

Study by Mass Spectrometry of Amino Acid Sequences in Peptides containing Histidine

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The choice of a method for the preparation of peptide derivatives suitable for mass spectrometry depends to a certain extent on the composition of the peptides. It is now common practice, besides blocking of the functional groups, to permethylate the peptide bonds. The permethylation of peptides containing histidine, however, gives rise to some problems.

Our procedure is the following. We prepare a protected peptide by dissolution of 0.01 mmol of a peptide in 2 ml of water and subsequently add 0.01 mmol of diethyl pyrocarbonate/acylatable group. The pH is kept constant at 8.0 with a pH-stat for 30 min. After acidification with 1 M-HCl the ethoxycarbonylated component is extracted with ethyl acetate (Kamerling, Heerma & Vliegenthart, 1968). Besides the amino group, the phenolic hydroxyl group of tyrosine, the thiol group of cysteine and the *N*-imidazole group of histidine are also ethoxycarbonylated. The peptide derivative is subsequently permethylated with methyl iodide and methylsulphonyl carbanion as base in dimethyl sulphoxide (Vilkas & Lederer, 1968). The application of this method to histidine-containing peptides leads to the formation of a quaternary base, which remains in the water phase during the extraction with chloroform. We were able to circumvent this problem by cleavage of the imidazole ring of histidine with an excess of diethyl pyrocarbonate. After the reaction of the peptide with the initial amount of diethyl pyrocarbonate an excess of 4 mol of reagent/mol of histidine residue is added in four 1 mol portions at 30 min intervals at constant pH 8.0. This operation splits off the C-2 atom of the imidazole ring as formate, and converts the remaining part of the ring

into the 1,2-bis(ethoxycarbonylamino)ethylene group. After acidification the reaction product can be isolated by extraction with ethyl acetate and subsequently be permethylated. The product is now soluble in chloroform and suitable for mass spectrometry.

Histidine itself can also be cleaved by exhaustive treatment with diethyl pyrocarbonate at pH 8.0, yielding 2,4,5-tris(ethoxycarbonylamino)pent-4-enoic acid. This product contains a small percentage of bis(ethoxycarbonyl)histidine. The cleavage reaction is comparable with cleavage of the imidazole ring in histidine methyl ester with benzoyl chloride under Schotten-Baumann conditions (Ashley & Harington, 1930). After permethylation of 2,4,5-tris(ethoxycarbonylamino)pent-4-enoic acid, the mass spectrum showed the presence of exclusively methyl 2,4,5-tris-(*N*-ethoxycarbonyl-*N*-methylamino)pent-4-enoate. We applied the exhaustive treatment with diethyl pyrocarbonate followed by permethylation with success to the following peptides containing histidine: Ala-His; β -Ala-His; His-His; Glu-His-Phe; His-Phe-Arg-Trp-Gly. Ala-His and β -Ala-His could easily be distinguished from each other. Before the reaction of the pentapeptide with diethyl pyrocarbonate it was treated with hydrazine (Shemyakin *et al.* 1967) to convert arginine into ornithine.

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