Isolation and Characterization of a Small Intestinal Surfactant-like Particle Containing Alkaline Phosphatase and Other Digestive Enzymes*

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Digestive brush-border enzymes in particulate form have been reported in the intestinal lumen in vivo and in medium from organ explants in vitro. It has been suggested that these particles derive from membrane shedding of the apical brush border. This study describes the isolation and characterization of particles derived from the 105,000 × g supernatant fraction of intestinal luminal washings and from light scrapings of the mucosa itself after fat feeding of rats. These fractions were separated in a continuous NaBr gradient, producing a visible band of 1.07–1.08 g/liter density and resulting in a 15-fold enrichment of intestinal alkaline phosphatase in the band fraction. Other brush-border hydrolases were represented in the banded fraction, but at specific activities only 1/4th to 1/36th that of the brush border. The major phospholipid in the fraction was phosphatidylcholine (58 ± 15%), containing 75% saturated fatty acids. In contrast, the major brush-border phospholipid was phosphatidylethanolamine. These characteristics showed that the particles derived from the lumen and mucosal surface were not identical to fragments of the brush border. Electron microscopy of the banded fraction revealed partially coiled membrane fragments. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blots, some proteins (e.g. surfactant protein B, collagenous protein 4) were found in common between the intestinal particles and rat pulmonary surfactant. These data suggest the production of a particle secreted by rat intestine that differs from brush-border membranes and that shares some morphological and biochemical similarities with pulmonary surfactant.

Intestinal alkaline phosphatase has been characterized as a membrane-bound enzyme attached to the apical microvillus membrane by a phosphatidylinositol-glycan anchor (1). The enzyme has also been described in the intestinal lumen in particulate form after fat feeding and cholecystokinin administration (2, 3), consistent with its origin from the apical membranes. In the rat, however, intestinal alkaline phosphatase appears in serum not bound to membranes in fasting and fat-fed states (4). In addition, the enzyme has been visualized by immunohistochemistry both on the basolateral membranes and within the cell interior, as well as in the expected location on the apical brush-border membrane. As a result of a search for additional pathways of intestinal alkaline phosphatase release from the cell, we have described the appearance of lamellar bodies morphologically similar to pulmonary surfactant both in the enterocyte itself and in the intercellular spaces and adjacent to the apical membrane (5). We have also reported that intestinal alkaline phosphatase is associated with these particles, as demonstrated by immune localization at the electron microscopic level (6). These studies suggested that these lamellar bodies could function as the vehicle for secretion of intestinal alkaline phosphatase bidirectionally from the enterocyte.

The purpose of this study was to isolate and characterize these lamellar bodies. Because of their presence in increased numbers on the surface of the enterocyte after fat feeding (5), we chose to use luminal washings and light scrapings of the mucosal surface of fat-fed rats as starting material for isolation. We found that the purified particulate bodies are enriched in intestinal alkaline phosphatase and resemble pulmonary surfactant much more than enterocyte brush-border membranes.

EXPERIMENTAL PROCEDURES AND RESULTS1

DISCUSSION

This paper describes the isolation and chemical composition of an intestinal lipoprotein containing brush-border hydrolases. This particle was derived from the layer of mucus and fluid on the luminal side of the enterocyte. In previous work (29–30), others have noted by electron microscopy the presence of vesicles in this extracellular compartment. The origin of these vesicles was felt to be from the brush border due to the similarity of their appearance by electron microscopy and to their proximity to the apical brush border. It was assumed that they were produced by a process of membrane shedding (31–33), although such shedding had been directly demonstrated infrequently. None of these particles, however, had been isolated or characterized. In contrast with the prior hypothesis, the particles isolated in this study from rat intestinal light scraping were not found to resemble brush-border membranes. They migrated with a different buoyant density (Fig. 1), had a different protein (Fig. 4) and enzymatic com-

1 Portions of this paper (including “Experimental Procedures,” “Results,” Figs. 1 and 2, and Tables 1 and 2) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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† Recipient of an American Physician fellowship.
position (Table 1), and displayed a different lipid composition (Table 2) than brush-border membranes. The presence of mostly saturated phosphatidylcholine in the intestinal particles is characteristic of lung surfactant and different from cellular membranes in which unsaturated fatty acids predominate (34).

The buoyant density of these intestinal luminal particles was lighter than that of brush-border or basolateral membranes (33). All the enzyme activities measured were probably associated with the vesicular structures seen in the gradient band (Fig. 3a, inset). After treatment of the gradient fraction with phosphatidylinositol-specific phospholipase C, intestinal alkaline phosphatase migrated with a more acidic pH, consistent with its release by the enzyme (Fig. 8). Moreover, intestinal alkaline phosphatase already released from the enzyme migrated at a density \( d = 1.057 \) g/liter different from that of the particles.

The high percent of lysophosphatidylcholine in the particles is unusual for any cell membrane and suggests that the isolated particles have been modified by exposure to luminal phospholipases. These particles did not appear, however, to be fragments of brush-border membranes with some of the hydrolases removed because the protein patterns were quite different (Fig. 4). In addition, antisera against whole rat brush borders identified mostly a few large proteins (Fig. 5), as would be expected from the presence of hydrolase activity in the particles. The particles were, moreover, markedly enriched in intestinal alkaline phosphatase when compared to brush borders (Table 1). One possible explanation for this result might be the preferential release from the particles of enzymes like sucrase and maltase by the action of luminal pancreatic proteases. On the other hand, trehalase, another enzyme attached like intestinal alkaline phosphatase by a phosphatidylinositol-glycan anchor, was not enriched in the particle as was intestinal alkaline phosphatase. If the hydrolase composition were merely the result of differential release from brush borders, then one would expect trehalase and intestinal alkaline phosphatase activities to parallel each other, as phosphatidylinositol-specific phospholipase C releases them equally well (1). The enrichment of intestinal alkaline phosphatase in the isolated particles recalls its enrichment in luminal particles after cholecystokinin administration in vivo (2, 3).

If the particles are not derived from brush borders, what then is their origin? They have some properties in common with lung surfactant, a secreted particle with an intracellular counterpart, the lamellar body (35). The intestinal particles are enriched for saturated phosphatidylcholine (Table 2) and
have surface tension-lowering activity. We have reported the presence of surfactant-like vesicles by electron microscopy on the surface of and within enterocytes (5). These lamellar bodies stain positively for intestinal alkaline phosphatase by protein A-gold immunolabeling (6). These intracellular lamellar bodies and extracellular vesicles are likely candidates for the surfactant-like particles isolated in this study. It is of interest that we have found tissue-nonspecific alkaline phosphatase to be present in lung surfactant. Edelson et al. (36) have found alkaline phosphatase in alveolar type II cells of rat lungs. Alkaline phosphatase expression by these cells was regulated in concert with phospholipid and apoprotein synthesis by these cells (36), a situation paralleled by the response of the intestinal particle following fat feeding.

Features of lung surfactant are common to the particles seen by electron microscopy (Fig. 3a, inset; and Ref. 5), including a lamellar structure with 4-nm periodicity, preference for phospholipid stains, and lack of a bilayered membrane appearance (27). Intestinal particles and lung surfactant are also similar biochemically, with high phosphatidylycerol content, mostly containing saturated fatty acids (Table 2). In addition, the particles contain two proteins found in surfactant, one (55 kDa) possibly corresponding to collagenous protein 4 (14) and the other identified as surfactant protein B (Fig. 7), one of the proteins which confers surface-active properties to surfactant phospholipids (20). The high percentage of lysophosphatidylcholine in the vesicles may represent degradation of phosphatidylcholine by luminal pancreatic phospholipase A2. The total amount of lysophosphatidylcholine and phosphatidylcholine in the gradient band is 90%, which is even higher than pulmonary surfactant. The difference in phospholipid composition between brush borders and intestinal vesicles makes it unlikely that the vesicles are derived from brush borders, but rather suggests that they are synthesized de novo. Because the tight junction is an effective barrier to lipid diffusion in the outer (but not cytoplasmic) leaflet of the plasma membrane (37), the particle presumably arises from secretion of intracellular particles and not from shedding of apical cell-surface membranes. These particles may play a role in protection or lubrication of the enterocyte surface (38) or in secretion of enzyme hydrolases.

The release of an intracellular particle from the enterocyte may not be the only mechanism by which particles are produced from these cells. The appearance of the particles reported here (Fig. 3a, inset; and Ref. 5) differs from earlier reports, in which particles looked like fragments of brush-border membranes (28, 30). In organ explants of mouse intesti-
be needed to establish the function of these particles and their prevalence in the lumen, as well as to identify the factors that stimulate their secretion from the enterocyte.

Acknowledgements—We wish to thank Dr. E. Crouch for providing various antibodies to rat lung surfactant, Jeffrey H. Whitsett (University of Cincinnati) for providing the antisera directed against surfactant proteins B and C, and C. L. Goodwin for excellent technical assistance.

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Supplementary Material:

ISOLATION AND CHARACTERIZATION OF A SMALL INTESTINAL SURFACANT-LIKE PARTICLE CONTAINING ALKALINE PROTEINS AND OTHER LIPOIDIC ENZYMES

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Intestinal Surfactant-like Particle

Surface activity measurement: Light intestinal mucosal scrapings enter the band fraction of the multi gradient were analyzed using the pulsed field technique described by Edwards (25). Twenty to 20 to 2 of plasmanolysis equivalents were loaded in separate 1 ml aliquots in a Pharmacia multi-gradient (Fig. 7). The surface activity of the mucosal scrapings entrapped in the band fraction of the multi gradient was determined by the surface tension method (17). The protein concentration of the mucosal scrapings was determined using the Biuret method. The protein concentration of the surfactant fraction was found to be about 0.5 mg/ml. The surfactant suspension was spread on a glass plate and the surface tension was measured using a tensiometer (Digid颜值). The surface tension was measured at 25°C and the mucosal scrapings were added to the surfactant suspension. The surface tension was measured using a tensiometer (Digid颜值). The surface tension was then measured at 25°C and the mucosal scrapings were added to the surfactant suspension. The surface tension was then measured at 25°C and the mucosal scrapings were added to the surfactant suspension. The surface tension was then measured at 25°C and the mucosal scrapings were added to the surfactant suspension.
Intestinal Surfactant-like Particle

Figure 2: Digestive enzyme activity in NABO gradients: fractions of mucosal scrapings. Light mucosal scrapings were obtained and processed as non-immune NABO gradient. Particles were collected at each gradient and assayed in duplicate for various enzyme activities as described in Methods.

| Enzyme                                     | Particle (mg protein) | Brush Border (mg protein) | Lung Surfactant (mg protein) | Particle (ratio enzyme/milligram) | Brush Border (ratio enzyme/milligram) |
|--------------------------------------------|-----------------------|---------------------------|-------------------------------|-----------------------------------|---------------------------------------|
| Alkaline Phosphatase                       | 7.6 ± 1.96            | 1.43 ± 0.16               | 0.16                          | 37.0 ± 0.35                        |
| Lactate Mannosidase                        | 0.03 ± 0.12           | 0.7 ± 0.001               | 0.16                          | 37.0 ± 0.35                        |
| Aseptic Lactase                           | 0.13 ± 0.006          | 0.97 ± 0.006              | 0.16                          | 37.0 ± 0.35                        |
| Serumbra                                  | 0.92 ± 0.001          | 0.55 ± 0.001              | 0.16                          | 37.0 ± 0.35                        |
| Trypsin                                   | 0.10 ± 0.005          | 0.53 ± 0.006              | 0.16                          | 37.0 ± 0.35                        |
| Retnae/Gлюконолаза                        | 0.23 ± 0.07           | 0.05 ± 0.01               | 0.16                          | 37.0 ± 0.35                        |

Particles and ret: pulmonary surfactant were purified in NABO gradients, and brush borders by hypoxic rupture as described in Methods. Results are the mean ± S.E. of 3 separate determinations.

Table 2

Phospholipid Content (% of total)

| Type                        | Right mucosal scrapings | Light scrapings | Non-immune NABO gradient | Brush Border |
|-----------------------------|-------------------------|-----------------|---------------------------|--------------|
| Lysophosphatidylinolein     | 26 ± 5.13               | 37 ± 3.7        | 18.46 ± 2.7               | 1            |
| Phosphatidylinolein         | 11.8 ± 5.5              | 7 ± 1.4         | 14 ± 2.1                  | 18           |
| Phosphatidylinositol        | 16.8 ± 5.4              | 5 ± 2.8         | 14.1 ± 3.4                | 14           |
| Phosphatidylinositol/Calculated PC | 37.2 ± 2.7%         | 56 ± 3.8%       | 70 ± 5%                   | 22           |
| Phosphatidylinolein         | 0.1 ± 0.01              | 0 ± 0.01        | 13 ± 2.1                  | 13 ± 2.1     |
| Phosphatidylinositol        | 7.9 ± 3.6               | 0 ± 5.3         | 24 ± 5.0                  | 24 ± 5.0     |
| Phosphatidylcholine         | N.D.                    | N.D.            | N.D.                      | N.D.         |

Light mucosal scrapings were extracted before and after NABO gradient purification as described in Methods. Brush border were extracted as prepared in Methods. Results represent the mean ± S.E. of 3 separate determinations. N.D. = not done.