phosphodiesterase in the RNA degradation was also excluded by the fact that the activity of phosphodiesterase was completely inhibited by phosphate ion under the assay conditions employed. Recently, the presence of nucleases which preferentially degrade single-stranded nucleic acids in *B. subtilis* has been reported[4-7]. The S-105 fraction, however, contained little or no deoxyribonuclease activity. These observations strongly suggest that the intracellular ribonuclease activity observed was really that of ribonuclease[8].

Extracellular (alkaline) ribonuclease of *B. subtilis* K was not influenced by ATP and ADP at either pH 5.7 or 7.5.

An intracellular inhibitor against extracellular ribonuclease has been reported[9], but no inhibitor of intracellular ribonuclease is known.

In conclusion, ATP and ADP probably act as natural regulators or inhibitors of intracellular ribonuclease in *B. subtilis*. The purification of the intracellular ribonuclease is now in progress.

The authors are grateful to Drs. G. TAMURA and T. BEPPU for their valuable discussion.

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Received February 27th, 1967

Biochim. Biophys. Acta, 139 (1967) 202–204

PN 61135

Localization of lysozyme activity in a Paneth cell granule fraction

The identity of the content of the Paneth cell granules is still largely unknown[1]. Histochemical studies indicate that these granules are rich in protein[2,3], tryptophan[4] and arginine[5], that disulfide bridges are present[6] and that the overall isoelectric point exceeds 10.3 pH units[5]. Administration of pilocarpine is known to produce a discharge of granules into the intestinal lumen[7]. Following the administration of pilocarpine we observed a rapid increase of lysozyme (mucopeptide *N*-acetylmuramylhydrolase, EC 3.2.1.17) in the small intestinal fluid. As there is some analogy between

the histochemically determined characteristics of the Paneth cell granule content and lysozyme, it was presumed that the Paneth cell granules contain this enzyme. In an attempt to localize the lysozyme activity, homogenates of the small intestinal mucosa of mice and cats were fractionated by differential centrifugation. A fraction rich in Paneth cell granules, and containing a maximum of lysozyme activity, was separated from other specific fractions of the intestinal homogenate from mice but not from cats. Paneth cells are known to be present in the former and absent in the latter species. This report summarizes results concerning the specificity of this finding.

The animals used in this study were mice of the NMRI strain and mongrel cats. The animals were killed by a sharp blow on the head. A segment from the middle part of the small intestine was quickly removed and washed in ice-cold 0.25 M sucrose. All subsequent manipulations up to the chemical and enzymatic analyses were carried out in a cooled chamber at a temperature of $2\pm1°$. The mucosa was scraped off and homogenized in 50 vol. (w/v) of 0.25 M sucrose in a Potter–Elvehjem homogenizer at 465 rev./min for 30 sec. The homogenate was diluted to twice its original volume with 0.25 M sucrose and centrifuged for 450 $g \cdot$ min (fraction 1), 12 000 $g \cdot$ min (fraction 2), 47 200 $g \cdot$ min (fraction 3), 180 000 $g \cdot$ min (fraction 4), and $4.5 \cdot 10^6$ $g \cdot$ min (fraction 5 and the supernatant, fraction 6). Half of fraction 2 was made up in 0.1 M Tris buffer at pH 7.8 and passed over a basic ion-exchange resin (Merck III) in the same buffer. The eluate was recentrifuged for 12 000 $g \cdot$ min. The corresponding sediment was called the "purified fraction 2". Histological sections and sedimented subcellular fractions were stained with haematoxylin–eosin and Gram stains and examined microscopically. Granules exhibiting the morphological and staining characteristics of Paneth cell granules were found to be present mainly in fraction 2 and, to a lesser degree, in fraction 1 of the mouse small intestinal homogenates. The "purified fraction 2" contained relatively less nuclei and unidentified material than the original fraction 2.

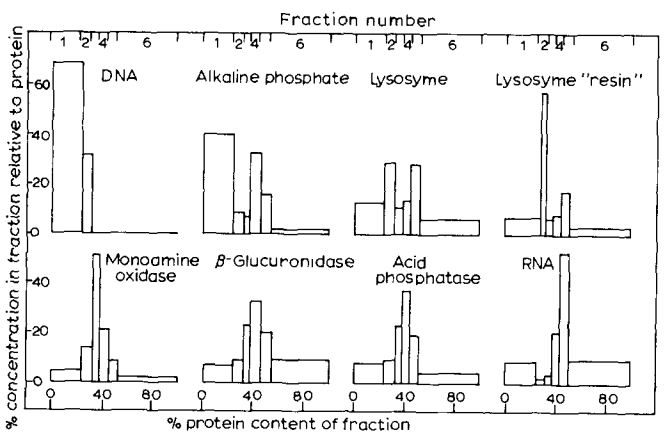

Fig. 1. Distribution of nucleic acids and enzyme activities in fractions isolated from mouse small intestinal mucosa by differential centrifugation. The lysozyme resin-treated fractions correspond to the sedimentable material in the eluates obtained after passing the fractions over a basic ion-exchange resin (Merck III) in 0.1 M Tris buffer (pH 7.8), except fraction 1. The protein material released on the resin from the original fraction 2 was added to fraction 1. The other fractions did not release any appreciable amount of protein material. Means of 8 analyses.

The fractions were analyzed for their content of protein[8], DNA[9] and RNA[10]. They were also assayed for activities of lysozyme[11], monoamine oxidase (EC 1.4.3.4)[12], β-glucuronidase (EC 3.2.1.31)[13], acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1)[14].

Fig. 1 summarizes the results obtained by the fractionation of mouse small intestinal mucosa. Microscopic examination and analysis of the distribution of nucleic acids and of enzyme activities permitted the identification of the following fractions[15,16]: Fraction 1: nuclei, cell debris and microvillus sheets; fraction 2: Paneth cell granule-like granules, nuclei and unidentified material; fraction 3: mitochondria; fraction 4: lysozomes and microvillus fragments; fraction 5: microsomes and microvillus fragments; fraction 6: $4.5 \cdot 10^6$ $g \cdot$min supernatant. A peak of lysozyme activity was present in fraction 2. A second peak was found in the microsome fraction. The specific lysozyme activity of fraction 2 increased about three times on passing the fraction over the ion-exchange resin. When treated in the same way, the microsome fraction did not show any change of lysozyme activity. Osmotic shock experiments with these two fractions indicated that, in a hypotonic medium, lysozyme is released from fraction 2 and not from the microsome fraction. The addition of NaCl or spermine to the

TABLE I

EFFECT OF OSMOTIC SHOCK AND SALT TREATMENT ON THE SOLUBILIZATION OF LYSOZYME FROM FRACTIONS 2 ("GRANULES") AND 5 (MICROSOMES)

A, fractions washed with NaCl at given concentrations; B, fractions washed with spermine previously neutralized with HCl; C, fractions washed with 0.15 M NaCl followed by sedimentation; the fractions were redispersed in bidistilled water and NaCl was added to a final concentration of 0.15 M. The percentage of total enzyme activity, which did not sediment after the various treatments, is indicated. Means of 3 experiments.

Treatment	*Fraction*	
	2	*5*
A. NaCl 0.100 M	26	39
0.150 M	38	60
0.225 M	41	79
B. Spermine, HCl (2 mM)	42	77
C. Osmotic shock	55	1

fractions resulted in a greater release of lysozyme from the microsome fraction than from fraction 2 (Table I). These experimental data suggest that the localization of lysozyme activity in fraction 2 is specific and probably bound in the Paneth cell granules. The presence of lysozyme activity in the microsome fraction could probably be explained by an electrostatic binding with microsomes. Similar observations have been made concerning ribonuclease distribution in pancreas subcellular fractions[17].

Lysozyme activity was absent or very low in the subcellular fractions of cat intestinal mucosa. If present, it did not have a definite distribution pattern. This low activity was probably derived from polymorphonuclear leucocytes. When the supernatant fluid of fractionated mouse mucosa was added to a homogenate of cat mucosa and submitted to differential centrifugation, it was found that the mouse lysozyme activity was redistributed and, to a large degree, bound to the cat microsome fraction (Table II).

TABLE II

LYSOZYME ACTIVITIES IN FRACTIONS ISOLATED FROM MOUSE AND CAT SMALL INTESTINAL MUCOSA

Results are expressed in μg of crystalline egg white lysozyme equivalents per mg protein. The fractions were obtained as described in the text. In experiment C, the supernatant (fraction 6) obtained from mouse mucosa homogenate served as the medium for the homogenization of the cat mucosa. The number of animals analyzed is indicated in parentheses.

	Fraction						
	1	*2*	*2 "resin"*	*3*	*4*	*5*	*6*
A. Mice (15)	0.75	1.74	5.39	0.61	0.82	1.68	0.36
B. Cats (4)	0.04	0.02	0.00	0.04	0.03	0.04	0.01
C. Cat, homogenate in mouse fraction 6 (2)	0.04	0.02	0.00	0.06	0.10	0.32	0.27

In summary, a fraction has been isolated from mouse intestinal mucosa (containing Paneth cells) characterized by the presence of Paneth cell-like granules and by a maximum of particle-bound lysozyme activity. In the corresponding fraction of cat intestinal mucosa, which do not contain Paneth cells, neither Paneth cell-like granules nor a peak of lysozyme activity was found. These results strongly suggest that lysozyme is a constituent of the Paneth cell granules. A histochemical study confirming these results will be published elsewhere.

We wish to thank Professor J. VANDENBROUCKE for his encouragement and continued interest in this work.

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Received March 16th, 1967