SELECTIVE MODIFICATION OF THE TRYPTOPHAN RESIDUE IN PEPTIDES AND PROTEINS USING SULFENYL HALIDES.

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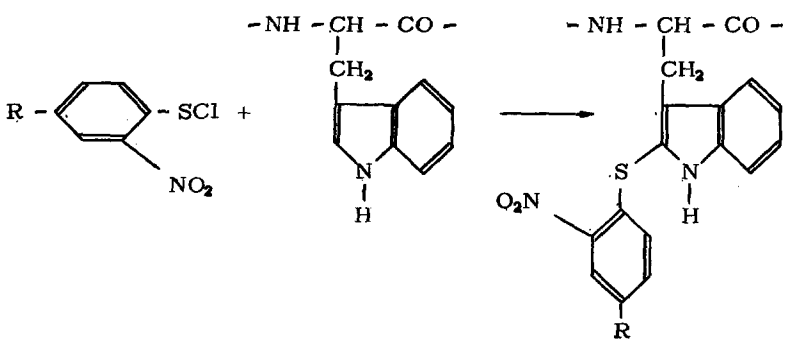
Since selective reagents are a powerful tool in the study of the relationships between structure and function in proteins, we have examined a new class of reagents for tryptophan modification.

The role of tryptophan in proteins has been ascertained through N-bromosuccinimide [1] and ozone [2,3] oxidation, photoxidation [4], iodination [5]. Recently Koshland et al. [6,7] have shown that 2-hydroxy-5-nitrobenzyl bromide reacts at low pH with good selectivity for tryptophan residues.

In previous papers [8,9] we have reported that 2-nitro (NPS-Cl) and 2,4-dinitrophenylsulfenyl chloride (DNPS-Cl), known to yield sulfenamides with amino acids in basic aqueous-organic solution, react selectively in acid media at the 2-position of the indole nucleus of tryptophan allowing the introduction of chromophoric groups as shown below:



with R = -H; $-NO_2$.

This general reaction for indole derivatives [9,10] occurs very cleanly with high yields in chloroform, ethyl ether as well as in glacial acetic acid or 99% formic acid.

In order to prove the potential usefulness of sulfenyl halides in modifying the indole residue in peptides and proteins, we first ascertained their selectivity toward tryptophan.

In a typical experiment a mixture of 1 μmole of all the amino acids normally found in proteins except tryptophan and cysteine in 1 ml of glacial acetic acid or 99% formic acid, was treated with NPS-Cl or DNPS-Cl (1:1 molar ratio for each amino acid) at room temperature for 15 hours. The reagent was destroyed by adding water, the solution filtered and lyophylized. The residue was dissolved in citrate buffer pH 2.2 and placed directly on the amino acid analyzer. All tested amino acids were recovered in 95-100% yields.

The behaviour of cysteine in this procedure was not clear and further experimental work is needed.

The selectivity toward tryptophan was further confirmed by titration of the sulfenyl halide [11] after a 15 hour reaction with the amino acids in glacial acetic acid.

The reactivity of sulfenyl halides was then checked with several tryptophan peptides. For example, the modified peptides listed in Table I were obtained by reaction with NPS-Cl, DNPS-Cl and phenylsulfenyl chloride (PS-Cl) in 99% formic acid.

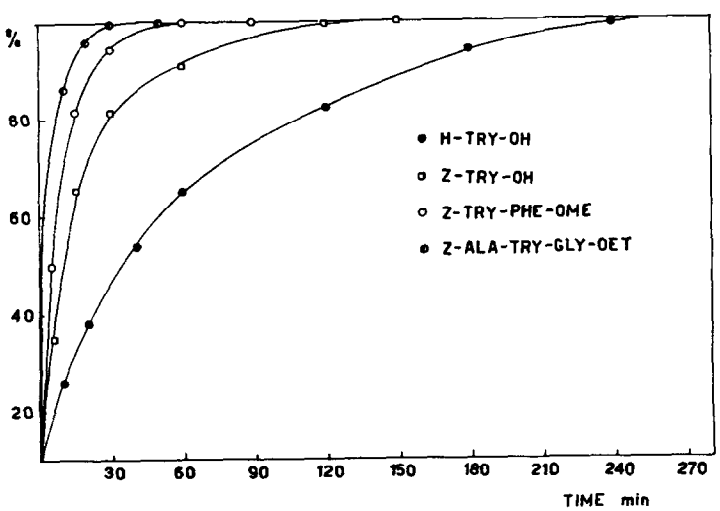


Figure I. – Reaction of NPS-Cl with tryptophan derivatives in glacial acetic acid at $21^{\circ} \pm 0.1^{\circ}$ (tryptophan deriv. 1×10^{-2} M; NPS-Cl 2×10^{-2} M).

By titration of the sulfenyl halide, it was possible to ascertain that in glacial acetic acid the reaction of NPS-Cl with tryptophan derivatives is practically quantitative in 2-4 hours (Fig. I) .

Table I. – Tryptophan modified peptides.

N°	Peptide (a)	Yield % (b)	Mp. °C	$[\alpha]_D$ c 0.5% MeOH
1	Z-Leu-Try(NPS)-OMe	77	93-95°	- 22.6°
2	Z-Leu-Try(DNPS)-OMe	83	100-102°	- 25.6°
3	Z-Leu-Try(PS)-OMe	83	136-137°	- 13.6°
4	Z-Try(NPS)-Met-OMe	71	73-75°	+ 2.8°
5	Z-Try(NPS)-Phe-OMe	76	75-77°	+ 18.8°
6	Z-Pro-Try(DNPS)-OMe	69	98° dec.	- 36.8°

(a) The abbreviations are those recommended by the Committee on Nomenclature for Amino Acids and Peptides; G.T. Young, Peptides, Proceedings of the Fifth European Symposium, Pergamon Press, Oxford (1963), 261-269. The amino acids are of the L-configuration. (b) Yields were calculated on analytically pure compounds.

The absorption spectra of the 2-NPS-tryptophan peptides (Fig. II) are noteworthy, with two maxima at 280 and 365 mμ (ε_{280} 16,700 and ε_{365} 4,400). The maximum at 365 mμ is in a region of the spectrum where proteins are transparent, allowing an easy calculation of the number of the modified residues on the basis of spectral data.

In order to test the procedure with a protein, lysozyme was reacted with NPS-Cl (1:20 molar ratio) in 99% formic acid for 3 hours at room temperature, water was added, the solution filtered and lyophylized. The protein was redissolved in water and further purified by gel filtration on Sephadex G 25.

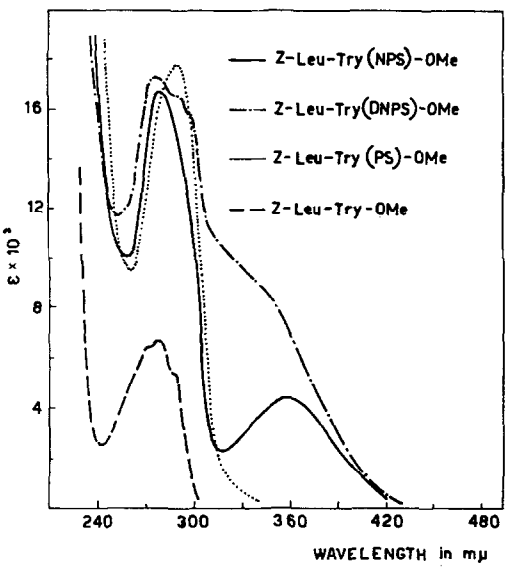


Figure II. Absorption spectra of tryptophan modified peptides in methanol.

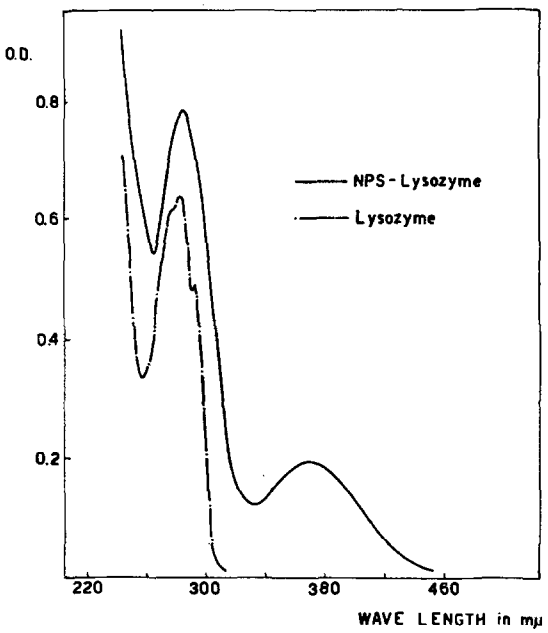


Figure III. Absorption spectra of lysozyme (1.78×10^{-5} M) and of the NPS–lysozyme (7.3×10^{-6} M) in water.

As shown in Fig. III the NPS-modified lysozyme has a spectral behaviour similar to that of NPS-tryptophan peptides. Assuming that the extinction coefficients in NPS-protein and in NPS-peptides are the same, it was possible from the experimental $O.D._{365}$ value to calculate 6.2 residues of modified tryptophan. This number is in good agreement with the known content of tryptophan in lysozyme (6 residues) [12].

The method proposed above seems of particular value in protein-modification studies. The noteworthy features of this procedure are i) selectivity that will aid in studying the role of tryptophan in proteins, ii) the easy calculation of the number of modified residues on the basis of spectral data, iii) the mild conditions of the reaction and the possibility of using formic acid, a solvent in which proteins are usually very soluble and only reversibly [13,14] inactivated.

It should be pointed out that this class of reagents allows a wide range of possibilities. For example sulfenyl halides carrying different chromophoric or fluorescent groups or containing heavy atoms can be employed.

Further details of this research will be reported elsewhere.

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