Purification and Partial Characterization of Lysozyme from Mouse Small Intestine

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Summary. Lysozyme was isolated from the small intestine of mice by combined ion-exchange and molecular sieve chromatography. This lysozyme differs from that isolated from the urine of mice with monocytoma in amino acid composition, and migration rate in cellulose acetate electrophoresis. As intestinal lysozyme originates at least in part from the Paneth cell, our results point towards the existence of isozymes of lysozymes in mice.

In avian egg-whites two markedly different forms of lysozyme ('chick-type' and 'goose-type') have been described[2-4]. Mammalian lysozymes belong to the 'chick-type' lysozyme, and although it has been suggested that granulocytic and macrophage isozymes of lysozyme exist in man[5], it is generally assumed that within one mammalian species lysozymes from different sources have identical structures[6]. The presence of lysozyme in the Paneth cells is now well documented[7-11] and intestinal lysozyme is, at least in part, produced by the Paneth cell. During our immunohistochemical studies[10], lysozyme was isolated from mouse small intestine. The characteristics of this lysozyme, described in this paper, differ from those reported for mouse monocytic lysozyme[12]. These results suggest that lysozyme isozymes exist in mice.

Materials and methods. Purification. Mice of the NMRI strain, approximately 6 weeks old, were killed by a sharp blow on the head. The small intestine was quickly removed, homogenized, and the homogenate centrifuged at 100,000 *g* for 60 min at 4°C. Lysozyme was isolated from the supernatans by ion-exchange and molecular sieve chromatography according to standard procedures.

Characterization. Amino acid analyses were performed on a Technicon Model 100 amino acid analyzer, following hydrolysis of the samples for 8, 15 and 24 h in 6 *N* HCl under vacuum at 110°C. The UV-spectra of hen's egg-white lysozyme (HEL) and mouse intestinal lysozyme (MIL) in 0.1 *M* HCl and 0.1 *M* NaOH were recorded on a Unicam SP-800. The molecular weight was estimated by comparing the migration rates of HEL and MIL on thin layers of Sephadex G-50 (grade superfine). Lysozymes were localized by spraying the plates with a *Micrococcus lysodeikticus* suspension of 10 mg/ml. Cellulose acetate electrophoresis was carried out with the Microzone equipment. (Beckman Instruments Inc., Palo Alto, Calif., USA) at two different pH-values.

Results. Purification. A supernatans obtained from the homogenate of the small intestine of 20 mice, containing approximately 7.5 mg lysozyme was loaded on a column of amberlite CG-50, equilibrated with 0.2 *M* phosphate buffer pH 6.5. A stepwise gradient of NaCl from 0.0 to 0.5 *M* was applied in 5 steps of 0.1 *M*, to elute absorbed proteins. Lysozyme activity was eluted at 0.3 *M* and concentration of this eluate was achieved by ammonium sulfate precipitation (90% saturation at 25°C). The precipitate was collected by centrifugation and chromatographed on Sephadex G-50. The overall yield at this point was 30%, especially due to a low recovery on amberlite (44% only). Therefore, the lysozyme containing fractions of several such preparations were combined, again precipitated by ammonium sulfate and rechromatographed on Sephadex G-75 yielding an almost symmetrical peak with constant specific activity. The center fractions were desalted on Sephadex G-10 with 0.1 *N* acetic acid as the eluate, and lyophilized.

Characterization. The purified enzyme was dissolved into 2 buffer solutions (acetate buffer 0.1 *M*, pH 5.5 and barbital buffer 0.1 *M*, pH 8.6) and subjected to cellulose acetate electrophoresis at both pH values. At each pH, the enzyme moved as a single band, but the progression of MIL was slightly faster than that of HEL. At pH 8.6, MIL was found at 2.0 cm from the application point in the cathodic direction, after electrophoresis for 20 min at 250 V, whereas HEL was found at 1.8 cm from the

Amino acid composition of mouse intestinal lysozyme (15 h) amino acid residues per molecule

Amino acid	MIL[a]	HEL	Human milk[18]
Aspartic acid	21	21	18
Threonine	7	7	5
Serine	8	10	6
Glutamic acid	13	5	9
Proline	5	2	2
Glycine	11	12	11
Alanine	13	12	14
Valine	6	6	9
Cystine (half)	8	8	8
Methionine	2	2	2
Isoleucine	7	6	5
Leucine	8	8	8
Tyrosine	5	3	6
Phenylalanine	3	3	2
Tryptophan	9[b]	6	5
Lysine	13	6	5
Histidine	2	1	1
Arginine	13	11	14

[a] Assuming 2 histidine residues per molecule.
[b] Determined spectrophotometrically [13].

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[2] F. J. MORGAN and N. ARNHEIM, in *Lysozyme* (Eds. F. F. OSSERMAN, R. E. CANFIELD and S. BEYCHOK; Academic Press, New York 1973), p. 81.
[3] N. ARNHEIM and R. STELLER, Arch. Biochem. Biophys. *141*, 656 (1970).
[4] N. ARNHEIM, in *Lysozyme* (Eds. E. F. OSSERMAN, R. E. CANFIELD and S. BEYCHOK; Academic Press, New York 1973), p. 153.
[5] A. MOUTON and J. JOLLES, FEBS Lett. *4*, 337 (1969).
[6] E. F. OSSERMAN, New Engl. J. Med. *292*, 424 (1975).
[7] R. J. DECKX, G. R. VANTRAPPEN and M. M. PAREIN, Biochim. biophys. Acta *139*, 204 (1967).
[8] Y. GHOOS and G. VANTRAPPEN, Histochem. J. *3*, 175 (1971).
[9] T. L. PEETERS and G. R. VANTRAPPEN, Gut *16*, 553 (1975).
[10] S. L. ERLANDSEN, J. A. PARSONS and T. D. TAYLOR, J. Histochem. *22*, 401 (1974).
[11] M. KLOCKARS and E. F. OSSERMAN, J. Histochem. Cytochem. *22*, 139 (1974).
[12] R. J. RIBLET and L. A. HERZENBERG, Science *168*, 1595 (1970).
[13] G. H. BEAVEN and E. R. HOLIDAY, Adv. Prot. Chem. *7*, 319 (1952).

application point. At pH 5.5, the figures were 2.2 cm for MIL and 1.9 cm for HEL. Amino acid analyses performed on 2 different preparations of MIL, yielded almost identical results. The similarity with HEL is striking, although MIL is still more basic than HEL (Table), and the total number of residues is higher. From the amino acid composition a molecular weight of 17,800 is computed, slightly more than accepted molecular weight of hen egg-white lysozyme 14,600. Both lysozymes had identical migration rates in thin layer-gel chromatography.

Discussion. In normal mice the small intestine is a relatively rich source of lysozyme (about 375 µg per mouse). We decided to isolate mouse intestinal lysozyme as a preliminary step in the study of the function of the Paneth cell[9]. A much more convenient model was used by RIBLET and HERZENBERG[12], who isolated lysozyme from the urine of mice bearing a type A reticulum sarcoma, in which the urine contained up to 25 mg/ml. Probably, this urinary lysozyme, as well as that found in some types of human leukaemia[14] is produced by monocytes.

According to RIBLET and HERZENBERG[12], the extinction coëfficient of mouse lysozyme is lower than that of egg white lysozyme, due to a lower amount of tryptophan residues (4 against 6), although more residues of the less absorbing tyrosine (7 against 3) are present. The mouse intestinal lysozyme which we isolated has a high extinction coëfficient due to the presence of 9 tryptophan residues and 5 tyrosine residues. Mouse monocytic lysozyme has the same total number of residues (130) and the same number of basic residues (18) as HEL, but there are 29 acidic residues against 26 in HEL. This monocytic lysozyme moved more slowly than HEL in cellulose acetate electrophoresis. In contrast, MIL has a total of 154 residues, 34 acidic and 28 basic. The surplus of charged residues probably explains why this lysozyme moved faster than HEL in cellulose acetate electrophoresis. It is difficult to estimate how much of the lysozyme in an intestinal homogenate orginates from the Paneth cell, and how much is derived from white blood cells in the intestinal wall. It is possible that monocytic lysozyme was eliminated during the purification procedure (this could explain the low recovery we obtained in the amberlite eluate), or that scraping off mucosa from muscle layers eliminates most of the monocytes. In this context it is worth noting that rabbits, deficient in lysozyme in serum and most other tissues still contain 50% of the lysozyme of normal rabbits in their intestine[15]. The suggestion that structural differences exist between human granulocytic and macrophage lysozymes[6], should be investigated in the light of our observations, as Paneth cells may be considered to function as fixed macrophages[10]. Further research is needed to clarify these points, particularly because the existence of isozymes in sera of patients with inflammatory bowel disease could be the cause of the divergent results obtained by different methods[16–18].

[14] E. F. OSSERMAN and D. P. LAWLOR, J. exp. Med. *124*, 921 (1966).
[15] D. S. PRIEUR, H. M. OLSEN and D. M. YOUNG, Am. J. Path. *77*, 283 (1974).
[16] T. L. PEETERS, K. GEBOES and G. R. VANTRAPPEN, New Engl. J. Med. *292*, 1349 (1975).
[17] T. L. PEETERS, G. R. VANTRAPPEN and K. GEBOES, Gut *17*, 300 (1976).
[18] J. JOLLES and P. JOLLES, Helv. chim. Acta *52*, 2671 (1969).

Galactose Transfer and UDP-Galactose Hydrolysis in Urine from Normal Balb/c and Balb/c YC8 Mice

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Summary. By chromatographic method we have shown the existence of a complex system for galactose transfer from UDP-galactose and for nucleotide hydrolysis in urines from Balb/c YC8 and normal Balb/c mice. By action of sera from normal and ascitic mice as source of enzyme, we have been able to detect transfer for galactose in urines from ascitic mice and an important inhibitory effect of the nucleotide sugar hydrolysis by the sera with urines from normal mice.

In previous studies, we have demonstrated the presence of soluble glycoprotein-galactosyl transferase activity in sera and in ascitic fluids from Balb/c mice bearing a lymphoma transmitted in ascitic form by i.p. injections of YC8 cells[1]. These results confirm those of BODOLSKY and WEISER[2] who detected galactosyl-transferase activities in human sera and a cancer associated isoenzyme in cancer sera. In our experiments, we have detected, in association with galactosyl-transferase activity, an important ratio of endogeneous transfer in sera and ascitic fluids from YC8 mice.

In the present study, we have investigated a glycoprotein-galactosyl-transferase activity in urines from healthy and ascitic mice on endogeneous and exogeneous acceptors, and we have demonstrated a galactose transfer in urines from ascitic mice by use of enzymes from sera. Hydrolysis of UDP-galactose has also been studied.

Materials and methods. UDP [(^{14}C)]-galactose (specific activity 274 mCi/m*M*) was purchased from New England Nuclear Corporation. Radioactivity was counted in a toluene PPO-POPOP as scintillant liquid. Glycoprotein acceptor in these studies was ovomucoid prepared as indicated by JAKUBCZAK and MONTREUIL[3]. Strain of YC8 ascite cells was carried in adults Balb/c mice by i.p. injections of 2×10^5 cells. Cells suspensions were harvested weekly. Sera from ascitic mice and from normal Balb/c mice were collected.

Urines from ascitic and normal Balb/c mice were collected for 12 h daily. Experiments were carried with 80 ascitic mice and 20 normal mice. Protein and sugars were determined in total urines according to PIRONNEAU et al.[4].

The assay mixture for galactosyl-transferase activity included, 10 µl urine (60 µg protein), 10 µl 1 *M Tris* pH 7.2, 5 µl 0.4 *M* $MnCl_2$, 100 µg ovomucoid for exogeneous