

The Amino Acid Sequence of Quail Lysozyme

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Hen egg-white lysozyme [EC 3.2.1.17] is one of a few enzymes whose primary, secondary and tertiary structures have been completely determined by the chemical (1—3) and the X-ray crystallographic (4—7) analyses. Much structural information is also available on this enzyme with regards to its active and substrate-binding sites (8, 9). It would be expected that the structural study on lysozyme from other species provides further information concerning the relationship between structure and function, and also the chemical basis of evolutionary changes in this enzyme.

In the present preliminary note we report the tentative amino acid sequence of lysozyme from Japanese quail (*Coturnix coturnix*) egg-white. Quail lysozyme was purified on a CM-cellulose column, and crystallized in a way similar to that for hen lysozyme (10). Hen and quail lysozymes showed qualitatively the same lytic activity against *Micrococcus lysodeikticus* as the substrate (11). Quantitative amino acid analysis showed that quail lysozyme contains one more residue each of valine, tyrosine, histidine, and lysine, and one less residue of aspartic acid (or asparagine), glycine, phenylalanine, and arginine than hen lysozyme. In order to avoid modification of methionine residues which might occur to some extent during the S-carboxymethylation of proteins, the intact quail lysozyme was first treated with cyanogen bromide (1% protein, and 1% BrCN in 70% formic acid, 27°C, 24 hr) (12). Although no peptide fragment

could be liberated by cyanogen bromide treatment of lysozyme, three peptides, BrCN-I, II, and III, were separated by gel filtration of the reaction mixture on a Bio-Gel P-10 column with 1N formic acid after reduction of the four disulfide bonds and subsequent S-carboxymethylation (13). Quantitative amino acid analysis showed that three peptides have the following composition in the molar ratio; BrCN-I: Cys(cm)₁, Glx₁, Gly₁, Ala₃, Val₁, Leu₁, Tyr₁, Lys₁, Arg₁, and homoserine (+homoserine lactone)₁. BrCN-II: Cys(cm)₅, Asx₁₆, Thr₆, Ser₁₀, Glx₄, Pro₂, Gly₈, Ala₆, Val₄, Ile₅, Leu₆, Tyr₃, Phe₂, Trp₃, Lys₅, His₂, Arg₅, and homoserine (+homoserine lactone)₁. BrCN-III: Cys(cm)₂, Asx₄, Thr₁, Gly₂, Ala₃, Val₂, Ile₁, Leu₁, Trp₃, Lys₁, and Arg₄. Peptide BrCN-III must be the C-terminal portion of the whole molecule because it lacks homoserine or homoserine lactone as the constituent. The Edman degradation on the reduced and S-carboxymethylated derivative of the whole protein showed that BrCN-I must occupy the N-terminal position. The BrCN peptides, consequently, can be assigned the order I-II-III. The amino acid sequence of three peptides has been completely or so far partially determined by the Edman degradation technique (14), and by considering the results of the Edman degradation of their tryptic and/or chymotryptic peptides. These results are consistent with the prediction that hen and quail lysozymes are analogous to each other in the amino acid sequence. The amino acid interchanges be-



FIG. 1. The amino acid sequence of lysozymes from hen and quail.

The sequence of hen lysozyme was taken from the paper by CANFIELD (2). The portions of the undetermined sequence of quail lysozyme are shown in parentheses. BrCN peptides were numbered in order from the N-terminus in the whole molecule. Asx and Glx are used when the acid or the amide form of aspartic and glutamic acid residues has not been established.

tween hen and quail lysozymes have been so far established at the residue Nos. 3, 19, 21, 102, 103, and 121.

Residue No.	3	19	21	102	103	121
Hen	Phe	Asn	Arg	Gly	Asp	Gln
Quail	Tyr	Lys	Gln	Val	His	Asn

No more interchange appears to be expected, since the above interchanges are compatible with the difference between hen and quail lysozymes in the amino acid composition described above.

It seems to be interesting that interchanges at the residue 102 and 103 are ad-

jacent to aspartic acid at the residue 101 which is believed to be involved in the substrate binding in hen lysozyme (7, 8), while others are far from the active and the substrate binding sites. Our results on Japanese quail lysozyme appear to be different from those on American quail lysozyme recently published by ARNHEIM *et al.* (15).

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