PROTEINS OF CHICK DUODENAL BRUSH BORDERS DURING DEVELOPMENTAL CHANGES1

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Summary Duodenal brush border membrane proteins were studied in chicks at different developmental stages. The protein pattern obtained from polyacrylamide gels with 2-day-old chick preparations was distinctly different from that obtained with 20-day embryos. The most remarkable changes were seen in the region of a protein with an Rf of 0.25, an area with high sucrase and maltase activity, and in the region of a protein with an Rf of 0.28, which was characterized by alkaline phosphatase activity. These proteins reacted strongly with carbohydrate stain after hatching.

It is known that the intestinal brush border is the site of terminal digestion and absorption of nutrients. Hydrolytic enzymes, such as disaccharidases, alkaline phosphatase, dipeptidases, etc., are located on the brush border membranes. These enzymes are induced during development, at the time of weaning in mammals (1-4) and at the time of hatching in birds (5-7).

The chick embryo obtains its nutrients from the yolk by a direct absorption via blood vessels surrounding the yolk sac. Shortly before hatching, the intestinal transport systems became activated and transport across the intestine is initiated at the time of hatching. A comparison of chick intestines before and after hatching thus provides a good model system for the study of transport mechanisms.

Although solubilized membrane proteins have been examined by gel electrophoresis (8), very little is known about the developmental changes of the chick intestinal brush border membranes. In this paper, we report our work on duodenal brush border membrane proteins from prehatched and newly hatched chicks.

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Abbreviations used in this paper: SDS, sodium dodecyl sulfate; Tris, tris-(hydroxymethyl)-aminomethane; DTT, dithiothreitol; PAS, periodic acid Schiff; EDTA, ethylenediaminetetraacetic acid; 1,25-(OH)2-D3, 1,25-dihydroxyvitamin D3.
METHODS

1. Animals. Fertilized eggs of White Leghorn hens were obtained from Kazusa Hatchery (Chiba, Japan) and incubated at 37.5°C and 60% relative humidity. After hatching, chicks were raised on commercial laboratory chow (Oriental Yeast Co., Osaka).

2. Preparation of brush borders from duodenum. Brush borders were prepared from duodena of 20-day chick embryos and 2-day-old chicks according to the method of Förster et al. (9). For those preparations, 50 embryos and 10 chicks were used. When used for enzyme assays, the brush borders were washed 3 times with 0.01 M Tris-HCl-5 mM MgCl₂ (pH 8.0).

3. Enzyme assays. Maltase and sucrase were assayed by the method of Dahlqvist (10). Substrate concentration was 28 mM in 50 mM sodium maleate buffer (pH 6.0). Activity was expressed as μmol disaccharide hydrolyzed/hr/mg protein. Alkaline phosphatase was assayed using p-nitrophenylphosphate as a substrate at pH 10.0. Substrate concentration was 8 mM in 0.5 M 2-amino-2-methyl-1-propanol buffer (pH 10.0) (11). Enzyme activity was expressed as μmol p-nitrophenol produced/min/mg protein. L-Leucyl-β-naphthylamide was assayed by the method of Goldberg and Rutenberg (12), and the activity was expressed as μmol substrate hydrolyzed/hr/mg protein.

4. Polyacrylamide gel electrophoresis. The brush borders were solubilized in 1% SDS solution containing 10% sucrose, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0), 40 mM DTT, and 10 μg/ml of pyronin Y (tracking dye) for 30 min at 37°C. The brush border protein concentration was approximately 1 mg/ml. The solubilized proteins, approximately 50 μg, were separated on polyacrylamide gels (5.6% in acrylamide) in the presence of 1% SDS, according to the method of Fairbanks et al. (8). Electrophoresis was performed for 2 hr at 6 mA/gel. Three different gels of the same sample were run simultaneously. One gel was stained for protein with Coomassie blue. The second gel was stained for carbohydrate with the periodic acid-Schiff (PAS) procedure as described by Fairbanks et al. (8). The third gel was immediately frozen and then cut into 1 mm slices by Yeda macro-tome (Yeda Scientific Instruments, Rehovot-Israel); they were soaked in 0.3 ml of redistilled water for 1 hr at room temperature; then maltase, sucrase, and alkaline phosphatase activity was assayed as described under “Enzyme Assays.” Enzyme activity was expressed as μmol substrate hydrolyzed or product produced/hr/slice.

A rough calibration of the gels was done comparing the relative mobility of the following standard proteins: apoferritin (Schwarz/Mann Y1292), albumin (Schwarz/Mann X1422), ovalbumin (Schwarz/Mann Y1011), chymotrypsinogen (Schwarz/Mann X3595), and cytochrome c (Schwarz/Mann Y1097).

5. Protein determination. Protein concentration was determined by the method of Lowry et al. using bovine serum albumin as the standard (13).

6. Chemicals. SDS, 2-amino-2-methyl-1-propanol, and Coomassie bril-
liant blue were purchased from Tokyo Kasei Industries Co., Ltd. Tris, DTT, and l-leucyl-β-naphthylamide were obtained from Sigma Chemical Co. N, N, N’, N’-Tetramethylethylenediamine was obtained from Daiichi Pure Chemicals Co., Ltd.; acrylamide from Eastman Kodak Co.; N, N’-methylenebisacrylamide from Nakarai Chemicals, Ltd.; EDTA and sucrose from Kokusan Chemical Works, Ltd.; ammonium persulfate from Kishida Chemicals, Ltd.; glucose oxidase from Worthington Biochemical Co.; and pyronin Y, maltose, p-nitrophenyl-phosphate, and the other chemicals from Wako Pure Chemical Industries, Ltd.

RESULTS

1. Comparison of chick duodenal brush border membrane proteins by SDS polyacrylamide gel electrophoresis

Chick duodenal brush border enzymes are known to show increased activity at the time of hatching (5–7), which suggests that considerable changes must have occurred in the brush border proteins just before hatching. Thus, to visualize these developmental changes, the brush borders were isolated from 20-day chick embryos and 2-day-old chicks. Brush borders were solubilized in SDS solution and subjected to electrophoresis in the presence of 1% SDS. The protein pattern of 2-day-old chick duodenal brush borders was distinctly different from that of 20-day embryo. The differences were found in 8 protein bands: the protein bands

![Fig. 1. Chick brush border membrane protein patterns on polyacrylamide gels. A and C, 20-day embryo; B and D, 2-day old chick. A and B, Coomassie blue staining; C and D, carbohydrate staining.](image-url)
of \( Rf 0.12, 0.18, 0.25, 0.28, 0.44, \) and 0.7, all of which were increased after hatching; and the protein bands with \( Rf \) of 0.84 and 0.89, which were more prominent before hatching (as measured by Coomassie blue staining). The most conspicuous difference was seen in the region of protein with \( Rf 0.25-0.28 \) (Fig. 1A, B).

When the gels were stained with carbohydrate specific stain, the brush border proteins from 20-day embryos showed only a very weak reaction at the region of \( Rf 0.05, 0.83, \) and 1.0, while those of 2-day-old chicks showed a quite different pattern. A strong glycosylation was observed in at least 3 protein bands. These proteins had \( Rf \) of 0.25, 0.28, and 0.44 (Fig. 1C, D) and their molecular weights were estimated to be approximately 240,000, 200,000, and 100,000, respectively. Furthermore, two PAS-positive bands were observed at \( Rf 0.33 \) and 0.37 in 2-day chicks. On the other hand, the regions of \( Rf 0.25, 0.28, \) and 0.44 on the gels of 20-day embryos were not stained with the PAS reagent. The density of protein of these bands was faint, but comparable to that of \( Rf 0.33 \) and 0.37 in 2-day chicks. Thus, we can exclude the idea that the protein concentration of \( Rf 0.25 \) and 0.28 on the gels of 20-day embryos might be too low to detect its glycosylation on the gels by PAS-staining. These results suggest that glycosylation of membrane protein is important in the course of development.

2. Identification and assay of enzymes associated with brush border proteins separated on SDS-gels

Since digestive enzymes of the duodenal brush border are induced at the time of hatching in birds (5–7), it seemed very likely that some of the differences that we had observed in the brush border proteins could be ascribed to these enzymes. Enzymatic assay revealed that the protein with \( Rf \) of 0.25 had maltase and sucrase activity, the protein with \( Rf \) of 0.28 had alkaline phosphatase activity and the

| Table 1. Enzymatic activities of chick duodenal brush borders. |
|---------------------------------------------------------------|
| 20-day embryo | 2-day-old chick |
| Sucrase\(^a\) | 1.63 | 2.96 |
| Maltase\(^a\) | 7.94 | 51.85 |
| Alkaline phosphatase\(^b\) | 1.67 | 17.50 |
| L-Leucyl-\(\beta\)-naphthylamidase\(^a\) | 11.72 | 44.62 |

\(^a\) \(\mu\)mol/hr/mg protein.

\(^b\) \(\mu\)mol/min/mg protein.

| Table 2. Recovery of enzyme activity after polyacrylamide gel electrophoresis in the presence of SDS. |
|---------------------------------------------------------------|
| 20-day embryo | 2-day-old chick |
| Sucrase | 50% | 52% |
| Maltase | 56 | 52 |
| Alkaline phosphatase | 7 | 0.5 |
| Leucyl-\(\beta\)-naphthylamidase | 0 | 0 |
protein with $R_f$ of 0.44 had maltase activity (Fig. 2). L-Leucyl-β-naphthylamidase activity was not detected in any of the protein bands examined as reported by MAESTRACCI et al. (14).

The enzyme activities were much higher in the brush borders from 2-day old chicks than in those from 20-day embryos (Table 1); sucrase activity was increased 1.8-fold; maltase 6.5-fold; alkaline phosphatase, 10-fold; and L-leucyl-β-naphthylamidase 3.8-fold.

About 50% of the sucrase and maltase activity from both the 20-day embryos and 2-day-old chicks could be recovered after electrophoresis, but no L-leucyl-β-naphthylamidase could be recovered. The recovery of alkaline phosphatase was 7% from the 20-day embryo preparations but only 0.5% from those of 2-day-old chicks (Table 2).

3. Effect of EDTA and SDS on alkaline phosphatase activity

Since EDTA is known to be a potent inhibitor of alkaline phosphatase, and since we used SDS and EDTA for the solubilization of brush border membranes,
it was necessary to study the effect of those compounds on the activity of alkaline phosphatase. These studies confirmed the strong inhibitory effect of EDTA on alkaline phosphatase activity, especially on the alkaline phosphatase from the 2-day-old chick duodenal brush borders (Fig. 3). SDS, on the other hand, did not affect the alkaline phosphatase from either preparation.

**DISCUSSION**

Recently Weiser (15) has demonstrated that, as the intestinal epithelial cell differentiates and matures during the migration from the crypt into villus, there is a corresponding increase in the incorporation of precursors into membrane glycoproteins. Our comparative studies with chicks at different levels of development showed that the duodenal brush border proteins, having sucrase, maltase, and alkaline phosphatase activity, increased in quantity and glycosylated after hatching. This suggests that duodenal brush border proteins of the 20-day embryo are not yet fully differentiated and that glycosylation of these proteins occurs after hatching and corresponds to an increased activity in hydrolytic enzymes. The activity and levels of disaccharidases and alkaline phosphatase were most markedly affected during these developmental changes. These findings lend support to the theory that cell surface membrane glycoproteins are important in the regulation of differentiation.

Another role for the carbohydrate-rich material in the brush border membranes might be that of an absorption barrier, since it is in the membranes of the
fully differentiated intestinal cell that the hydrolytic enzymes are located (16).

One of the proteins which we found to be increased in concentration and activity at hatching was a protein with alkaline phosphatase activity. This is of great interest because of the similarity of this protein to a protein that was found to be modified by vitamin D₃ and related to Ca transport in the intestine (17).

On the other hand, our studies with brush border alkaline phosphatase suggest that the difference in sensitivity of this enzyme to EDTA would be due to an altered isozyme pattern (as the result of increased glycosylation) during developmental changes, as reported by CHANG and MOOG (18). It is well known that the Ca transport mechanism is activated at the time of hatching in birds. Thus, comparison of brush border membrane proteins at various developmental stages seems to offer a useful system for the elucidation of membrane transport mechanisms.

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