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A New Reductive Modification of Hen's Egg White Lysozyme with Pyridine-borane

A reductive modification of tryptophan residues in hen's egg white lysozyme was carried out in trifluoroacetic acid with pyridine-borane. Six tryptophan residues were fully reduced to the corresponding 2,3-dihydro compounds without any detectable change in the molecule. Enzymic activities of the reduced lysozyme was found to be completely lost.

Keywords——lysozyme; 2,3-dihydrotryptophan; pyridine-borane; trifluoroacetic acid; chemical modification

We report a new chemical modification of hen's egg white lysozyme by reducing tryptophan residue selectively to 2,3-dihydro compound without affecting other functional groups of the protein. This reduction procedure proved to be effectively utilized in the study of structure-activity relationships of biologically active peptides and proteins containing tryptophan residue when they are stable in trifluoroacetic acid for few minutes under cooling. The modification of tryptophan residue in peptides has been achieved only through oxidative procedures[1] with the limitation due to the undesirable oxidation of other functional groups. Although the literature dealing with the chemical modification of tryptophan residue in proteins is extensive,[2] the more specific reactions between the reagents used and the tryptophan residue in proteins have been required. One of us has recently reported the novel reductive modification of tryptophan residue in dipeptides[3] and in octapeptide, Xenopsin.[4] In this communication, we applied this method to the modification of hen's egg white lysozyme. The method gives a good yield of conversion and shows specificity to the reduction of tryptophan residue.

Hen's egg white lysozyme was reduced by the method published previously.[3] To 30 mg of commercially available lysozyme in 2 ml of ice-cooled trifluoroacetic acid, 10 μl of pyridine-borane[5] was added with vigorous stirring. After 2 min of stirring, tris(hydroxymethyl)aminoethane was added to neutralize the solution. The resulting solution was dialyzed and lyophilized as usual to give 27 mg of the reduced lysozyme (yield, 90%). To exchange the trifluoroacetate anion for acetate anion, the reduced lysozyme (2 mg) was dissolved in a small volume of 0.01 M $AcONH_4$ buffer (pH 8.0) and passed through a column of DEAE-Sephadex A-25 (0.9×15 cm, acetate form) with the same buffer as the eluting solvent. The fractions were monitered for absorption at 280 nm. Amino acid analyses of the reduced lysozyme thus obtained were performed with JEOL (JLC-6AH). The reduced lysozyme (1 mg) was hydrolyzed in 200 μl of 6N HCl containing 2% thioglycolic acid[6] in an evacuated, sealed glass tube at 110° for 22 hrs. The amount of 2,3-dihydro-L-tryptophan was calculated from the area of the peak corresponding to the authentic compound (mp 267—268 (dec.)), recryst. from H_2O–EtOH; *Anal.* Calcd. for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found:

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TABLE I. Amino Acid Compositions of the Reduced Lysozyme

Amino acid	Reduced lysozyme	Native lysozyme	Published amino acid composition[7]
Asp	21.5	20.1	21
Thr	7.0	6.7	7
Ser	8.9	8.6	8
Glu	4.9	4.7	5
Pro	1.8	1.9	2
Gly	12.3	12.5	12
Ala	12[a]	12[a]	12
Val	5.4	5.4	5
Met	2.2	2.6	2
Ile	5.2	5.7	5
Leu	7.9	8.2	8
Tyr	2.9	2.9	3
Phe	2.8	3.0	3
His	0.9	0.9	1
Lys	6.0[b]	5.8	6
Arg	10.6	11.5	10
Trp	0	5.8	6
2HTrp[c]	5.9[b]	—	—

a) Calculations of values of amino acids are based on the content of 12 alanine residues per molecule of the reduced lysozyme.
b) One of the diastereomers of 2HTrp eluted with lysine and lysine was taken as 8.0 residues in calculations. The value of 2HTrp was recalculated to be 5.9 by assuming that the value of lysine was 6. Accordingly the ratio of the diastereomers of 2HTrp is 1.9: 1.0.
c) 2,3-Dihydro-L-tryptophan.

C, 64.04; H, 6.85; N, 13.43. MS *m/e*: 206 (M^+) which was obtained by the reduction of L-tryptophan with pyridine-borane.[3] The six tryptophan residues of hen's egg white lysozyme were fully reduced to the corresponding dihydrotryptophans and the amino acid compositions of the reduced lysozyme were within reasonable agreement with the corresponding theoretical values. Spectrophotometric assay for the reaction of N-ethylmaleimide[8] with the reduced lysozyme was performed to check the reductive cleavage of disulfide bonds and showed negative results. Enzymic activities of the reduced lysozyme was examined by use of *Micrococcus lysodeikticus* cell[9] and found to be completely lost, while 91% of the activities retained in the trifluoroacetic acid treated control experiment. As the tryptophyl residue is ofter found in the active site of peptides and plays an important role in the enzymic activities, this reduction procedure will find wide application as a general approach to protein modification.

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