The C-terminal amino-acid sequence of lysozyme

Leucine has been identified as the C-terminal amino acid in lysozyme by the carboxypeptidase[1], reduction[2] and hydrazinolysis[3] techniques. Evidence that the penultimate residue is basic has been presented[4] in the fact that leucine is liberated during tryptic digestion of lysozyme. Conflicting evidence regarding the C-terminal sequence has been reported[5] from a study of the peptides present in partial hydrazinolysates of lysozyme. The sequence, according to OHNO, is Asp.Gly.Ala.-Asp (NH_2).Leu. If this were the sequence, it would be expected that asparagine, alanine and preceding amino acids would be released in addition to leucine by carboxypeptidase, whereas the liberation of amino acids other than the C-terminal leucine from both heat-denatured lysozyme and oxidised lysozyme is extremely slow and no asparagine is detectable. If a basic residue preceded the C-terminal leucine, the slow release of the penultimate and preceding residues would not be surprising since recrystallised carboxypeptidase splits off C-terminal basic amino acid residues very slowly[6]. In experiments on the hydrazinolysis of lysozyme, BRADBURY[7] detected no C-terminal peptides, but from the rate of liberation of leucine concluded that a penultimate lysine residue was unlikely. In an attempt to confirm that a basic residue preceded the C-terminal leucine, the following experiments with lysozyme oxidised with performic acid were successful in establishing the C-terminal sequence arginylleucine. The oxidised lysozyme (106 mg) was esterified with methanol-HCl[8] so that the C-terminal leucine was now labelled specifically as leucine methyl ester. Digestion of the esterified protein in 20 ml 0.2 *M* phosphate buffer, pH 7, with 2 mg Armour crystalline trypsin (containing 50% $MgSO_4$) for 8 h at 25° C released leucine methyl ester, which was extracted into ethyl acetate (3 × 10 ml) after saturation of the solution with K_2HPO_4. The leucine methyl ester was identified by comparison with an authentic sample on paper chromatograms developed with butanol/acetic acid/water (4 : 1 : 1 aged) and 75% aqueous phenol/NH_3/HCN. After treatment *in vacuo* with 0.1 *N* NaOH overnight the extract was neutralised with 0.1 *N* HCl and aliquots were examined by paper chromatography. A single spot running with leucine in both solvents was obtained. The yield of leucine estimated by the ninhydrin method[9] was 24%. Unoxidised lysozyme methyl ester, following digestion with trypsin, also gave leucine methyl ester although the yield was somewhat lower (16%).

In view of the insolubility of the substrate, the ease of saponification and polymerisation of amino acid esters, and difficulty of quantitative extraction, these low yields are not surprising.

To obtain further evidence regarding the penultimate residue, the oxidised lysozyme was treated with reagents which would substitute the free amino groups and consequently make bonds involving the carboxyl group of the substituted lysine residues no longer susceptible to trypsin[10]. Acetylation of the amino groups and simultaneous esterification was carried out with a mixture of methanol and acetic anhydride[11]. Digestion of the product with trypsin gave leucine methyl ester in 20% yield. Similarly the dinitrophenyl-substituted protein[12] after esterification and tryptic digestion gave leucine methyl ester in 11% yield. Since blocking of the amino groups would be expected to be almost complete, the failure of these substituents to prevent liberation of leucine methyl ester shows that arginine is the penultimate residue.

This reasoning assumes that trypsin will attack only those peptide bonds involving the carboxyl group of a basic amino acid residue. This has been the case with both synthetic substrates and those polypeptide chains whose amino acid sequence is known[13].

Since this work was completed a note by THAUREAUX AND JOLLES[14] has come to hand in which they have established the C-terminal sequence Gly.$CySO_3H$.Arg.Leu in oxidised lysozyme.

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