (8, 15), there has been only one cursory examination of the effects of ADX on trehalase enzyme activity in rats (26) and no such studies at the mRNA level in either rats or rabbits. In view of these gaps in...
our knowledge, the goals of the present work were fivefold: 1) to clone rat trehalase cDNA to have a sensitive probe for detecting the mRNA, 2) to determine the tissue distribution of trehalase mRNA in the adult rat, 3) to quantify the steady-state levels of trehalase mRNA along the length of the rat intestine and during normal development, 4) to study the influence of exogenous glucocorticoid administered at various postnatal ages, and 5) to investigate the effect of ADX on the ontogenic pattern of intestinal trehalase mRNA.

MATERIALS AND METHODS

Cloning of rat trehalase cDNA. A λZAP II cDNA library from adult rat jejunum (2) was screened with the entire 1.8-kb rabbit trehalase cDNA (pTre14), generously provided by N. Mantei (29). An initial plating of 5,000 plaques yielded two putative positives (TOTre1 and TOTre2). The isolated cDNA clones were excised into pBluescript and sequenced (both strands) using an Applied Biosystems model 373A DNA sequencer together with vector and internal primers. The sequences from all fragments were assembled using Genetics Computer Group software and then aligned to rabbit trehalase. Searches for identity at the nucleotide (nt) and amino acid (aa) levels were performed using multiple sequence databases (including GenBank, Swiss-Prot, etc.).

Animals and tissue collection. Adult males and timed-pregnant dams of the Sprague-Dawley strain (Charles River Crl:CD(SD)BR) were obtained from Charles River Breeding Laboratories (Portage, MI) and were maintained as described elsewhere (20). To study the tissue distribution of trehalase mRNA, two male rats (age 36 days) were subjected to bilateral ADX or sham operation using standard methods (25). Trunk blood was subsequently stripped and reprobed with elongation factor 1α (EF-1α) as a constitutive marker (2). For the dexamethasone study, two male rats were also reprobed with rat sucrase-isomaltase using the linearized plasmid GC4.5 (2).

Quantitative analyses. Signals from Northern blots were quantified by phosphorimaging using a linear scan through the most representative portion of each band. To correct for loading variations, these data first were expressed as a ratio of the hybridization signal of the band of interest (trehalase or sucrase-isomaltase) to that of the constitutive marker EF-1α. This ratio then was expressed as a percentage of the P25 jejunal standard from the same blot. These percentages are shown graphically as means ± SE for the number of animals in each experimental group. Statistical analysis was by either a one-way or two-way analysis of variance (ANOVA). A P value < 0.05 was considered significant, and post hoc Fisher’s least-significant difference tests were then performed when appropriate.

RESULTS

Cloning of a partial rat trehalase cDNA. To obtain a cDNA probe that could be used efficiently on Northern blots of rat RNA, we screened a rat intestinal cDNA library with the rabbit trehalase cDNA. Two clones (TOTre1 and TOTre2) were obtained, each having inserts of ~1 kb. Initial sequence confirmed the identity of both by comparison with the published rabbit trehalase sequence (29). Complete sequencing revealed that the two clones were overlapping and represented 1,710 nt of rat trehalase cDNA, in comparison to the rabbit cDNA, which has been reported as 1,788 nt.1 Sequence alignment showed that the two clones together covered nt 69–1,778 of rabbit trehalase cDNA. Thus, at the 5’ end, the rat sequence is missing the initiation codon and those for the next 21 aa, whereas at the 3’ end the rat sequence includes the stop codon and 35 nt of the untranslated region. The 1,671 nt of the rat trehalase coding region showed overall sequence identity of 80% with the corresponding region of rabbit trehalase (29).

The predicted aa sequence derived from the rat trehalase cDNA is shown in Fig. 1. Comparison with the rabbit sequence shows an overall identity of 81% and a similarity of 86%. The only other protein in the databases with high sequence similarity was a partial human trehalase (31) which showed 84% identity over 308 residues. One region (aa 160–230) of the predicted rat protein showed high similarity with a domain that has been identified in trehalases from a variety of lower...
organisms, including bacteria, yeast, and insects. This span of 70 aa residues of rat trehalase (Fig. 1) has identities ranging from 41 to 54% with the trehalases of lower organisms and similarities ranging from 56 to 70%. Within this broad region, the rat sequence includes a 14-aa stretch (165–178) that shows 100% agreement with a consensus sequence that has been designated as a trehalase signature pattern (16) and that distinguishes trehalases from other classes of glycosyl hydrolases. This region (Fig. 1) is believed to be part of the catalytic domain (19) and includes conserved Asp (D) and Glu (E) residues that commonly participate in catalysis by glycosyl hydrolases (16). Various other features of the predicted aa sequence for rat trehalase show concurrence with those for rabbit. Overall, the protein is highly hydrophilic, consistent with its location on the exterior of the brush border membrane and its function as an ectoenzyme in the intestinal lumen. There is a hydrophobic stretch at COOH-terminus (Fig. 1), which is believed to function as a temporary membrane anchor before replacement by a glycosylphosphatidylinositol anchor (29). Finally, four potential N-glycosylation sites identified in rabbit trehalase are conserved in rat trehalase (Fig. 1).

Tissue distribution of trehalase mRNA in adult rats. To assess the expression pattern of trehalase, RNA was prepared from 11 tissues collected from adult rats. Northern blotting using the TOTre1 insert showed an abundant transcript in the jejunum (Fig. 2). No trehalase signal was detectable in brain, heart, kidney, liver, lung, skeletal muscle, smooth muscle, spleen, or testes. In both rat (Fig. 2) and rabbit (data not shown), the
trehalase mRNA was readily detectable in jejunal RNA using total cellular RNA, despite a previous report in rabbit indicating the need for poly (A)⁺ RNA (10). On the other hand, because trehalase mRNA runs very close to 18S RNA, for purposes of sizing, gels included lanes with poly (A)⁺ RNA from both rat and rabbit jejunum. These lanes showed the trehalase transcript to be ~1.9 kb in both cases, in good agreement with published values for rabbit trehalase mRNA (10, 29). Comparison of the size of the mRNA with that of the partial cDNA (1,710 nt) suggests that our rat cDNA is missing ~200 nt.

Longitudinal and developmental expression of trehalase mRNA in rat intestine. Because trehalase enzymatic activity typically has been reported to be significantly higher in proximal than distal regions of the rat intestine (4, 28), it was of interest to examine the longitudinal distribution of trehalase mRNA. The Northern blot (Fig. 3A) shows an abundant signal in both duodenum and proximal jejunum, a marked decline in the distal jejunum and ileum, and no detectable signal in the cecum, proximal colon, or distal colon. The quantitative data (Fig. 3B) show that the gradient of expression is highly significant (P < 0.001 from overall ANOVA) and that, as indicated by the letters above the bars, the levels of trehalase mRNA in individual regions are as follows: duodenum = proximal jejunum > distal jejunum > proximal ileum = distal ileum. On the basis of this distribution, for all future experiments we chose to use the entire jejunum. This choice represented a compromise between the need for peak levels of expression and that for adequate amounts of tissue from younger animals.

The pattern of trehalase mRNA expression in rat jejunum during postnatal development is shown in Fig. 4. As seen in the Northern blot (Fig. 4A), trehalase mRNA was first detected on P19 and increased markedly by P22 to a level just slightly less than that seen at P25. Quantitative analysis (Fig. 4B) shows a significant overall effect of age on trehalase mRNA expression (P < 0.001 by ANOVA). Comparisons of specific ages are shown by the letters and indicate P19 < P22 < P25.

Influence of exogenous glucocorticoid on trehalase mRNA expression. Previous studies have shown that administration of exogenous glucocorticoid to rats starting at P10 for 4 days (7) or at P13 for 3 days (9) causes a precipitous increase of trehalase enzymatic activity. For other intestinal enzymes, there is evidence that effects

Fig. 3. Distribution of Tre along intestinal tract of adult rats. A: representative Northern blot showing signal obtained for Tre and for constitutive marker elongation factor 1α (EF). D, duodenum; PJ, proximal jejunum; DJ, distal jejunum; Pi, proximal ileum; Di, distal ileum; C, cecum; Pco, proximal colon; Dco, distal colon. Lane labeled S shows postnatal (P) day 25 (P25) pooled standard from jejunum. B: quantitative data for Tre expressed as percentage of P25 standard. Bars show means ± SEM for 2 animals. Letters above bars show results of Fisher’s least-significant difference (LSD) post hoc tests; values with no letter in common are significantly different from each other.

Fig. 4. Postnatal development of Tre in rat jejunum. A: representative Northern blot of jejunal RNA collected from individual rats every third day from P4 to P22. Postnatal ages (days) are shown above each lane. Lane labeled S shows P25 pooled standard. Blot was probed for Tre and EF as indicated. B: quantitative data for Tre expressed as percentage of P25 standard, showing means ± SE for 3 animals. Letters to right of points show results of Fisher’s LSD post hoc tests; values with no letter in common are significantly different from each other.
of glucocorticoid differ during the first, second, and third postnatal weeks (8, 22, 23). Thus, to study the responsiveness of trehalase mRNA, we used three series of animals with early, middle, and late administration of the synthetic glucocorticoid dexamethasone. Jejunal RNA was collected 1 and 4 days after treatment was initiated to assess the rapidity of the response to dexamethasone. Representative Northern blots from each series are shown in Fig. 5A. In the early series (with injections beginning on P4), trehalase mRNA was undetectable after 1 day (P5) but clearly detectable after 4 days (P8). In the second series (with injections beginning on P10), a weak trehalase mRNA signal was detected after 1 day (P11) and a much stronger one by the fourth day (P14). In both of these series, control animals receiving vehicle injections displayed no trehalase mRNA. In the final series, in which injections began on P18, control animals showed trehalase mRNA at both P19 and P22, consistent with the developmental pattern of untreated animals shown in Fig. 4. Dexamethasone appeared to have little effect at these ages. Quantitative analysis (Fig. 5B) confirmed the above impressions. For the early and middle series, the effect of dexamethasone was statistically significant (P < 0.001 in both cases). The response to the glucocorticoid treatment was quite slow, however, with little or no trehalase mRNA being seen after 1 day of treatment, in contrast to significant levels after 4 days of treatment. Direct comparison of the early and middle series showed the dexamethasone-induced levels of trehalase mRNA to be significantly greater in the middle series (P < 0.001 by 2-way ANOVA), indicative of increasing responsiveness. In contrast, in the late series there was a significant effect of age (P < 0.001 by 2-way ANOVA) but no significant effect of dexamethasone. Thus, once the developmental increase of trehalase mRNA had begun, it was no longer influenced by exogenous glucocorticoid. Loss of responsiveness at this time appears to be a general phenomenon, having been previously reported for numerous aspects of intestinal maturation at the functional level (8, 15) and for rat sucrase-isomaltase (25) and rabbit trehalase (10) at the mRNA level.

A striking feature of the data shown in Fig. 5B is that, although dexamethasone is clearly capable of inducing trehalase mRNA as early as the first postnatal week, the levels elicited fall far below those normally seen during the fourth postnatal week. In the early series, trehalase mRNA levels reached only 19 ± 5% of the P25 standard after 4 days of dexamethasone treatment; in the middle series, the corresponding value was 44 ± 3%. This is in contrast to previous studies on sucrase-isomaltase mRNA that had demonstrated a much stronger response to exogenous glucocorticoid during the second postnatal week, in fact reaching 100% of mature levels (20, 25). Thus it appeared that there may be a difference in the responsiveness of trehalase mRNA compared with sucrase-isomaltase mRNA. To address this directly, the blots from Fig. 5 were reprobed with sucrase-isomaltase cDNA. The results for the early and middle series are shown in Fig. 6. As can be seen from both the Northern blot (Fig. 6A) and the quantitative data (Fig. 6B), sucrase-isomaltase mRNA displayed a robust response to dexamethasone. In both series, the dexamethasone effect on sucrase-isomaltase mRNA was evident after only 1 day of treatment and after 4 days had reached or exceeded the level of the P25 standard. Statistical analysis shows that the difference between the response of trehalase mRNA and sucrase-isomaltase mRNA to dexamethasone is significant (P < 0.001 and P < 0.005 by ANOVA for both 1 and 4 days of treatment, respectively).

Effect of ADX on trehalase mRNA expression during intestinal maturation. During normal development, endogenous concentrations of circulating corticosterone (the principal glucocorticoid in the rat) are low through P12 and then increase markedly over the next week (12). Because this surge precedes the normal increase in expression of trehalase mRNA, as shown in Fig. 4, our next goal was to assess the role of endogenous glucocorticoid in the developmental pattern of jejunal trehalase mRNA. To this end, rat pups were ADX at P9, i.e., before the developmental increase of plasma corticosterone. The data are shown in Fig. 7. In
the representative Northern blot (Fig. 7A), trehalase mRNA was not detectable at P17 in either control or ADX animals. The developmental increase of expression is seen at P20 in both animals but with the ADX animal showing a weaker signal. The same pattern is present at P23, but by P26 there is no apparent difference between control and ADX animals. The quantitative data from multiple animals (Fig. 7B) show that ADX caused a modest suppression of the developmental increase of trehalase mRNA. The effects of both age and treatment were statistically significant (P < 0.001 and P < 0.04, respectively, by 2-way ANOVA). Thus elimination of the ontogenic surge of endogenous glucocorticoid resulted in a reduced rate of accumulation of trehalase mRNA during the third and fourth postnatal weeks. The effect of ADX is consistent with a prior study on trehalase enzymatic activity (26) that examined only the early part of the developmental rise (through P20).

DISCUSSION

The studies described in this paper represent the first analysis of trehalase expression in rat intestine at the mRNA level. Overall, the data reveal an interesting array of both similarities and differences to prior work with rabbit trehalase mRNA. First, with respect to the cloning, our two overlapping rat trehalase cDNAs represent almost the full coding region and thus permit extensive comparison with the rabbit sequence. In contrast, the only other mammalian trehalase cloned to date is a partial human cDNA (31) that represents only 308 out of the total of 578 aa. As indicated in RESULTS, the predicted aa sequence of rat trehalase has many similarities with rabbit (29). Of particular note is the fact that subsequent to the cloning of the rabbit trehalase cDNA, the analysis of trehalases from a variety of lower organisms has led to the identification of a conserved domain that has now been defined as the trehalase signature (16, 19). The predicted aa sequences for both rat and rabbit trehalases show 100% agreement with this consensus. Its conservation in organisms ranging from bacteria to mammals strongly supports the suggestion (19) that this domain is essential for the catalysis of trehalose hydrolysis.

In contrast to the sequence similarities between rat and rabbit trehalase cDNAs, examination of the tissue distribution of trehalase mRNA showed a striking difference, with the rat showing detectable trehalase mRNA only in the small intestine (Fig. 2), whereas in the rabbit, the mRNA is almost as abundant in kidney as in small intestine (29). This difference correlates with prior studies that have demonstrated trehalase enzymatic activity in rabbit kidney but not in rat kidney (27, 30). Thus it is clear that the latter reflects lack of accumulation of trehalase mRNA in the renal epithelium.
Our study of trehalase mRNA levels in various regions along the length of the adult rat intestine revealed a dramatic gradient from proximal to distal small intestine. This gradient correlates well with prior reports for trehalase enzyme activity (4, 28), indicating that the latter is regulated at a pretranslational level. The longitudinal gradient of expression of trehalase mRNA appears to be somewhat different in rat compared with rabbit, the latter displaying peak expression in midjejunum and only a modest decline distally, such that levels in the terminal ileum are still 40–50% of maximum (10). The quantitative rat data (Fig. 3) showed peak levels in the duodenum and proximal jejunum, quantified as 228 to 247% of our P25 standard RNA, and a steady decline to the distal ileum, where levels were only 1%. The steep gradient of expression in the rat intestine makes the rat trehalase gene an attractive candidate to further our understanding of the molecular mechanisms of such gradients.

The developmental pattern observed for trehalase mRNA in rat jejunum is strikingly similar to that previously reported for sucrase-isomaltase mRNA (20). From P4 to P16, trehalase mRNA was below the limits of detection (Fig. 4). At P19, a clear signal was seen for all animals, and the abundance of the mRNA increased markedly over the next 6 days. The lack of detection of trehalase mRNA in the suckling jejunum contrasts with reports in the literature that low levels of trehalase enzymatic activity are detected at this time (7, 9, 26). This discrepancy may reflect very low levels of trehalase transcripts that could not be detected on our Northern blots. Alternatively, it is possible that the low levels of trehalase hydrolysis observed with preparations from the small intestine of suckling rats is due to some other enzymatic activity. Because lysosomal hydrolyases are generally upregulated in the suckling intestine (12), cross-reactivity by one of the members of this family would seem a likely possibility (e.g., acid maltase). By analogy with yeast, which has both acid and neutral trehalases (19), it is also possible that mammalian intestine expresses an acid trehalase during the suckling period.

The ability of exogenous glucocorticoid to cause precocious elevation of trehalase mRNA in rat jejunum (Fig. 5) correlates with prior reports at the level of trehalase enzyme activity (7, 9, 26). By administration of the dexamethasone at three different ages, an interesting pattern of changing responsiveness was revealed. The observed increase in responsiveness of trehalase mRNA to dexamethasone between the first and second postnatal weeks represents a novel finding, as does the contrast between trehalase mRNA and sucrase-isomaltase mRNA. These observations raise a number of intriguing questions. First, because this dose of dexamethasone had previously been shown to be saturating (25), it would be expected that the glucocorticoid receptors in the target cells would be fully occupied by hormone. Thus the reduced responsiveness of trehalase mRNA compared with sucrase-isomaltase mRNA in the young animals suggests that either the target cells or the molecular pathway is different. For sucrase-isomaltase, the effects of glucocorticoids are known to be mediated via the intestinal crypts (14, 17), although it is not yet clear whether it is the crypt epithelial cells or the pericryptal fibroblasts (18) that are the actual targets. For trehalase, the slow time course indicates that the effect of glucocorticoids is not only indirect but perhaps even more circuitous than for sucrase-isomaltase.

The ADX experiment showed that ablation of the normal circulating rise of endogenous glucocorticoid did not prevent the developmental upsurge of trehalase mRNA but merely slowed the rate of its rise (Fig. 7). Such a pattern has been observed previously for sucrase-isomaltase mRNA (25) and for various other aspects of functional maturation (8). It appears to be mediated by the ability of glucocorticoid to enhance the rate of emergence of enterocytes bearing the mature phenotype from the crypts (25). Taken in the context of the literature, the modest effects of ADX on trehalase mRNA suggest that the ontogenic changes of trehalase gene expression (like those of many other aspects of intestinal development) may be initiated by an intrinsic timing mechanism (5, 8, 15). On the other hand, although the weaning diet generally has been excluded as a trigger for intestinal maturation (12, 34), in the case of trehalase, a role for dietary control must be kept as a possibility. In this regard, because trehalase is likely to be a sporadic and minor component of dietary carbohydrate, its specific hydrolysis may have been subject to different evolutionary pressures compared with other disaccharidases such as sucrase-isomaltase and maltase-glucoamylase.

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