BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 50 (11), 3073—3074 (1977)

Chemical Synthesis of Antigenic Determinants of Hen Egg-White Lysozyme. II. Tetradecapeptide Corresponding to Positions 1—14 in the Primary Structure but with Ala^{6} and Met(O)^{12} *

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(Received August 1, 1977)

Synopsis. For characterizing one of the antigenic determinants of hen egg-white lysozyme in delayed hypersensitivity, the tetradecapaptide, H-Lys-Val-Phe-Gly-Arg-Ala-Glu-Leu-Ala-Ala-Ala-Met(O)-Lys-Arg-OH, was synthesized by the solid phase method using ultrasonic waves. This peptide corresponds to positions 1—14 of the amino acid sequence of hen egg-white lysozyme but with alanine and methionine *S*-oxide residues at positions 6 and 12, respectively.

The antigenic determinant on a macromolecule recognized by immunocytes for circulating antibody is topographically distinct from that for cellular immunity or delayed hypersensitivity induced with glucagon[1,2] as an immunogen. Recently, Miyagawa *et al.*[3,4] investigated the structural relations between the antigenic determinants of hen egg-white lysozyme for circulating antibody and those for delayed hypersensitivity, induced by hen egg-white lysozyme as an immunogen. They found that the peptide [sequence[5,6] Lys^{1} to Hse^{12} and Trp^{123} to Leu^{129} linked by a disulfide (Cys^{6} and Cys^{127})], named here peptide A, obtained by treatment of the peptide [sequence Lys^{1} to Asn^{27} and Trp^{123} to Leu^{129} linked by a single disulfide (Cys^{6} and Cys^{127})], named here peptide B, with cyanogen bromide, reacted both with circulating antibody to hen egg-white lysozyme and with cells sensitized with hen egg-white lysozyme in delayed hypersensitivity. However, when peptide A was reductively alkylated, the resulting N-terminal peptide [Lys^{1} to Hse^{12}] showed no reactivity with circulating antibody of hen egg-white lysozyme elicited to peptide B,[7] but retained its ability to induce delayed hypersensitivity in cells sensitized with hen egg-white lysozyme. These results show that the specificity of the antigenic determinant elicited to peptide B for circulating antibody is distinct from that for delayed hypersensitivity, induced with hen egg-white lysozyme as an immunogen.

For characterizing this determinant, we synthesized the N-terminal fragment of hen egg-white lysozyme, H–Lys–Val–Phe–Gly–Arg–Ala–Glu–Leu–Ala–Ala–Ala–Met(O)–Lys–Arg–OH, by the solid phase methode using ultrasonic waves.[8] The sequence contained alanine and methionine *S*-oxide residues instead of cysteine and methionine residues at positions 6 and 12 of hen egg-white lysozyme, respectively, because it seemed unlikely that a disulfide linkage between positions 6 and 127 and the methionine residue at position 12 were essential for reactivity. The crude peptide cleaved from resin with hydrogen fluoride was purified on a column of carboxymethylcellulose, as shown in Fig. 1. The tetradecapeptide thus synthesized inhibited migration of macrophages from a peritoreal exudate from guinea pigs immunized with hen egg-white lysozyme.[9] Furthermore, this peptide was found to be immunogenic in guinea pigs.[9]

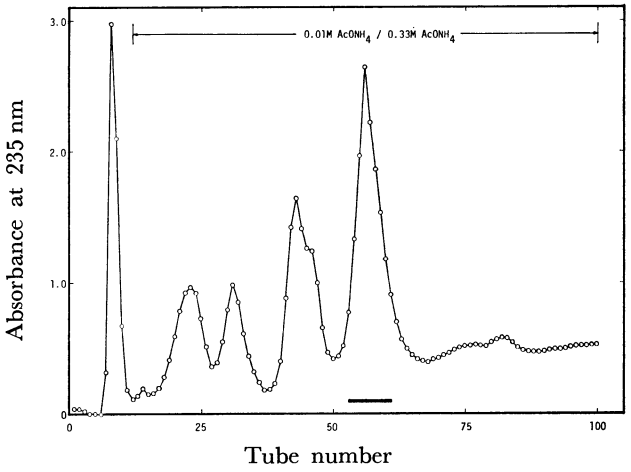

Fig. 1. Chromatogram of the crude product on carboxymethylcellulose (ammonium cycle). The column was eluted with a linear gradient of 0.01 M to 0.33 M ammonium acetate, pH 7.0, at a flow rate of 25—30 ml per h. Fractions of 3 g were collected.

Experimental

Boc–Arg(NO_2)–resin (2.00 g; 0.18 mmol of arginine per g of resin) prepared by the method of Kishida *et al.*[10] was used as starting material. The peptide was synthesized on the solid support using ultrasonic waves, as reported previously,[8] except that a mixture of trifluoracetic acid and dichloromethane (v/v, 1/1) was used instead of 1M HCl in acetic acid for removing the Boc group. The Boc amino acid derivatives were coupled in the following order: Lys(Z), Met(O), Ala, Ala, Ala, Leu, Glu(OBzl), Ala, Arg(Tos), Gly, Phe, Val, and Lys(Boc). After introductions of the Boc derivatives of Lys(Z), Met(O), and Arg(Tos), respectively, the resin was treated with acetic anhydride.[11] Peptide resin (520 mg) was weighed with anisole (0.6 ml) in the Daiflon cylinder of the HF-reaction apparatus.[12] HF (10 ml) was distilled into the cylinder previously cooled to −78 °C in a Dry-Ice-methanol bath. The sample was transfered from the bath at −78 °C to that at 0 °C and stirred for 60 min. Then the HF was evaporated off from the reaction mixture as fast as possible. The free peptide cleaved from the resin was extracted into 0.1 M acetic acid by treatment with ultrasonic waves for 3 min. The extract was passed through a column of IR-45 (acetate cycle). The eluate was lyophilized; 100 mg (*ca.* 75 %). This material (80 mg) was dissolved in 0.01 M am-

* All the amino acids used except glycine were of the L-configuration. The abbreviations used are those recommended by IUPAC-IUB: *J. Biol. Chem.*, **247**, 977 (1972).

monium acetate and charged on a column of carboxymethylcellulose (ammonium cycle, 1×33 cm). The column was eluted with 700 ml of a linear gradient of 0.01 M to 0.33 M ammonium acetate at pH 7.0. The fractions shown by a bar in Fig. 1 were collected and lyophilized; yield 30 mg; R_f (TLC) 0.34 in 1-butanol : acetic acid : pyridine : water (15 : 3 : 10 : 12, by volume); $R_{f,arg}$ (paper electrophoresis) 0.67 (0.2 M pyridinium acetate, 30 V/cm). The amino acid ratio after hydrolysis in 6M HCl for 24 h at 105 °C was Lys, 1.99 (2): Arg, 2.01 (2): Glu, 1.02 (1): Gly, 1.00 (1): Ala, 3.85 (4); Val, 0.92 (1): Met, 0.82 (1): Leu, 1.00 (1): Phe, 1.06 (1).

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