Intestinal Lactase
SHIFT IN INTRACELLULAR PROCESSING TO ALTERED, INACTIVE SPECIES IN THE ADULT RAT*

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The regulatory mechanism of decline in catalytic activity for intestinal lactase (lactase-phlorizin hydrolase, β-galactosidase) as mammals mature has not been defined. Solubilized intestinal brush-border membranes from adult male rats (>4 months of age, 200–400 g) were examined by high performance liquid Zorbax GF-450 chromatography, subjected to denaturing acrylamide electrophoresis, blotted to nitrocellulose, and identified by specific polyvalent anti-lactase. Three major species were present within the 235-kDa active lactase peak (225, 130, and 100 kDa). The 100-kDa moiety was also prominent in the ~300-kDa region of the GF-450 effluent, suggesting it is a catalytically inactive oligomer. In vivo synthesis and assembly of lactase by intraintestinal pulse (35S)methionine, 5 min and chase (15–120 min) revealed rapid (15 min of chase; maximum, 60 min) intracellular synthesis in the endoplasmic reticulum-Golgi fraction of multiple species (64, 100, 130, 175, and 225 kDa). The 64-kDa species disappeared from the intracellular membrane compartment and was not transferred to the brush-border surface. The 175-kDa moiety appeared to be processed to the 225-kDa unit prior to relocation to the surface membrane. By 120 min, the 100-kDa species became the predominant (~60%) radiolabeled unit in both endoplasmic reticulum-Golgi and brush border.

In the adult rat, lactase is assembled in multiple molecular forms that are differentially processed: (a) intracellular degradation (64-kDa unit) or (b) transfer to the brush-border surface as catalytically active (225 and 130 kDa) or inactive (100 kDa) species. Although substantial synthesis of lactase proteins prevails, major changes in processing appear to serve as an important regulatory mechanism producing the maturational decline of catalytic activity. The accompanying article (Castillo, R. O., Reisenuer, A. M., Kwong, L. K., Tsuibo, K. K., Quan, R., and Gray, G. M. (1990) J. Biol. Chem. 265, 15889–15893) extends our studies to synthesis and assembly during the neonatal period of maturation.

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1 The abbreviations used are: SDS, sodium dodecyl sulfate; DTT, dithiothreitol; ER, endoplasmic reticulum; HPLC, high performance liquid chromatography.

Intestinal lactase (lactase-phlorizin hydrolase, β-galactosidase), a glycoprotein localized at the enterocyte's brush-border surface membrane, is essential for the hydrolysis of the lactose component of milk in young mammals. Unlike other intestinal digestive carbohydrases such as sucrase and maltase, which are present at birth or shortly after weaning and are maintained at maximal levels throughout the life of mammals (1, 2), intestinal lactase wanes abruptly either at the time of weaning or, in most human population groups, in young childhood or adolescence (3, 4). Several theories have been proposed to explain this phenomenon, but the exact molecular mechanisms responsible for the abrupt maturational loss of activity have not been defined.

The study of the structure and function of intestinal lactase has been difficult because its purification is associated with appreciable loss of activity, and multiple putative subunits have been identified by acrylamide electrophoresis (5–7). The enzymatically active native enzyme has been characterized after solubilization as a macromolecule of 250–320 kDa in rat (9), pig (9), and human (6, 10–13) small intestine. Analysis by denaturing acrylamide gel electrophoresis has revealed several protein subunits, the most predominant being a ~130-kDa species in suckling or neonatal rats (5, 14–16). Hence, the enzyme is generally believed to be present in the brush border as a dimer, but there has been no systematic analysis of the subunit structure or synthesis and assembly of the lactase glycoprotein in the adult mammal where catalytic activity is markedly reduced.

Studies of lactase synthesis have involved in vitro radiolabeling of short-term intestinal explant cultures where rapid synthesis of a large lactase species (>200 kDa) was found in pig (17) and human (13) explants by SDS-acylamide electrophoresis of solubilized crude microsomal membranes. This suggested that lactase may be synthesized initially as a single polypeptide and then cleaved to the 130-kDa species either within the cell or after its insertion into the brush-border membrane. Analogous studies in sucking rat intestinal explants indicated that lactase appears as a 220-kDa moiety in association with intracellular membranes and is transported to the brush-border surface where it is processed in sequence to 180- and 130-kDa forms (18).

We have found that both the structure and in vivo synthesis and assembly of intestinal lactase are modified dramatically in the adult rat, a catalytically inactive 100-kDa species being the predominant moiety synthesized in vivo. These post-translational alterations, along with the known shortened life span of the enterocyte (16), appear to account for the decline of lactase catalytic activity during maturation. The mecha-
nisms of functional lactase decline in the neonatal intact rat is considered in the accompanying article (19).

**EXPERIMENTAL PROCEDURES**

**Chemicals and Reagents**—Horseradish peroxidase, gluco oxidase, amino-9-ethylcarbazole, Tris, 1-methionine, diethrealcohol, Coomassie Blue, leupeptin, aprotinin, 2-mercaptoethanol, p-nitrophenol mannose, p-nitrophenol, phenylmethylsulfonyl fluoride, Nonidet P-40, N,N-dimethylformamide, p-choloromercuribenzene, and leucyl β-naphthylamidane were obtained from Sigma. Emulphogene BC-720 was from Perkin-Elmer, leupeptin just distilled to the dimethylformamide, SDS from Drabkin (Lab Diagnostics). Sodium perborate was from Abbott. N',N',N'-Methylenebisacrylamide, acrylamide, ammonium persulfate, and N',N',N',N'-tetramethylethylene diamine were obtained from Bio-Rad. Lactose, cellulose, sucrose, and glucose were obtained from Pfannstiel. Goat anti-rabbit horseradish peroxidase was obtained from Tago (Burlingame, CA). Staphylococcus aureus cells were obtained from Calbiochem-Behring. All other chemicals were purchased as reagent grade.

**Animals and Membrane Preparations**—Male adult Wistar rats (>4 months of age, 200-400 g) were kept in individual cages and fed a commercial rat chow ad libitum. After being allowed only water for 12 h, the animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and killed by decapitation. The isolated loop was removed and washed with 150 mM NaCl, 5 mM Tris (pH 7.0), 200 mM NaCl (Blocking Solution) at 22°C for 30 min with constant agitation and then washed twice with the same solution. Monospecific polyvalent anti-lactase serum was raised in rabbits as described previously (8). Biochemical Assays—Leucyl-β-naphthylamidane hydrolysis, sucrose, and lactase activities were assayed as previously described (20). Samples were diluted prior to assay to eliminate the inhibitory effects of Tris. Sodium-potassium ATPase was determined by modification of the methods of Fujita et al. (21) and Fiske and SubbaRow (22). Mannosidase II activity was assayed as described previously (23). Protein was determined by the Bio-Rad assay (24); bovine serum albumin was the standard protein.

**RESULTS**

**Molecular Forms of Lactase in the Steady State Brush-Border Pool**—HPLC gel filtration of the detergent-solubilized brush-border membrane preparations on GF-450, GF-250, and brush-border preparations at 22°C by HPLC on 250 X 9.4-mm DuPont Zorbax GF-450 and GF-250 columns, either alone or coupled in series, and eluted with 0.010 M sodium-potassium phosphate (pH 7.0), 2 mM Tris, 0.02% Nonidet P-40 at 0.3 ml/min. Estimation of molecular mass was based on standards: γ-globulin, ovalbumin, myoglobin, vitamin B12, rat succrase—a-dextrinase, and rat amino-oligopeptidase.

After assay of fractions for lactase activity, cold trichloroacetic acid was added to achieve a final concentration of 7%, and precipitates, recovered by centrifugation at 10,000 × g for 5 min, were washed four times with cold acetone and solubilized in 62.5 mM Tris-HCl (pH 6.8) containing 2% SDS, 10% glycerol, 1 mM EDTA, 10 mM DTT. Proteins were separated on a 6.5% acrylamide gel and transferred to nitrocellulose as described by Towbin et al. (27). Residual binding sites on the nitrocellulose matrix were blocked with 2% fetal bovine serum in 50 mM Tris (pH 7.5), 200 mM NaCl (Blocking Solution) at 22°C for 30 min with constant agitation and then washed twice with the same solution. Monospecific polyvalent anti-lactase serum, raised in rabbits as detailed previously (8), was diluted 1:150 and incubated with 100 µl of buffer changes over 1 h and in Blocking Solution for 15 min. Goat anti-rabbit globulin linked with horseradish peroxidase was diluted 1:1,000 in Blocking Solution and overlaid at 22°C for 1 h. The nitrocellulose sheet was then washed three times with 200 mM NaCl, 50 mM Tris (pH 7.5), followed by 0.010 M phosphate-buffered saline (pH 7.4), 0.05% Tween 20. The peroxidase was localized by the freshly prepared chromagen (4 mg of 3-amino-9-ethylcarbazole in 1 ml of N,N-dimethylformamide, filtered, and diluted 1:5 with 0.05 mM sodium acetate buffer, pH 5.0, containing 0.036% hydrogen peroxide) which was reacted in the dark for 10 min and then flushed with distilled water five times to remove excess soluble chromagen.

**Immunoprecipitation and Acrylamide Gel Electrophoresis**—The purification and recovery of ER-Golgi and brush-border fractions in individual rats was monitored by specific markers (mannosidase II for ER-Golgi; sucrose and leucyl-β-naphthylamidase for brush border), as detailed previously (23, 28). The isolated ER-Golgi and brush-border membranes were solubilized by sonication in Detergent Buffer, separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, transferred to nitrocellulose, and sectioned by immunostaining with specific polyclonal antibody (binding capacity 1 milliunit of lactase/µl antiserum), raised by injection of rabbits with pure lactase from neonatal rats as detailed previously (8). This anti-lactase precipitates all lactase activity but no maltase, sucrase, alkaline phosphatase, or aminopeptidase. Aliquots of subcellular fractions (5-100 µg of proteins (5-100 milliunits of lactase activity) were incubated with anti-lactase for 15 min at 37°C in phosphate-buffered saline, 0.5% Nonidet P-40, and then overnight at 4°C with constant slow rotation of the tube. Fixed S. aureus cells were then added (0.06-0.50 ml of 10% suspension in 50 mM sodium phosphate, pH 7.5) to bind immune complexes, and the mixture was incubated for 15 min at 37°C in a shaking water bath and then for 5 h at 4°C in a rotator. The post-purification supernatant was assayed to verify that all lactase had been immunoprecipitated. Immune pellets were washed four times in 0.0625 M Tris (pH 6.8); the final precipitate was suspended in the same buffer containing 2% SDS, 10% glycerol, 1 mM EDTA, 10 mM DTT (Denaturing Buffer) and heated to 100°C for exactly 2 min. Preliminary experiments (data not shown) revealed that this heat treatment resulted in incomplete solubilization, while more vigorous treatment (3-5 min, 100°C) cleaved the subunits variably to smaller fragments. SDS-electrophoresis autoradiography and quantitation of radioactivity in the individual lactase species was carried out as detailed previously (28), except that immune pellets were treated as described above, and the separating gel was 6.5% total acrylamide with 0.3% cross-linking.

**RESULTS**

**Molecular Forms of Lactase in the Steady State Brush-Border Pool**—HPLC gel filtration of the detergent-solubilized brush-border membrane preparations on GF-450, GF-250,
and coupled GF-450 and GF-250 columns revealed a single major peak of lactase activity having a mass of ~235 kDa (Fig. 1). Proteins from individual fractions were precipitated by trichloroacetic acid, separated by SDS-polyacrylamide gel electrophoresis under denaturing and reducing conditions, and transferred to nitrocellulose sheets. Immunoblotting with the polyclonal anti-lactase antibody revealed several molecular species of lactase, as shown in Fig. 1 (insets). Three major bands of immunolactase were identified consistently across the lactase activity peak: 225 kDa, 130 kDa, and a relatively diffuse 100-kDa species. Notably, the 100-kDa band was not distributed as symmetrically across the lactase peak, and the most intensely staining fractions were present in those that eluted prior to the lactase activity peak. This suggested that it was in an inactive and aggregated species, perhaps in the form of a homotrimer or oligomer in association with other macromolecules having an overall molecular mass of 300 kDa or greater.

When immune pellets of lactase from detergent-solubilized brush-border membrane samples were placed in Denaturing Buffer at 100 °C for 2 min and applied directly to acrylamide gels, three molecular forms were observed: the major 130- and 225-kDa moieties and the more diffuse 100-kDa species. Fig. 2 shows this pattern for seven individual adult rats. Hence, the 100-kDa moiety, while a minor component of the catalytically active lactase (Fig. 1), was found uniformly as a prominent species in the brush-border membrane compartment.

The in Vivo Synthesis and Assembly of Lactase—Previous work in this laboratory on the in vivo intraluminal radiolabeling of adult rats with radioactive amino acid precursors revealed a stoichiometric pattern of synthesis and assembly of other intestinal hydrolases in association with ER, rapid movement to Golgi, and directional transport to the brush-border surface membrane (20, 28). This same experimental approach was used to study lactase. As expected for the adult animal which has relatively low levels of catalytic activity, the extent of lactase synthesis was about 25–30% of that previously found for other, more abundant, digestive hydrolases. Yet the dynamics of synthesis and assembly of multiple molecular species of lactase could be quantified in individual adult rats after the intraintestinal [35S]methionine pulse.

A typical SDS-electrophoresis-autoradiogram from a series of intraluminal [35S]methionine pulse-chase experiments is shown in Fig. 3. Several labeled species (175, 130, 100, and 64 kDa) appeared simultaneously in the ER-Golgi compartment just 15 min after the [35S]methionine pulse and were maintained until 60 min of chase. With the possible exception of the 175-kDa moiety, these newly synthesized species then

![Fig. 1.](http://www.jbc.org/content/early/1977/158/4/515/F1.large.jpg)  
**Fig. 1.** High performance liquid chromatography of detergent-solubilized brush-border membrane proteins (see "Experimental Procedures" for details). Purified brush-border membranes from four adult rats were solubilized in 1% BC-720, applied to the Zorbax GF-450 column, and eluted with 0.01 M sodium phosphate (pH 7.0), 150 mM NaCl, 2 mM Tris. Fractions (0.2 ml) were collected at 1 ml/min and assayed for lactase activity, given on the ordinate as absorption at 495 nm produced by the released glucose product from incubation with lactose substrate; recovery of added lactase activity was 90%. The void volume, determined by thyroglobulin, was at 5.5 min. The insets display the lactase subunit patterns of three pooled fractions that were subjected to denaturation and acrylamide electrophoresis, blotted to a nitrocellulose membrane, and probed with anti-lactase. The molecular mass of the lactase peak, determined from log-linear plots of standard proteins, was 225 kDa. Note the presence of the prominent 100-kDa species in the larger mass fractions (A and B, ~300 kDa and larger) that are devoid of catalytically active lactase.

![Fig. 2.](http://www.jbc.org/content/early/1977/158/4/515/F2.large.jpg)  
**Fig. 2.** SDS-acrylamide electrophoresis of lactase immunoprecipitates from rat brush-border membranes. Brush borders, prepared from seven different adult animals, were detergent-solubilized with Nonidet P-40, immunoprecipitated with anti-lactase, treated with Denaturing Buffer at 100 °C for 2 min, and subjected to acrylamide electrophoresis and stained with Coomasie Blue (see "Experimental Procedures"). Notably, all animals displayed the same pattern of the three species of lactase.

![Fig. 3.](http://www.jbc.org/content/early/1977/158/4/515/F3.large.jpg)  
**Fig. 3.** Molecular species of lactase by SDS-electrophoresis-autoradiography in adult rats pulsed intraintestinally with [35S]methionine. After the 5-min pulse, animals were perfused intraluminally with unlabeled methionine for the periods shown. Each chase time represents data from an individual rat. ER-Golgi and brush-border (BB) fractions were prepared and the lactase immunoprecipitated, denatured, and applied to the acrylamide gel, as detailed under "Experimental Procedures." The mass of each major lactase species is based on log-linear plots (see "Experimental Procedures"); the prominent, most rapidly moving band in ER-Golgi at 15–60 min is the 64-kDa species (cf. Fig. 4).
declined appreciably by 120 min. In contrast to the findings for other intestinal digestive hydrolases such as sucrase or amino-oligopeptidase in the adult (28) or lactase in the neonate (18), there was no readily identifiable precursor-product sequence of intracellular assembly for lactase in the purified ER-Golgi membrane pool of adult rats. With the exception of the 64- and 175-kDa moieties which represented minor bands in the brush-border autoradiograph (Fig. 3, far right lane), several species appeared to be transferred to the brush-border surface membrane at 120 min. Notably, the 130-kDa and particularly the 100-kDa species of newly synthesized lactase predominated in the brush border at 120 min, and the 225-kDa moiety, although less prominent, was always detected readily at the enterocyte’s surface at a time when ~25% of the radioactivity in lactase had been transferred from ER-Golgi to brush border (Fig. 3, 120-min lane). The relative predominance of the 175-kDa form as the intracellular species having the largest mass (Fig. 3, ER-Golgi lanes) and its apparent replacement with the 225-kDa species later in the brush border (Fig. 3, right lanes) suggests that it was processed to the 225-kDa species during the assembly and transfer from ER-Golgi to brush border. The 64-kDa form was evanescent in the ER-Golgi (compare 15 and 120 min, Fig. 3) and was barely detectable in the brush border at 120 min. Notably the 225-kDa band was a component of the detergent-solubilized lactase (Fig. 1) and was always readily identifiable on Coomassie-stained SDS gels of brush border (Fig. 2). In contrast, although not identifiable by protein staining, the 175-kDa band was prominent in the autoradiogram of ER-Golgi membranes. Overall, this in vivo study of lactase synthesis and assembly in the adult rat suggests that the several forms of lactase are synthesized very rapidly and perhaps proteolytically processed in association with ER and Golgi prior to their transport to the luminal membrane, thereby producing a complex array of lactase molecular structures.

The stoichiometry of in vivo lactase synthesis and assembly is considered further in Figs. 4 and 5. The protein bands were excised from dried SDS-acrylamide gels and the radioactivity quantified for the periods after the intraintestinal pulse. Because total incorporation into lactase was variable between sets of experimental animals but consistent within an experimental group, comparisons were made by expressing the incorporation into individual lactase immunoprotein species as a percentage of the total radioactivity in lactase immunoprecipitates. Fig. 4 shows the data from a typical set of four animals. Maximal radioactivity was incorporated into lactase in ER-Golgi by 60 min, while appreciable transfer of the radiolabeled lactase to the brush border was apparent by 120 min, when it represented about 20–30% of the maximal incorporation achieved for lactase in the ER-Golgi compartment.

Fig. 5 shows the relative kinetics of [35S]methionine incorporation into the individual lactase species in ER-Golgi membranes. Adult rats were pulse-chased intraluminally, and ER-Golgi fractions were prepared and run on acrylamide electrophoresis, as detailed under “Experimental Procedures.” Radioactive lactase bands, localized by the gel autoradiogram as a template (cf. Fig. 3), were excised, solubilized, and the radioactivity determined. Each plotted value (mean for four animals) represents the relative fractional distribution of radioactivity in the lactase species, where 100% is the sum of all of the subunit species. (S.E. is ±2–5 at each data point omitted for clarity except for the 100 and 130 kDa values at 120 min). The labeling of the 100-kDa species was significantly greater than that for the 130-kDa subunit at 120 min (p < 0.05; paired analysis).

Fig. 4. Kinetics of incorporation of [35S]methionine into lactase in intact adult rats. Conditions were as detailed in the legend to Fig. 3. Total radioactivity in lactase immunoprecipitates from ER-Golgi (ERG) and brush-border membranes, based on recovery of specific marker enzymes (see “Experimental Procedures”), was determined at each chase period for four rats. Note that 2 h after the pulse, ~25% of the maximal radioactivity present in the ER-Golgi (60 min) had been transferred to the brush border.

Fig. 5. Kinetics of [35S]methionine incorporation into individual lactase species in ER-Golgi membranes. Conditions were as detailed in the legend to Fig. 3. Total radioactivity in lactase immunoprecipitates from ER-Golgi (ERG) and brush-border membranes, based on recovery of specific marker enzymes (see “Experimental Procedures”), was determined at each chase period for four rats. Note that 2 h after the pulse, ~25% of the maximal radioactivity present in the ER-Golgi (60 min) had been transferred to the brush border.
becoming a prominently labeled species that is transferred to the brush border at 120 min. Indeed, the incorporation of the \(^{35}\)S\text{methionine precursor into the 100-kDa species in ER-Golgi at 120 min was uniformly greater than that found for the 130-kDa subunit, the 100/130 ratio being 1.7 (Figs. 3 and 4; p < 0.05; paired t analysis, \(t\) test).

The relative incorporation of \(^{35}\)S\text{methionine into the lactase species that are inserted into the brush border at 120 min is shown in Table I. Notably, the 100- and 130-kDa units predominated, and the \(-2:1\) 100/130 ratio present intracellularly in the ER-Golgi was maintained after insertion into the surface membrane. The 225-kDa species accounted for less than 10% of the radiolabeled species.

**DISCUSSION**

**Subunit Structure in the Adult Rat**—Because lactase readily denatures after solubilization and purification under optimal storage conditions (29), it has been the most difficult digestive hydrolase to isolate and characterize. Although stability of its catalytic activity has been enhanced by the addition of Tris (8) and \(p\)-chloromercuribenzoate (14), our data indicate that this does not protect the enzyme from extensive proteolytic cleavage during isolation when papain is used to remove it from the membrane, even though there is maintenance of hydrolytic activity (cf. “Experimental Procedures”). For this reason, we have restricted our experiments to the detergent-solubilized enzyme in the presence of protease inhibitors.

Gel filtration by HPLC showed that lactase, when detergent-solubilized from brush-border membranes, had an estimated molecular mass of 235 kDa, similar to that reported by Neu et al. (15) who used low pressure gel filtration on TSK, a vinyl polymer (EM Science, Gibbstown, NJ). The larger molecular mass (300-400 kDa) found for the human enzyme (12, 13) is probably due to species differences in post-translational glycosylation, since the lactase cDNA codes for a protein chain of nearly identical mass for vastly disparate mammalian species (30).

Probably because of the relative sensitivity to endogenous proteases and the common use of papain for solubilization, variable results for lactase subunit structure have been reported. Birkenmeier and Alpers (14) identified five bands for papain-solubilized suckling rat lactase under denaturing conditions, the major one being approximately 100 kDa. Later, Neu et al. (15) confirmed that purified suckling rat lactase had a major 130-kDa species under reducing conditions; Tsuibo et al. (8) raised a polyvalent antiserum to purified lactase from infant rats and found multiple subunits by immunoprecipitation under denaturing and reducing conditions (10).

Using the same antibody as Tsuibo and a modified electrophoresis system to examine the enzyme preparation that was detergent-solubilized in the presence of protease inhibitors, we now show that the steady state lactase pool in brush border in the adult rat consists of 295-, 130-, and 100-kDa units (Fig 2) and that the two larger species predominate in the native detergent-solubilized 235-kDa active moiety isolated from HPLC-gel filtration (Fig. 1). Thus, despite the use of intestinal pre-perfusion to remove luminal pancreatic proteases and inclusion of protease inhibitors during all preparative steps, a complex subunit pattern is present in the adult rat. The 225- and 130-kDa species were identified consistently in the symmetrical peak of active purified lactase isolated from gel filtration experiments (cf. Fig. 1) and hence appear to be closely associated with the active, native enzyme in the enterocyte’s surface membrane. Although the exact relationship of the different molecular forms will require further study, we suggest that the active lactase, having a molecular mass of \(~235\) kDa (Fig. 1), is present in the intestinal surface membrane both as a monomer and as a dimer consisting of two 130-kDa subunits or possibly of a 130-100-kDa hybrid.

Although the 100-kDa species is a component of the catalytically active lactase protein, the bulk of this form is present as an inactive, aggregated moiety with a mass of \(~300\) kDa (Fig. 1). Analysis of immunoblots (Fig. 1, insets) and autoradiograms (Figs. 3 and 5) of denaturing acrylamide electrophoretic gels reveals that the 100-kDa unit represents a major fraction of the total lactase immunoprotein in the adult rat. Because parallel examination of lactase subunit structure in neonatal rats has shown very little of the 100-kDa species (accompanying article, 19), we suspect that the 100-kDa inactive unit is produced from immediate post-translational intracellular processing (cf. Fig. 3, 15-min ER-Golgi lane) that occurs prior to brush-border insertion in the adult animal. The combination of rapid intracellular processing of the lactase protein to the inactive 100-kDa form and to the transiently expressed 64-kDa form appears to play an important role in the decline in lactase activity after the neonatal period. Although previous studies had not revealed the 100-kDa species in the pig (17) or rat (18), these were carried out on intestinal explants which may have altered regulation of protein synthesis and processing as compared to the intact animal. Also, the 100-kDa species may not possess the particular epitope recognized by the monoclonal antibody used in the rat explant studies (18).

Nsi-Envo et al. (31), using mild denaturing conditions, found an inactive 300-kDa lactase species in a calcium-precipitated membrane fraction of adult rat intestine which was not present in the suckling animal, and these authors suggested that this macromolecule represented an inactive lactase protein that accumulates and is degraded inside the cell. However, the calcium-precipitated intracellular membrane fraction used by these investigators is heavily contaminated with brush-border membranes which could harbor the 300-kDa unit, and, in highly purified intestinal organelle preparations, we could not identify a 300-kDa glycopeptide subunit despite finding easily detectable lactase activity in ER-Golgi membranes. Furthermore, the treatment of intestinal membranes at \(29^\circ\)C for 10 min as used by Nsi-Envo et al. (31) prior to acrylamide electrophoresis does not release a true subunit (see Ref. 5 for denaturation requirements for lactase).

We suspect that the putative 300-kDa species of Nsi-Envo et al. (31) is actually an aggregate of the inactive 100-kDa moiety which we have identified in the solubilized brush-border fraction (cf. Figs. 1 and 2) and which constitutes the major species of newly synthesized lactase in both the ER Golgi and brush-border membrane compartments of the adult rat (Figs. 3 and 5 and Table I).

**Molecular Species of Lactase during Intracellular Synthesis, Processing, and Transfer to the Brush Border**—Previous stud-
ies of mammalian lactase synthesis and assembly in short-term culture of pig intestinal explants revealed an early-labeled polypeptide precursor that undergoes post-translational carbohydrate chain modification and is eventually cleaved to the brush-border form. Initially, a 225-kDa form containing immature N-linked carbohydrate chains of lactase was found in calcium-precipitated membranes (17). This eventually matured to yield 245- and 160-kDa bands, both of which appeared to contain complex N-linked carbohydrate chains. The predominant 160-kDa form appeared to be a cleavage product of the 250-kDa unit. Similar studies of Bülter et al. (18) in infant rat explants revealed that a 220-kDa form was synthesized initially and then converted in sequence to a 180- and 130-kDa species. In contrast, a detailed analysis has revealed discrete but distinctly different sequence of events in intact neonatal rats (accompanying article, 19).

In contrast to the apparent orderly procession of events for lactase assembly in the neonatal period, our in vivo experiments suggest a very different mode of intracellular assembly in the adult rat. Analysis of highly purified ER-Golgi membranes revealed that newly synthesized lactase exists in a variety of molecular species (225, 175, 130, 100, and 64 kDa) early in intracellular membrane-associated synthesis only 15 min after an intraintestinal pulse of $[^35]S$methionine to intact rats (Fig. 3). Although, at first analysis, these forms might be considered to represent fragments produced during membrane isolation, pre-perfusion of the intestinal lumen and inclusion of protease inhibitors in the isolation buffers has prevented fragmentation of the single-chain succrase-a-dextrinase in our laboratory. Hence we believe that lactase is either synthesized de novo in several molecular forms in the adult rat, or perhaps more likely, undergoes much more rapid and complex post-translational intracellular processing than in the infant rat. The smallest of these lactase species, the 64-kDa moiety, appears to disappear rapidly from the intracellular membrane synthetic compartment and is not transferred to any extent to the brush-border surface (cf. Figs. 3 and 5). In contrast, substantial amounts of the 130-kDa subunit are transported to the brush border as early as 120 min. The prominent 175-kDa unit in the ER-Golgi (Fig. 3, left) appears to be a minor form in the brush border but is probably processed to the larger 225-kDa unit during its assembly (Fig. 3, right). Although the use of protease inhibitors during incubation of explants from infant rats prevented the appearance of the 130-kDa species (18), this moiety was prominent in the ER-Golgi compartment in the intact adult rat at only 15 min of chase (Fig. 3, left lanes), despite inclusion of protease inhibitors in the intestinal pre-perfuse and at all stages of membrane isolation.

Clearly, the initial lactase synthesis in the adult rat is brisk and several molecular species are transferred to the brush border (Figs. 3 and 4); indeed, nearly one-third of the lactase glycoprotein initially synthesized is transferred to the brush border by 2 h (Fig. 4). This timing of assembly is similar to that found in our laboratory for other intestinal hydrolases in the adult rat (20, 28) and is also compatible with preliminary reports that lactase mRNA levels may be maintained in the adult rat even when there is low catalytic activity of the enzyme (30, 32).

Mechanism of Catalytic Decline of Lactase in the Adult Mammal—Tsuboi et al. (16) suggested that decline in lactase activity in the post-weaned animal, rather than being due to a decrease in its synthesis, involved a reduction of the enterocyte’s life span and a consequent loss of time for its expression. In contrast, Smith and James (53), while agreeing that the enterocyte’s shortened life span is important, suggested there was also an inhibition in the rate of lactase synthesis. Freund et al. (32) recently reported that the decline of lactase in the adult rat and pig was not accompanied by a parallel decline of lactase mRNA; this suggests that a maturation decrease of lactase transcription may not occur and hence would not account for the decline in catalytic activity. This is compatible with our structural and kinetic findings for the lactase glycoprotein in vivo. Also, because the dynamics of lactase synthesis, assembly, and transport in the adult rat closely approximated that previously reported in infant rat explants (Fig. 3 and Ref. 18), it seems unlikely that the decline of lactase activity during maturation could be due to a delay in translation or transport to the brush border.

In this study, we have observed a previously unrecognized species of lactase in the adult rat. Notably, the 100-kDa moiety represents the major newly synthesized species present at the brush-border surface after 2 h of chase (cf. Figs. 3 and 5). Since the particular processing step that produces the 100-kDa species is associated with both a tendency to aggregate with itself or other macromolecules (see Fig. 1) and an appreciable loss of catalytic activity, it is likely to derive from degradation and denaturation of larger subunits. Such a process may constitute a major means of down-regulation of lactase catalytic activity in the adult mammal. This concept is supported by the findings that the 100-kDa species is absent in the nursing rat, when lactase activity is maximal (cf. accompanying article, 19).

These differences in lactase structure in neonatal and adult rats could be explained by a variety of mechanisms during co- and post-translational assembly. Intracellular proteolytic processing of the glycoprotein may change as the mammalian intestine matures. In particular, alterations in glycosylation with maturation of the animal may facilitate this by uncovering sites for post-translational proteolytic processing during intracellular assembly.

Although an alteration in the processing of mRNA transcripts or a slowing of translation and membrane-associated assembly could possibly contribute to the maturational decline of lactase activity, there is no need to implicate these as additional mechanisms. Our experiments provide strong evidence that an appreciable proportion of lactase protein synthesis in the adult rat has been redirected toward production of catalytically inactive molecules which are either degraded intracellularly (the 64-kDa species; Figs. 5 and 7) or transferred to the brush-border surface as major but inactive components (the 100-kDa species; Figs. 1–3, and 5). Whether there is a secondary physiological role for the 100-kDa unit will be of interest.

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Note Added in Proof—After this and the accompanying article (19) were submitted, documentation of the persistence of high levels of lactase mRNA in adult rats has been published (Bülter, H. A., Kothe, M. J. C., Goldman, D. A., Grubman, S. A., Sasak, W. V., Matsudaïa, P. T., Montgomery, R. K., and Grand, R. J. (1990) J. Biol. Chem. 265, 6978–6983). This finding is totally compatible with the persistence of appreciable proportion of lactase protein synthesis in the adult rat has been redirected toward production of catalytically inactive molecules which are either degraded intracellularly (the 64-kDa species; Figs. 5 and 7) or transferred to the brush-border surface as major but inactive components (the 100-kDa species; Figs. 1–3, and 5). Whether there is a secondary physiological role for the 100-kDa unit will be of interest.

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