Immunoelectron Microscopic Localization of Lactase-Phlorizin Hydrolase in Rat Small Intestine

Setsuko NODA and Toshinao GODA

Department of Morphology, School of Medicine, Tokai University, Isehara 259–11, Japan

1School of Food and Nutritional Sciences, The University of Shizuoka, Shizuoka 422, Japan

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Summary To provide insight into the intracellular translocation of lactase-phlorizin hydrolase, an immunoelectron microscopy was performed on rapidly embedded Lowicryl K4M sections of rat jejunum. Lactase-phlorizin hydrolase immunoreactivity was detected not only in the microvillous membranes and in the smooth apical vesicles, but also in the lateral membranes, suggesting an alternative route for intracellular transport of lactase-phlorizin hydrolase via the lateral membranes to the microvilli.

Key Words immunoelectron microscopic localization, Lowicryl K4M, lactase, microvillous membrane, lateral membrane, rat jejunum

Lactase-phlorizin hydrolase (LPH) belongs to a group of intestinal microvillous enzymes characterized as integral membrane proteins (1). Plasma membrane proteins of eukaryotic cell are commonly synthesized and assembled in the rough endoplasmic reticulum, pass through the Golgi apparatus, and are subsequently transferred to the cell surface (2–4). Previous studies on the biosynthesis and precursor forms of LPH demonstrated that LPH is initially synthesized as a large precursor protein (5–8). Molecular cloning of pre-LPH cDNA revealed that approximately 45% of amino acid residues in pre-LPH is lost before the mature form of LPH reaches the microvillous membrane (9). The studies on biosynthesis of LPH suggested a co-translational membrane insertion, high mannose glycosylation of the primary translation product in the rough endoplasmic reticulum and trimming and complex glycosylation of N-linked oligosaccharides in the Golgi apparatus before expressing in the microvillous membranes (5). However, most of previous experiments on intracellular processing of pre-LPH were carried out by pulse-labeling, followed by separation of Ca-precipitable intracellular membrane (ER-Golgi) fractions and the microvillous membrane fractions. In these methods, the treatment of basolateral membrane fraction was obscure and a complete separation between ER-Golgi fraction and basolateral membrane fraction seems
difficult. Therefore, it has been unclear whether newly synthesized pre-LPH pass from the Golgi apparatus directly to the microvillous membranes via the smooth apical vesicles as has been suggested for sucrase-isomaltase (10, 11) and for aminopeptidase N (12), or it is transferred to the lateral membrane before the insertion into the microvillous membranes.

In this study, we adopted immunoelectron microscopy to elucidate the intracellular localization of LPH in rat jejunum. At present, only a few reports are available regarding the distribution of LPH which was investigated by light microscopic immunohistochemistry (13, 14), and the detailed intracellular localization of LPH is still unclear. Because lactase activity is known to be sensitive to heat, we used the technique of rapid embedding in a low-temperature embedding medium, Lowicryl K4M (K4M), and we demonstrate in this study that LPH is localized not only in the microvillous membranes, but also in the lateral membranes.

MATERIALS AND METHODS

Suckling and weanling rats of Sprague-Dawley strain were used for the experiment. The rats remained with their mother until 12 or 28 days of age. The jejunum was dissected quickly, cut into small blocks and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2 (PBS) at 4°C for 1 h. After washing in PBS, tissues were dehydrated in a graded series of dimethylformamide (DMF) for 10 min in each step. Infiltration was completed in 1:2 Lowicryl K4M (K4M): DMF (10 min), 1:1 K4M: DMF (15 min), and 100% K4M (20 and 25 min) in order, and then the tissues were embedded in fresh 100% K4M in BEEM capsules followed by exposure to UV irradiation (UV polymerizer, EM-12A Nissin EM) for 2-4 h. All steps were performed at 4°C. It took about 6 to 8 h to finish the whole procedure by the way of rapid embedding (15). Silver and gold sections were cut and placed on nickel grids mounted with carbon-coated Formvar films and immunostained. The immunoelectron microscopic staining was a modification of the method of Lorenzsonn et al. (10). The grids were placed on a drop of 10% hydrogen peroxide, rinsed in PBS containing 1% filtered bovine serum albumin (BSA) for 5 min, and subsequently exposed to drops of primary antibody or control serum in PBS containing 1% BSA at 37°C for 2 h. The grids were then “jet washed” with PBS, placed in PBS, jet washed again, blotted on a filter paper, and immediately incubated with drops of gold-labeled goat anti-rabbit IgG conjugate as a secondary antibody probe for 45 min at room temperature. The grids were then jet washed with PBS, immersed for 5 min in PBS, jet washed again, placed in PBS, jet washed with distilled water, and blotted. The sections were stained with 4% aqueous uranyl acetate or both 2% uranyl acetate and lead citrate. Rabbit antiserum against LPH was used as primary antibody. The specific antiserum against LPH was prepared by immunizing rabbits with the purified rat enzyme as described previously (16). As an appropriate secondary antibody, goat anti-rabbit
immunoglobulin-collodial gold conjugate (10 and 15 nm, Auroprobe EM) from Janssen Life Science Products (Olen, Belgium) was used.

RESULTS AND DISCUSSION

Immunoelectron microscopic results for weanling rat jejunum are summarized in Fig. 1. The LPH immunoreactivity was always present in the microvillous membranes intensely, and it was also present in the smooth apical vesicles and in the apical membrane invaginations of the base of the microvilli (Fig. 1A–C), suggesting the presence of the route of intracellular transport of LPH directly from the Golgi apparatus to the microvillous membranes via the smooth apical vesicles, a common route suggested for various microvillous enzymes including sucrase-isomaltase (10, 11) and aminopeptidase N (12). However, not only the microvillous membranes, but also the lateral membranes often showed heavy staining for LPH immunoreactivity (Fig. 1A, C, F). As shown in Fig. 1B, gold particles were located at the extracellular side of the surface of the cross-sectioned microvilli. In addition, gold particles were also seen in the smooth vesicles nearby the lateral membranes or in the vesicles in cytoplasm except apical region of absorptive cells (Fig. 1A). However, negligible or no staining was observed in the Golgi apparatus, the basal membranes, the endoplasmic reticulum, the mitochondria, and nucleus (Fig. 1A, C, E). Results of suckling rats were similar to those obtained for weanling rats (data not shown).

As regards the biosynthesis and intracellular processing of LPH, the earlier reports have agreed in the points that LPH of pig (5), human (6, 7), and rat (8) is synthesized as a large precursor, and then cleaved into the mature form followed by dimerization (17). Molecular cloning of pre-LPH cDNA has revealed that approximately 45% of N-terminal region of newly synthesized pre-LPH is lost during the post-translational processing and that only the C-terminal part reaches the brush border membranes (BBM) (9). However, rat LPH might differ from those of human and pig in terms of post-translational processing of precursors. Büller et al. (8) suggested that a newly synthesized precursor of LPH in rat jejunum is transported to the BBM, where two subsequent cleavages occur, resulting in the accumulation of mature forms of LPH (13 kDa). In human and pig small intestine, the cleavage of pre-LPH to the mature form of LPH was reported to be leupeptin-sensitive, suggesting an intracellular processing, and the only form in the BBM was the mature 160 kDa form (5–7). These studies, however, did not elucidate the route of post-Golgi transport of pre-LPH and mature LPH. Therefore, we have investigated the intracellular localization of LPH immunoreactivity by immunoelectron microscopic technique.

Only a few reports have dealt with immunolocalization of LPH. A light microscopic study showed a staining of LPH immunoreactivity in the BBM (13, 14), consistent with the results of co-purification of lactase activity in BBM. However, this method was too insensitive to demonstrate the detailed intracellular
localization of the precursor forms. Recently, an immunoelectron microscopic study was reported on LPH in human enterocytes using monoclonal antibodies (18). They found that only BBM was stained with the monoclonal antibodies; no significant stain was seen in the Golgi and other intracellular membrane structures. Unfortunately, it was not clear whether the monoclonal antibodies used for the immunoelectron microscopy reacted with intracellular precursor forms of LPH as well. The monoclonal antibodies might have a strict antigenic specificity which might be restricted to the mature and/or dimeric form of LPH. The antibody used in the present study was a polyclonal antibody against rat lactase purified to a homogeneity (16). This antibody has been shown to react not only to mature form of LPH but also to large precursor forms (Goda et al., unpublished results). Thus, the discrepancies on the intracellular localization of LPH immunoreactivity between the previous report and ours might be ascribed to either the epitope specificity of the antibody used or the sensitivity for immunohistochemical detection. Because the replacement of anti-LPH antiserum with control rabbit serum produced no appreciable labeling in the microvillous membranes and lateral membranes, we consider that the anti-LPH antibody used in the present study specifically bound to LPH and/or its precursors located in the apical and lateral membranes.

Our rapid immunoelectron microscopic technique used in this study seemed to be a useful method to define the localization of pre- and mature forms of LPH because of a high degree of preservation of antigen by the work performed at low temperature, and the contraction of working time, and furthermore for reason that the direct demonstration of LPH on intestinal thin sections is technically feasible. The immunoelectron microscopic localization of LPH seems to be different from that of sucrase-isomaltase and aminopeptidase N; no significant staining was found in the lateral membranes for sucrase-isomaltase (10) and aminopeptidase N (19), whereas dense staining was present in BBM, smooth apical vesicles and in the Golgi apparatus. These electron microscopic results for sucrase-isomaltase were inconsistent with the proposed alternative transport route for microvillous glycoproteins.

Fig. 1. Electron micrographs of upper region (A–D and F) and Golgi region (E) of jejunal absorptive cell of weanling rat showing localization of lactase. Gold particles are seen on the microvilli (A–C) and the lateral membranes (A, C, F) intensely, and also in the electron-lucent spaces corresponding to apical vesicles (small arrows) (A, C) or apical invaginations of base of the microvilli (small arrowheads) (A–C). In addition, in A, gold particles are also seen in the electron-lucent spaces nearby the lateral membranes (large arrowheads) and vesicles in the cytoplasm except apical regions (large arrow). Negligible or no staining is seen in the endoplasmic reticulum (A, C), mitochondria (A, C, F), Golgi apparatus and nucleus (E). In D, negligible or no labeling is seen on the microvilli or other organelle when normal serum replaced anti-lactase serum as a control. er, endoplasmic reticulum; go, Golgi apparatus; lm, lateral membrane; lu, lumen; mt, mitochondria; mv, microvillus; n, nucleus. A, C, D, and E: ×15,000; B and F: ×23,000.
i.e., passing the Golgi apparatus to the BBM via the lateral membranes (20). The present study has demonstrated an evidence for a lateral membrane-associated post-Golgi transport of LPH, thus suggesting a distinct targeting mechanism for LPH apart from sucrase-isomaltase and other microvillous enzymes.

In adult rat small intestine, inactive form of LPH has been detected (21). Interestingly, this inactive form of LPH was found to be present in both ER-Golgi fractions and the BBM fraction, with an unexpectedly long half-life (28 h) which is close to the half-life of enterocytes themselves (22), suggesting either less susceptibility to proteolysis or distinct localization of this inactive form of LPH other than in the BBM. Since, it was not clear whether lateral membranes were completely separated from the ER-Golgi and the BBM fractions, it was possible that the inactive lactase was located in the lateral membranes. Further study is required to characterize the molecular form of LPH present in the lateral membrane, and to elucidate a possible targeting mechanism of pre-LPH destined to either the BBM or the lateral membranes.

We have found in a previous study that cytoskeletal composition of the lateral membrane changed under certain organ culture condition (Noda et al., unpublished results). This might be related to cellular polarity and the intracellular translocation of membrane glycoproteins. Thus, the organ cultured materials may be suited to gain more insight into the lateral membrane-associated membrane glycoprotein traffics.

In conclusion, the present study has demonstrated an evidence for a lateral membrane-associated post-Golgi transport of LPH precursor in rat small intestine.

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