

It is interesting that each of the major glycolipids occurring in plants (the sulpholipid and two β -D-galactosyl diglycerides^{8,9}) possesses a linkage which may be cleaved by β -galactosidase.

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Interaction between Lysozyme and a Phospholipid of Neoplastic Tissue (Oncolipin)

LYSOZYME is a basic protein, tending to form complexes with fatty acids, aliphatic long-chain alcohols¹, alkyl sulphates^{1,2}, colic acids³, and cephalins⁴. Interaction promoted by lysozyme towards Rous sarcoma phosphatides is of particular interest⁵.

We have examined the possibility of lysozyme producing insoluble complexes with a phospholipid (oncolipin) isolated in malignant tissues by Aoyama⁶.

Tests on interaction have been performed on phosphate 0.067 buffers, using Sørensen's technique.

Oncolipin was isolated from Yoshida's ascite sarcoma in rats. This sarcomatous phospholipid is suspended in saline solution and the above suspension is used for preparing the 0.01 per cent solution of the substance contained in phosphate buffers, at pH ranging from 5.9 to 8.2.

Table 1. TURBIDITY RELATED TO HYDROGEN-ION CONCENTRATION, IN SØRENSEN'S PHOSPHATE BUFFERS

Sample (per cent)	Hydrogen-ion concentration (pH)					
	5.9	6.6	7.1	7.6	8.0	8.2
Lysozyme 0.05	0.004	0.004	0.004	0.004	0.004	0.004
Oncolipin 0.01	0.022	0.020	0.024	0.027	0.029	0.027
Oncolipin 0.01 + Lysozyme 0.001	0.036	0.034	0.032	0.038	0.041	0.038
Oncolipin 0.01 + Lysozyme 0.005	0.051	0.053	0.061	0.065	0.068	0.068

The 0.01 per cent solution of oncolipin in phosphate buffers is slightly turbid. By addition of lysozyme, the turbidity becomes more evident, and proportionally to the added quantity of the enzyme.

It is concluded that lysozyme interacts with oncolipin, at saline hydrogen-ion concentrations, by promoting formation of insoluble complexes. This behaviour of lysozyme is like that of another basic

protein, namely salmine, being also an insoluble product interacting with oncolipin, at pH 6.8-7.0 (ref. 6).

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Lysozyme from Human Milk

CONTINUING our research on lysozymes from different origins¹, we were interested in the purification of a human lysozyme. Some preliminary experiments have indicated that human placenta, saliva and spleen contain lysozymes, approximately 4, 10 and 40 mgm./kgm. respectively (quantities expressed in mgm. of hen's egg white lysozyme). In contrast with cow's milk, we have found that human milk contains a lysozyme (40 mgm./l. expressed in mgm. of hen's egg white lysozyme).

We now report the isolation of this new lysozyme which we obtained in a chromatographically pure state.

The purification includes three steps:

(1) Adsorption of the lysozyme on 'Amberlite CG-50' buffered with a 0.2 M phosphate buffer of pH 6.5. 50 ml. of 'Amberlite' are added to 1 litre of milk and the mixture is stirred for 4 hr. The resin is then washed with water and the lysozyme is eluted with a 0.8 M phosphate buffer of pH 6.5. After dialysis against water at 2° C. and lyophilization, a 'primary material' is obtained: the lysozyme has undergone a 250-fold purification.

(2) Chromatography of the primary material on carboxymethylcellulose. The conditions are indicated in Fig. 1. 200 mgm. of the primary material are chromatographed on a 40 × 1.8 cm. column at 20°. Lysozyme activity was determined by its bacteriolytic action on *Micrococcus lysodeikticus*². After rechromatography of the lysozyme-containing fractions in the same conditions, a symmetrical peak, active against *M. lysodeikticus*, is obtained where the specific activity is constant. In this way, the primary material was again purified 7-8 times.

(3) Desalting of human lysozyme on 'Sephadex G-50'. The lysozyme-containing fractions after chromatography on carboxymethylcellulose are pooled, dialysed against water at 2° C. and lyophilized.

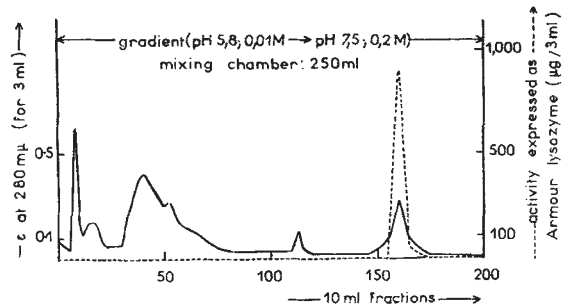


Fig. 1