LYSOSOMAL ENZYMES OF RAT INTESTINAL MUCOSA

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ABSTRACT

Six intracellular hydrolases known to be associated with lysosomes in rat liver were found in rat intestinal mucosa. The extent to which they were particulate-bound and the degree of enzyme release when the particulate fractions were suspended in hypotonic media followed the same pattern in both mucosa and liver. The specific activities of the mucosa enzymes were either comparable to or slightly smaller than those of the liver enzymes. These results suggest that the mucosa hydrolases belong to lysosome-like particles. However, differential fractionation of the mucosa indicated that the particles from the mucosa sediment at lower centrifugal forces than do those from the liver and are more heterogeneous in size, bearing a closer resemblance to kidney lysosomes. Possible physiological functions of particulate-bound digestive enzymes in intestinal mucosa are discussed.

INTRODUCTION

The absence of ingested food macromolecules in the blood has been generally interpreted as due to their being broken down to simple units in the lumen of the digestive tract by enzymes secreted into the lumen, prior to their absorption by the small intestine. Considerable work has gone into elucidating the mechanisms of entry into the intestinal epithelium of monosaccharides, fatty acids, and amino acids.

However, as early as 1911, Starling pointed out that when the mucous membrane of the small intestine was pounded in water it yielded a solution of maltase, invertase, and lactase which had a more powerful activity than the succus entericus itself, and he suggested that the main action of these enzymes occurs not in the lumen but in the epithelial cells of the small intestine (1). In 1933, Cajori reported that the hydrolytic activity of dog's intestinal contents on peptones, sucrose, and lactose was insufficient to be compatible with luminal breakdown preceding absorption (2). In recent years, evidence has accumulated to the effect that the intestinal mucosa is metabolically very active (3, 4) and that the intracellular hydrolysis of foodstuffs in this tissue may be significant (5). That this is true for sucrose, maltose, and glucose-1-phosphate was shown by Miller and Crane (6). Newey and Smyth demonstrated that dipeptides can enter mucosal cells and be hydrolyzed inside the cell before passage into the blood stream as amino acids (7).

Another indirect line of evidence for an intracellular locus of digestion stems from electron and fluorescence microscopy (8–11) indicating that certain types of cells, including the epithelial cells of the intestinal mucosa, have the ability to pinocytose macromolecules. Cells active in pinocytosis, such as liver and kidney cells, are also rich in lysosomes. The lysosomes, first biochemically identified in rat liver by de Duve (12), are

subcellular organelles characterized by a single membrane enclosing some twelve hydrolytic enzymes which have acid pH optima. They appear to be involved in the intracellular digestion of pinocytosed material and in the turnover of subcellular constituents (12–16). There have been isolated reports of the presence of acid hydrolases in the intestinal mucosa: acid phosphatase has been demonstrated by Shnitka (17), Dempsey *et al.* (18), Straus (10), Sheldon *et al.* (19), and Padykula *et al.* (20); β-galactosidase, by Doell and Kretchmer (21); and acid ribonuclease, by de Lamirande and Allard (22). This paper reports the measurements of the activity of six lysosomal enzymes and their subcellular distribution in rat intestinal mucosa.

EXPERIMENTAL

Male Sprague Dawley rats (200 to 250 gm) were sacrificed by decapitation after fasting for 24 hours. Liver and small intestine (the length extending from stomach to cecum) of each rat were immediately removed and chilled in ice-cold 0.25 M sucrose, and subsequent operations were carried out at 4°C. The luminal contents of the small intestine were washed off by squirting through one end of the intestine a steady stream of distilled water for 1 minute. The intestine was then slit open and the soft luminal coating scraped up with a razor blade and put into 0.25 M sucrose.

Homogenization of liver and mucosa tissues was done in 0.25 M sucrose for 20 seconds in a Waring blendor at top speed. For the comparative study of mucosa and liver hydrolases, the homogenates were centrifuged at 480 *g* for 10 minutes to yield a pellet which was termed "nuclear fraction." The supernatant was recentrifuged at 75,000 *g* for 45 minutes to yield a supernatant fraction and a particulate fraction consisting of mitochondria and microsomes as well as particles sedimenting in between, to which group the liver lysosomes belong. The 75,000 *g* particulate was resuspended in water to rupture the particle membrane and centrifuged at 75,000 *g* for 45 minutes to give two fractions, particulate membrane and particulate soluble.

For the differential fractionation of mucosa homogenate based on the scheme worked out for rat liver (23), six major fractions were obtained: a "nuclear and cell debris" fraction sedimented at 480 *g* for 10 minutes; a "heavy mitochondrial" fraction at 5000 *g* for 10 minutes; a "light mitochondrial" fraction at 16,300 *g* for 20 minutes; a "heavy microsomal" fraction at 30,000 *g* for 30 minutes; and a "light microsomal" fraction at 100,000 *g* for 1 hour. The supernatant fluid obtained after the last centrifugation was termed the soluble fraction. As suspending medium, 0.25 M sucrose was used throughout.

Assays of the lysosomal enzymes were run according to the procedures outlined by Sawant *et al.* (23). The activities of acid phosphatase, acid ribonuclease, aryl sulfatase, β-glucuronidase, and β-galactosidase were determined in acetate buffer, pH 5.0, with the following substrates: β-glycerophosphate, ribose nucleic acid, 2 OH-5-nitrophenyl sulfate, phenolphthalein glucuronide, and *O*-nitrophenyl-β-D-galactoside, respectively. Cathepsins were assayed in acetate buffer, pH 3.8, with hemoglobin as substrate.

Glutamic dehydrogenase served as mitochondrial marker, and its activity was measured as DPNH oxidized when glutamic acid was formed from α-keto glutaric acid and ammonia in phosphate buffer of pH 6.8 and 0.01 M in KCN. The enzyme marker for the microsomal fraction was a Mg^{++}-insensitive alkaline phosphatase (24, 25). It was assayed, at pH 10.3, with *p*-nitrophenyl phosphate as substrate. Protein values were obtained by Miller's method (26). The level of amino acids and derivatives was determined by the ninhydrin colorimetric assay of Rosen (27).

RESULTS

1. *Particulate-Bound Lysosomal Enzymes in Mucosa*

Homogenates and cytoplasmic fractions of rat mucosa and livers were assayed for acid phosphatase, cathepsins, acid ribonuclease, aryl sulfatase, β-glucuronidase, and β-galactosidase. All six enzymes are present in both tissues as indicated in Fig. 1. The particulate fraction (75,000 *g*) in both liver and mucosa has the highest concentration of all enzymes except acid ribonuclease.

A characteristic of liver lysosomes is that when they are subjected to a variety of treatments such as non-ionic detergent, successive freezing and thawing, and osmotic shock, their membrane is damaged and the enzymes are liberated in active form into the supernatant. When the 75,000 *g* particulate fraction was resuspended in water, all enzymes in both tissues were released into the supernatant in varying degrees, depending on the enzyme and the tissue (Fig. 1). Generally, about

half or more of the particulate enzyme was set free except for acid ribonuclease which was only 17 per cent released in liver. Over-all, the distribution pattern of the hydrolases between particulate and soluble fractions and the extent of release of bound enzyme after osmotic shock indicated no essential differences between mucosa particles and liver lysosomes.

would be expected if osmotic shock caused the liberation of bound enzymes into the supernatant.

There is, however, a major difference between liver and mucosa if one looks at the specific activities of the nuclear fractions. Whereas these specific activities consistently increase over those of homogenate in the case of mucosa, they consistently decrease in the case of liver.

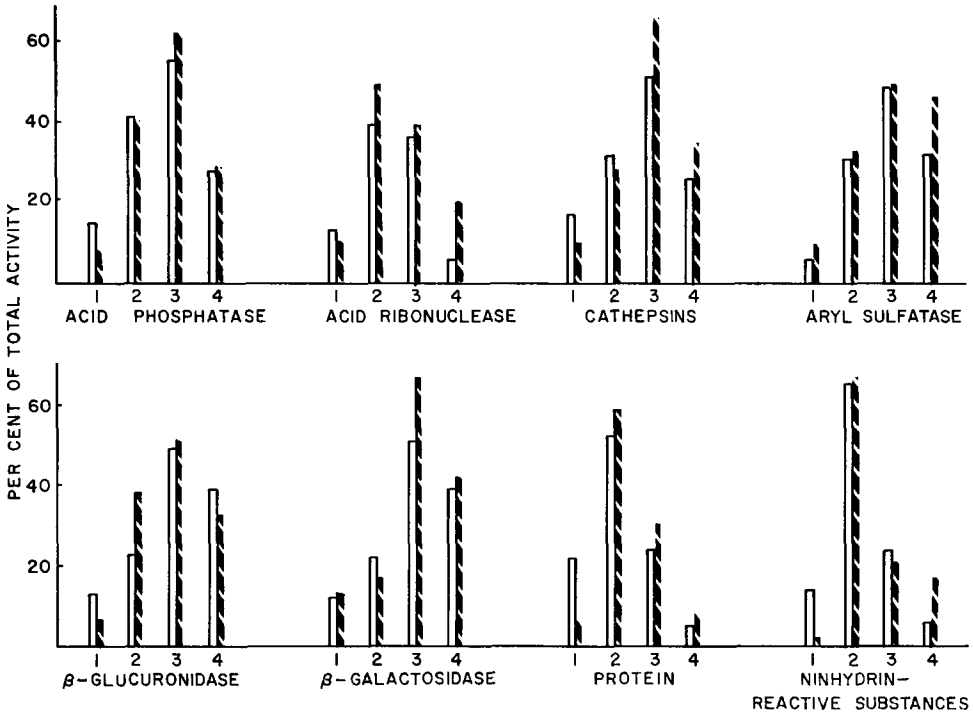

FIGURE 1 Comparative distribution of lysosomal enzymes and components in subcellular fractions of rat liver and mucosa. Open bars are for liver; black bars are for mucosa. Fractions 1, 2, 3, and 4 are respectively: nuclear and cell debris, supernatant, 75,000 *g* particulate, 75,000 *g* particulate soluble. Per cent activity is referred to the homogenate which is set at 100.

2. *Comparison of Specific Activities of Liver and Mucosa Hydrolases*

The specific activities of the six hydrolases in the mucosa are either comparable to, or slightly smaller than those of the liver enzymes (Table I). In contrast, glutamic dehydrogenase was found to be only about one-tenth as active as in liver homogenate. There is a definite increase in the specific activities of all lysosomal enzymes in the 75,000 *g* particulate fraction over that of the homogenate. The values are highest in the 75,000 *g* particulate soluble fraction in both tissues, as

3. *Ninhydrin-Reactive Substances of the Mucosa*

In the cathepsin assay which measures the amount of free amino acids, mainly tyrosine, released, the blanks of the mucosa were much higher than those of liver. When the level of ninhydrin-reactive substances of the mucosa was measured by the method of Rosen (27), it was found to be some three times higher than in liver, although the distribution in the various cytoplasmic fractions was similar (Fig. 1 and Table II). In both tissues, almost 70 per cent of the ninhydrin-reactive substances are concentrated in the supernatant.

TABLE I

*Specific Activities of Lysosomal Enzymes in Mucosa and Liver**

Enzyme	Homogenate		Nuclei and cell debris		Supernatant		75,000 *g* particulate		75,000 *g* particulate soluble		75,000 *g* particulate membrane	
	Liver	Mucosa	Liver	Mucosa	Liver	Mucosa	Liver	Mucosa	Liver	Mucosa	Liver	Mucosa
Acid phosphatase	40	28	27	39	25	21	74	58	202	97	40	55
Acid ribonuclease	43	27	27	40	32	19	55	36	49	62	29	26
Cathepsins	2.1	2.6	1.7	4.5	1.3	1.3	4.6	5.9	11	11	1.0	1.3
Aryl sulfatase	0.36	0.22	0.17	0.36	0.20	0.12	0.73	0.36	2.3	0.87	0.23	0.054
β-glucuronidase	25	8.6	15	9.5	11	5.5	51	15	192	45	14	4.0
β-galactosidase	1.0	1.0	0.55	1.8	0.42	0.20	2.2	1.8	7.7	4.0	0.68	0.73

* Specific activities are expressed as mμmoles of substrate released per minute per mg of protein.

4. *Differential Fractionation of Intestinal Mucosa*

In contrast to liver lysosomes which are concentrated in the light mitochondrial fraction (16, 23), lysosome-like particles of the mucosa sediment at lower centrifugal forces, much like kidney lysosomes (28). More than half of the particulate-bound hydrolases in the mucosa are about equally distributed between the two heaviest fractions in all cases (Table II).

Generally, the distribution of all enzymes in the mucosa is more diffuse than in liver, suggesting that the mucosa subcellular organelles are more heterogeneous in size. Alterations and permeabllity changes of the mucosa particles during the course of fractionation is likely, as indicated by the high soluble glutamic dehydrogenase activity.

The distribution profiles (Fig. 2) of all six acid hydrolases resemble one another more than they do the mitochondrial and microsomal markers; this would permit one to conclude tentatively that the bulk of the mucosa lysosomal enzymes belongs to the same type of particles, distinct from mitochondria and microsomes. The subcellular distribution of acid ribonuclease differs from that of the other hydrolases in that it suggests a second locus of enzyme concentration in the "light microsomal" fraction which has the highest acid ribonuclease activity of all. This "light microsomal" fraction is relatively low in alkaline phosphatase activity so that the acid ribonuclease of this fraction could actually be associated with free ribosomes. The relatively high activity of the enzyme in the supernatant would be in keeping with the observation of Reid and Nodes (29) on liver acid ribonuclease. Those authors believe that the activity in the supernatant may be due in part to an enzyme different from that in lysosomes.

TABLE II

*Concentration of Ninhydrin-Reactive Substances in Mucosa and Liver**

Tissue	Homogenate	Nuclei and cell debris	Supernatant	75,000 *g* particulate	75,000 *g* particulate soluble	75,000 *g* particulate membrane
Mucosa	2.1	0.78	2.4	1.5	4.2	0.45
Liver	0.56	0.35	0.69	0.56	0.63	0.50

* Concentrations expressed as μmoles of glutamic acid per mg of protein.

TABLE III

Distribution of Enzyme Activities in Subcellular Fractions of Mucosa

Enzyme	Nuclei	Heavy mitochondria	Light mitochondria	Heavy microsomes	Light microsomes	Soluble	Recovery
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Glutamic dehydrogenase	37	15	14	1.5	0	32	100
Alkaline phosphatase	16	20	17	13	14	2.5	83
Acid ribonuclease	17	16	2.9	2.8	21	28	88
β-glucuronidase	23	24	6.9	3.5	9.4	24	91
Acid phosphatase	31	39	11	7.5	9.6	19	117
Cathepsins	24	33	18	11	1.5	3.8	91
β-galactosidase	29	33	16	6.0	6.3	12	102
Aryl sulfatase	33	38	12	3.5	4.9	24	115

* Per cent of total activity is referred to the homogenate which is set at 100.

DISCUSSION

The presence of six lysosomal enzymes in the intestinal mucosa is further evidence for the digestive capacity of mucosa tissue. The particulate nature

of these acid hydrolases and their release into the supernatant after osmotic shock are preliminary data which must be supported with further investigation in order to determine whether the hydrolases are associated with lysosomes, such as defined by de Duve. The heterogeneity of particle size is not surprising if one takes into consideration the very rapid turnover of mucosal epithelium, which would result in the simultaneous presence of cells in various stages of differentiation (5), together with the variety of cell types composing this epithelium, each with different functions and probably different populations of subcellular organelles. Our biochemical data do not inform us as to the type of mucosal cells in which these particles are located. Other methods of study will be of great value in elucidating the origin of mucosa lysosomes. The electron micrographs of the crypt epithelium of human small intestine which Trier (30) has published depict dense bodies delimited by a single membrane, in the Paneth and enterochromaffin cells, which very much resemble liver lysosomes. The mucosa hydrolases could also be brought in by migratory macrophages of the reticulo-endothelial system associated with the lamina propria, or the lysosomes could be associated with all of these cells. Using histochemical methods, Padykula *et al.* (20) found that in the normal human intestinal mucosa there are three foci of acid phosphatase activity—the absorptive epithelium of the villi, the Paneth cells at the bottom of the crypts, and scattered cells in the lamina propria. In biochemical studies

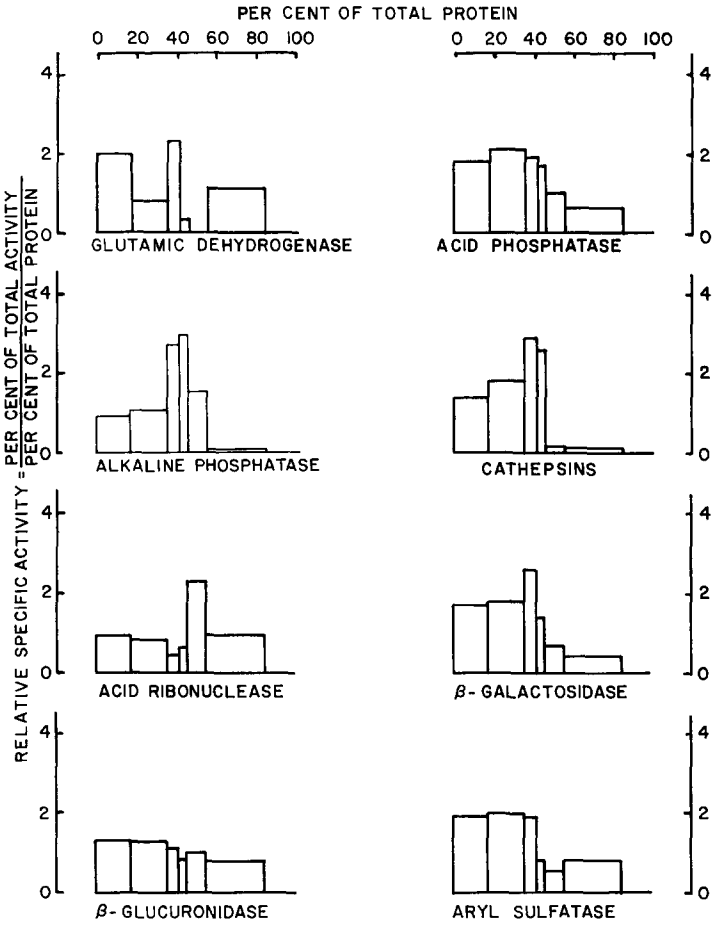

FIGURE 2 Distribution of enzyme activities among subcellular fractions of mucosa. Along the abscissa, fractions are from left to right: nuclear, heavy mitochondrial, light mitochondrial, heavy microsomal, light microsomal, and soluble. This fractionation is based upon the sedimenting pattern of rat liver subcellular components.

of liver lysosomes, for instance, one is actually dealing with a mixture of two different populations of lysosomes, those of the parenchymal or liver cells proper, and those of the Kupffer cells or macrophages lining the sinusoids of the liver.

Despite the limitations of this study, the fact that the specific activities of the six acid hydrolases in mucosa are of the same order of magnitude as those of the hydrolases of liver, which is one of the tissues richest in lysosomes, would justify some discussion of the possible physiological significance of these particulate digestive enzymes in the mucosa.

The work of Straus with kidney cells (10, 14) would indicate that vacuoles containing pinocytosed foreign material fuse with lysosomes to form discrete pockets in which digestion can proceed without disturbing the rest of the cell. It would be tempting to visualize a similar role for the mucosal lysosome-like particles, especially since they seem to sediment like those of kidney. On the basis of the enzymic activities which we found, macromolecules of food protein and nucleic acids, phosphate and sulfate compounds, and polysaccharides could be hydrolyzed, inside the mucosa cells, to amino acids, nucleotides, and monosaccharides prior to their entry into the blood. It is clear that our data do not permit us to evaluate the quantitative importance of such a mechanism of food digestion. However, such mechanism would be in agreement with the consistent finding that ingested food macromolecules must be broken down to simple units before their appearance in the portal system, (31, 32) and with the reports that the enzymes secreted into the lumen are not sufficient to break down the foodstuffs completely (31, 32).

Numerous studies will be needed to clarify the physiological role of particulate acid hydrolases in the mucosa, a role which might not be limited to the digestion of food. Spencer and Knox (3) report that the mucosa holds a generous pool of amino acids and derivatives, which is also what our data indicate. It was shown by Friedberg *et al.* (33) that the mucosa has a priority for amino acids, for when those authors administered sulfur-labeled methionine intravenously to fasted rats and dogs, the intestinal mucosa proteins showed the highest specific activity. Blood proteins can also find their way into the mucosal cells, as demonstrated by Straus (10). In the light of such evidence, it seems advisable to entertain the possibility that the particulate-bound hydrolases might be involved in the segregated and organized breakdown of endogenous protein stores as well as pinocytosed plasma proteins to assure the mucosa cells of a steady supply of amino acids for synthetic activities vital to the integrity and adequate functioning of the mucosa tissue. In this connection, it is significant that the greater part of ninhydrin-reactive substances of the mucosa is in the supernatant fraction.

However, cathepsins are but one group of lysosomal enzymes identified in the mucosa. Of some interest are the mucosa β-glucuronidase, aryl sulfatase, and β-galactosidase which would find ready substrates in the chondroitin sulfates, heparin, and other mucopolysaccharides of the mucus coating the luminal side of the intestine. The well documented rapid turnover of the mucosal epithelium is thought to proceed by a migration of the epithelial cells from the bottom of the villi, where they are produced, to the tip of the villi where they are then sloughed off into the lumen (5, 34). Hooper showed that in a closed-off segment of intestine the extruded epithelial cells and mucus accumulate in the lumen (34). It would be interesting to know whether at least some of these cells and mucus are reabsorbed farther down the intestinal tract, in which case they could be digested intracellularly by the lysosomal enzymes, which could also break down the incoming plasma mucoproteins, to furnish raw material for the synthesis of new mucus and new cells.

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