ISOLATION AND PURIFICATION OF THE BIOLOGICALLY ACTIVE DES-LYSYLVALYL PHENYLALANINE-LYSOZYME.*

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After the elucidation of the chemical structure of hen's egg-white lysozyme (N-acetylmuramide glycanohydrolase E.C.3.2.1.17) (Jollès et al., 1963,1964), some studies concerning the relationship between chemical structure and biological activity were undertaken. Some points concerning the C-terminal sequence Arg-Leu.OH, the importance of tryptophan and cystine residues (Jollès, 1964) and also the unic histidine residue were already reported (Jollès et al., 1965). No data concerning the N-terminal sequence were however available until now; the native lysozyme molecule was not digested by aminopeptidases like those described by Smith (1960) or Pfleiderer and Celliers (1963). Recently we purified and studied a microbial aminopeptidase (Uhlig et al., 1965) which was able to split off the N-terminal lysine and a part of the following amino acids from native lysozyme. Our aim was to purify from the reactive mixture a well defined shortened lysozyme.

Materials and Methods

Hen's egg-white lysozyme was obtained from Armour, Kankakee, Ill. (lot (N° 638040).

Action of aminopeptidase. Native lysozyme was digested at pH 7.6 (0.1 M veronal buffer; or trimethylamine) and 37° with

* 47th communication on lysozymes; 46th communication, see D. Charlemagne and P. Jollès, Nouvelle Rev.franç.Hématol., 1965, in the press.

the microbial aminopeptidase (Uhlig et al., 1965); enzyme/substrate: 1/100; different reaction times.

Determination of the liberated amino acids. In order to determine the extent of the enzymic reaction and to characterize the liberated amino acids, aliquots of the enzymic digest were withdrawn and analyzed with a Technicon Autoanalyzer after precipitation of the protein.

Chromatography of the enzymic digest. Native lysozyme as well as the enzymic digest were chromatographed on a 60x1.8 cm column of Amberlite CG-50 (Type II) with a 0.2 M phosphate buffer of pH 7.18. The peaks were characterized with the ninhydrin reagent of Moore and Stein (1954) after alkaline hydrolysis and desalted on Sephadex G-25 with water or 0.1 N acetic acid or 0.1 N formic acid as eluent.

Biological activity. The biological activity of lysozyme was determined as described by Jollès (1962).

Table I

Amino acids (%) liberated by the action of microbial aminopeptidase (Uhlig et al.,1965) on native lysozyme and peak 5 (see figure 1).

Substance	Reaction time (hour)	Lys	Val	Phe	Gly	Arg
native lysozyme	0	0	0	0	0	0
id.	0.5	12	6	6	6	3
id.	1	20	9	9	8	8
id.	6	60	27	21	8	4
id.	24	76	55	13	12	11
peak N° 5	6	0	0	5	5	3

Results

The N-terminal sequence of lysozyme is H.Lys-Val-Phe-Gly-Arg-Cys-... Table I indicates the amount of the different amino acids splitt off by the aminopeptidase after different reaction times. The biological activity remains quite stable during the first six hours and decreases of around 30% after 24 hours.

500 mg of native lysozyme were submitted during 24 hou to the action of the microbial aminopeptidase and the enzymic digest was chromatographed on Amberlite CG-50. Seven peaks were obtained (figure 1). Peaks 3 to 7 were biologically active. Peak 5, the most important one, was desalted on Sephadex G-25 and rechromatographed on Amberlite CG-50.

The amino acid composition of peak 5 was established: it contains the same number of amino acid residues as native lysozyme except for lysine and valine; one residue of both of these amino acids failed in peak 5. The N-terminal amino acid,

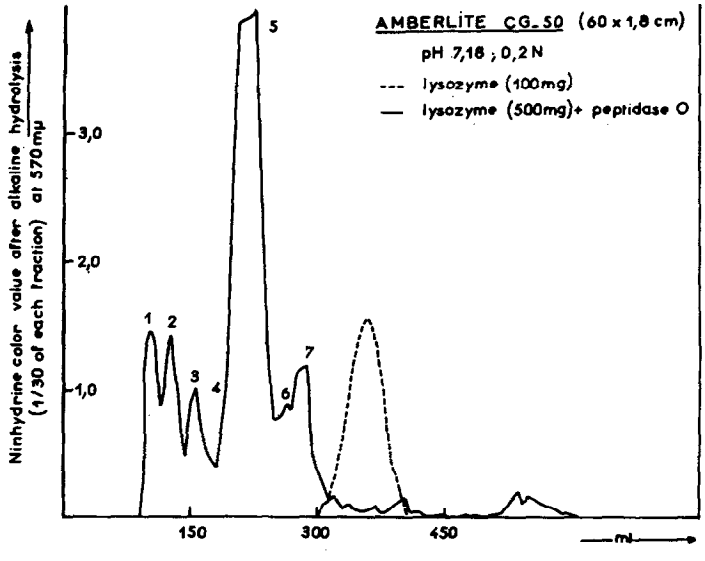

Figure 1

Chromatography on Amberlite CG-50 of native lysozyme (-----) and of the enzymic digest obtained by the action of the microbial aminopeptidase on native lysozyme (——).

phenylalanine, was determined by the method of Sanger and the N-terminal sequence by the action of aminopeptidase. This latter liberated no lysine nor valine but was able to split off small quantities of phenylalanine, glycine and arginine. These experiments indicate that peak 5 contains des-lysylvalyl phenylalanine-lysozyme. Activity determinations have shown that this new derivative is as active as native lysozyme.

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