PROTECTION OF TRYPTOPHAN DURING CLEAVAGE OF TYROSINE PEPTIDE BONDS BY N-BROMOSUCCINIMIDE

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N-Bromosuccinimide (NBS) has been widely used for cleavage of both tryptophyl and tyrosyl bonds in peptides and proteins (Witkop and Ramachandran, 1964). Differential cleavage has so far been possible only for tryptophan. For example, the C-tryptophyl peptide bonds in bovine hemoglobin are cleaved at pH 3.5, while tyrosyl residues are only brominated. At pH 4.5 both C-tryptophyl and C-tyrosyl bonds are cleaved (Sasakawa 1963). Tryptophyl residues are cleaved with NBS in 8.0 M urea at pH 4.0, while tyrosyl residues are stable (Funatsu et al., 1964). Tribromocresol cleaves only tryptophyl but not tyrosyl bonds (Patchornik 1965). Tyrosyl peptide bonds are stable to the action of NBS after treatment with mercuric acetate (Ramachandran and Witkop, 1964). O-Alkyl (Corey and Haefele, 1958) and O-acyl (Shaltiel and Patchornik, 1963) tyrosyl residues are not cleaved by NBS.

We have now found a method for the exclusive cleavage of tyrosyl residues with NBS by protecting the tryptophyl residues with 2-hydroxy-5-nitrobenzyl bromide (Koshland et al., 1964).

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Table I

Cleavage of Model Peptides with NBS[1]

Model Peptides	Cleavage yield[2] before alkylation	Cleavage yield after alkylation with 2-hydroxy-5-nitrobenzyl bromide
L-Try-L-Ala	37%	0
L-Try-Gly	38%	0
L-Try-L-Phe	40%	0
L-Try-Gly-Gly	38%	0
L-Tyr-L-Ala	75%	50 - 60%
L-Tyr-Gly	70%	45 - 65%
L-Tyr-L-Phe	75%	65%
L-Try-L-Ala Mixture[3] L-Tyr-L-Phe	37% 70%	0 55%
L-Try-Gly Mixture[3] L-Tyr-L-Ala	37% 70%	0 55%
L-Try-L-Phe Mixture[3] L-Tyr-Gly	37% 65%	0 52%

[1] Three moles of NBS/mole of peptide was added.

[2] Yield of cleavage was determined by paper chromatography of the reaction mixture and quantitative ninhydrin assay.

[3] Equimolar mixtures of the peptides were used.

When tryptophyl-alanine (50 μmole) was alkylated with excess of 2-hydroxy-5-nitrobenzyl bromide (500 μmole), the yellow solution showed no more starting material (blue Ehrlich reaction) on thin layer

chromatography, but a new compound which gave a deep yellow color with Ehrlich reagent. Excess reagent was extracted with ether in which the peptide, but not its N-acetyl derivative, is insoluble. At this point the modified peptide was acetylated with acetic anhydride[2] and treated with excess NBS (3-10 eq). No cleavage was observed, while in a control experiment tryptophyl-alanine, taken through the same steps without 2-hydroxy-5-nitrobenzyl bromide, was cleaved in 37% yield. Similar results were obtained with other model peptides (Table I). Tryptophyl peptides, after treatment with 2-hydroxy-5-nitrobenzyl bromide, consume 1.8-2.0 equivalents and, after acetylation, only 1.0 equivalent of NBS.

When tyrosyl peptides were taken through the same procedure, yields of cleavage averaged 45-65%. In the control experiments, i.e. in the absence of 2-hydroxy-5-nitrobenzyl bromide, tyrosyl peptides were cleaved in somewhat higher yield, 60-75% (Table I).

In order to ascertain the feasibility of the method, mixtures of tyrosyl and tryptophyl peptides were treated with 2-hydroxy-5-nitrobenzyl bromide, acetylated and reacted with NBS. Only tyrosyl, but not tryptophyl peptide bonds, were cleaved (Table I).

The method was now applied to a larger molecule, viz. hen's egg white lysozyme which contains 6 tryptophan and 3 tyrosine residues (Table II). On treatment of lysozyme with excess 2-hydroxy-5-nitrobenzyl bromide (100 moles/mole of tryptophan) in 30% 2-chloroethanol (Bewley and Li, 1965) we confirmed that complete alkylation of all the tryptophans occurred. The modified protein was cleaved with NBS and the liberated NH_2-terminal residues determined by the dinitrophenylation method. In addition to the bis-DNP-Lys from the NH_2-terminal residue of the enzyme, the ether-soluble DNP-Ser and DNP-Gly were found. The third amino acid following tyrosine, viz. the ether-insoluble DNP-Arg

(2) The acetylation is necessary because free tryptophyl and tyrosyl peptides are not cleaved by NBS (Wilchek and Witkop). The reason for this surprising resistance is now under investigation.

Table II

Cleavage of Tryptophan and Tyrosine Peptide Sequences in hen's egg white lysozyme (NH_2-Terminal:Lysine)

In Lysozyme	Ether-Soluble DNP-Derivative After NBS-Cleavage	In Lysozyme	Ether-Soluble DNP-Derivative After NBS-Cleavage
...Try-Val...	-	...Tyr-Arg...	
...Try-Try...	-	...Tyr-Ser...	+
...Try-Cys...		...Tyr-Gly...	+
...Try-Val...	-		
...Try-Arg...			
...Try-Ileu...	-		

was not determined because of interference with yellow alkylated tryptophan derivatives. Neither DNP-Val nor DNP-Ileu, the ether soluble DNP-amino acids expected from cleavage of tryptophan residues were present (Table II).

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