PURIFICATION AND CHARACTERIZATION OF A LYSOZYME FROM GOOSE EGG WHITE*

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An explanation of the mechanism of action of hen egg lysozyme (muramidase) that is based upon X-ray crystallographic studies of the structure of this enzyme has been proposed. Specifically, a site on the protein that binds a trisaccharide inhibitor of lysozyme has been located, and the details of the suggested mechanism of action have been deduced from observations of the region adjacent to this locus (Johnson and Phillips, 1965; Phillips, 1966). While the evidence is excellent for the general location of the site of catalytic activity, it is difficult to obtain evidence to substantiate the inferred mechanism of action. One indirect approach to this problem would be to determine the structure of another lysozyme that possesses a distinctly different amino acid sequence than hen egg lysozyme with the hope that examination of the three-dimensional structure of its active site would permit one to note those features common to both which are essential for enzyme activity and to note also those dissimilar features that might explain differences in the observed characteristics of the action of the two lysozymes. This report describes the initiation of a study of goose egg lysozyme, which is shown to have a significantly different amino acid composition than the enzyme from hen egg. In addition the rate of lysis of M. lysodeikticus cell walls by

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goose egg lysozyme is increased, while the capacity to hydrolyze a pentasaccharide from chitin is lost, indicating that the structure of the active site differs from that of hen egg lysozyme.

Experimental Procedure and Results:

Goose egg white (Embden Geese) was suspended in two volumes of 0.05 M NaH_2PO_4 and CM-cellulose (4 gms/liter) was added with constant stirring at 4°. After 12 hours the CM-cellulose was centrifuged and washed with 0.05 M NH_4HCO_3 and the supernatant discarded. Protein containing the lysozyme activity was eluted by washing the cellulose with 0.4 M $(NH_4)_2CO_3$. The solution was lyophilized to eliminate most of the salt, redissolved in water, and applied to a 2.5 X 12 cm column of microgranular CM-32 cellulose (Whatman). This column was developed at 4° with a two chamber varigrad running from 0.05 M $(NH_4)_2CO_3$ to 0.20 M $(NH_4)_2CO_3$ and lysozyme activity was determined in the effluent by techniques described earlier (Canfield, 1963a). The results are shown in Figure 1. The material in the center of the peak of lysozyme activity was lyophilized and when subjected to chromatography again under identical conditions, a single symmetrical peak was obtained.

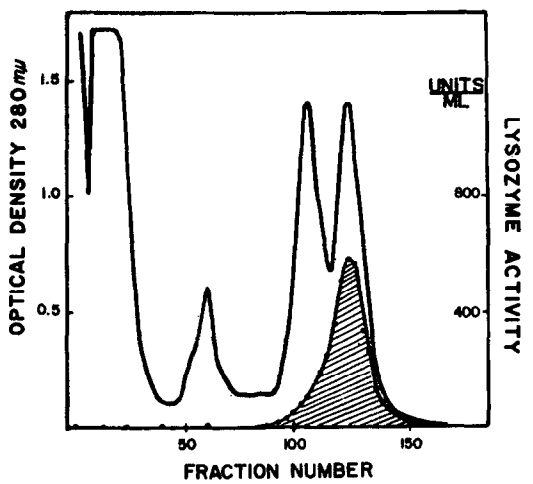

Figure 1. Chromatography of protein obtained from goose egg white on a 2.5 X 12 cm column of CM-cellulose. The shaded area depicts lysozyme activity which is plotted in arbitrary units (see text).

Goose egg lysozyme prepared by this procedure yields a single peak when passed over a column of G-75 Sephadex and a single band when subjected to gel electrophoresis. The molecular weight of the reduced, carboxymethylated protein determined by high speed sedimentation equilibrium is $14,700 \pm 1,000$, and trypsin digestion of this derivative yields approximately twenty unique peptides which is consistent with the number of lysine and arginine residues reported in Table 1.

Estimations of lysozyme activity based upon the initial rate of change of optical density at 650 mμ of a suspension of M. lysodeikticus cell walls in 0.06 M potassium phosphate, pH 6.2, reveals that goose egg lysozyme is three times as active as an equimolar amount of hen egg lysozyme. Following lysis by the goose enzyme there is an increased amount of reducing sugar in solution (Schales and Schales, 1945).

Equal aliquots of the goose egg lysozyme were subjected to acid hydrolysis at 110° in evacuated tubes for 24, 48 and 72 hour periods. The results of amino acid analyses obtained with the aid of a Beckman-Spinco Model 120 B amino acid analyzer are shown in Table 1. The estimation of tryptophan content was obtained by the method of Koshland (Horton and Koshland, 1965). An amino acid analysis of a sample of performic acid oxidized enzyme (Hirs, 195 yielded an estimated 3.2 residues of cysteic acid. In addition, an aliquot (15 mg) of the protein was dissolved in 2 ml of 8 M urea buffered by $(NH_4)HCO_3$ and reacted with 7 mg of ^{14}C iodoacetic acid at 20° for 15 minutes. The mixture was passed over a 2.5 X 15 cm column of G-25 sephadex and radioactivity determined in each of the effluent fractions. There was no radioactivity in the protein peak, and amino acid analysis did not reveal any S-carboxymethyl cysteine. On the basis of these results it is concluded that there is no cysteine and that the cysteic acid analysis is most consistent with two cystine disulfide links in the protein.

Jollès has studied the composition and substrate specificities of duck egg lysozymes, which exhibit fewer variations from hen lysozyme than

TABLE 1

AMINO ACID ANALYSIS OF GOOSE EGG LYSOZYME

	Time of hydrolysis (micromoles)			Estimated Residues	Hen Lysozyme[a]
	24 hr	48 hr	72 hr		
Lysine	.383	.387	.387	11	6
Histidine	.114	.117	.116	3-4	1
Arginine	.230	.232	.229	6-7	11
Aspartic Acid	.468	.476	.471	13-14	21
Threonine	.287	.279	.264	8-9	7
Serine	.208	.192	.170	6-7	10
Glutamic Acid	.345	.349	.349	10	5
Proline	.093	.094	.093	2-3	2
Glycine	.485	.489	.486	14	12
Alanine	.352	.358	.357	10	12
Half-cystine	.069	.061	.052	3-4[b]	8
Valine	.226	.249	.253	7	6
Methionine	.068	.068	.070	2	2
Isoleucine	.278	.305	.312	9	6
Leucine	.162	.164	.164	4-5	8
Tyrosine	.210	.208	.205	6	3
Phenylalanine	.072	.069	.070	2	3
Tryptophan				2-3[c]	6
Total				118-127	129

a) From data of Canfield, 1963 b.

b) Based upon recovery of 3.2 residues of cysteic acid in an analysis of a performic acid oxidized sample of protein.

c) Determined by reaction with 2-hydroxyl-5 nitrobenzyl bromide (Horton and Koshland, 1965)

the goose enzyme reported here (Jollès, 1965a; Sharon, 1966). The existence of lysozyme activity in goose egg white has been noted previously (Jollès, 1965 b).

Several results reported here suggest that the lysozyme from goose egg white differs significantly from that found in hen egg white. The amino acid composition differences, especially those for lysine, arginine, glutamic acid, half-cystine, tyrosine and tryptophan, indicate that large portions of the primary structure must be different, and preliminary results of the analyses of tryptic peptides support this conclusion. Furthermore, the finding that under the assay conditions reported here, goose lysozyme is several times more active than hen lysozyme in its action

upon M. lysodeikticus cell walls, and the preliminary finding of Rupley that goose lysozyme is not active against a pentasaccharide fragment of chitin that is hydrolyzed by hen lysozyme (Rupley, 1966) indicate that the structures of the sites of catalytic function on the two enzymes are different Since both lysozymes are found in bird egg white and since both appear to liberate reducing sugars, it is probable that they act at the same site in cell walls. However, this point is not certain and requires further investigation.

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