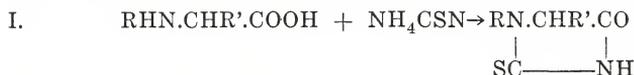


Condensation of Proteins with Thiocyanate¹

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While the N-terminal amino acid of a peptide chain can be determined by alkylation of its amino group and separation of the N-alkyl derivative from the protein hydrolysate, the methods used for the determination of the C-terminal amino acids are unsatisfactory. One of these methods is based on the condensation of terminal amino acid residues with thiocyanate in the presence of acetic anhydride and acetic acid (12) (reaction I).



Since the β -carboxyl groups of aspartyl and the γ -carboxyl groups of the glutamyl residues do not react with thiocyanate, the method has been used to determine the C-terminal amino acid of peptides and proteins (1, 3, 4, 14, 15, 16).

When proteins were treated in our laboratory with thiocyanate and acetic anhydride at 100°, it was found that the sulfur content increases by 10 or more S atoms per protein molecule (10). This was confirmed by experiments in which thiocyanate containing radioactive S³⁵ or C¹⁴ was used (7, 9, 17). The NCS residues are incorporated within 10-20 minutes; further heating causes very slow incorporation of a small number of NCS residues. The protein-bound NCS residues cannot be exchanged with nonradioactive thiocyanate; evidently they are bound by covalence. Table I shows that the molecules of insulin, salmine, ribonuclease and tyrocidin, which are poor in or devoid of α -carboxyl groups, combine with the expected small number of NCS residues. However, the number of such residues in the reaction products of the molecules of edestin, ovalbumin and beef serum γ -globulin is much higher than expected. Since it is well established that these proteins contain only one terminal α -amino group per molecule, it is difficult to attribute the high values for NCS groups to the presence of multiple terminal α -carboxyl groups.

In order to get more insight into the nature of the groups which combine with thiocyanate, proteins were exposed to acetylation, deamination, substitution with phenylisocyanate, sulfation (13) and to reduction by LiAlH₄ (6). The number of incorporated NCS residues was not significantly changed by the first three procedures, but was considerably lowered after sulfation and after treatment with LiAlH₄ (7, 17). Since

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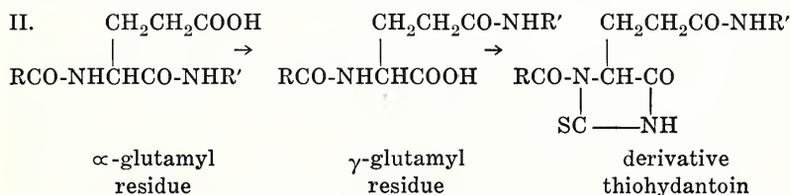
TABLE I

Condensation of proteins with thiocyanate (30 minutes, 100°)

Protein	Mol. weight	Free carboxyl groups per molecule	Incorporated NCS-residues per molecule
Edestin	50,000	62	10.5
Ovalbumin	44,000	51	7.8
γ -Globulin	156,000	67	39.4
Fibroin	(100,000) ²	20	3.3
Insulin	6,000	5	1.4
Salmine	8,000	0	0.2
Ribonuclease	15,000	7	1.6
Tyrocidin	1,300	0	0.2
Polyglutamic acid	12,000	93	7.8

sulfation affects the hydroxyl side chains of serine and threonine, hippurylserine isopropyl ester and hippurylthreonine ethyl ester were prepared and exposed to thiocyanate and acetic anhydride. Neither of these peptides combined with thiocyanate (17).

The low values of incorporated NCS residues observed after reduction of the proteins with LiAlH_4 suggested that thiocyanate reacts with carboxyl groups. Therefore, the ethyl esters of carbobenzoxy- α -DL-aspartylglycine, carbobenzoxy- α -L-glutamylglycine and carbobenzoxy- α -DL-aspartyl-L-tyrosine were prepared. None of these peptide esters combine with thiocyanate (17). Although it is well known that the carboxyl side chains of these peptide esters cannot react with thiocyanate, it has been shown recently (2, 11) that α -aspartyl and α -glutamyl residues can undergo rearrangement into β -aspartyl and γ -glutamyl residues, respectively. The free α -carboxyl groups of such residues can then combine with thiocyanate (reaction II).



γ -Glutamyl residues are present in glutathione, in folic acid and its derivatives, and in the capsular polyglutamic acid of certain bacilli. The ability of such groups to combine with thiocyanate is proved by the high number of NCS residues in polyglutamic acid after exposure to thiocyanate (Table I). The presence of γ -glutamyl residues in other proteins is indicated by the formation of succinylpeptides when partial hydrolysates are oxidized (8), and by the detection of 5-hydroxy-4-aminovaleric acid in the hydrolysate of LiAlH_4 -treated proteins (5).

² The molecular weight of fibroin is not known. The values refer to an equivalent weight of 100,000.

Table I shows that the number of incorporated NCS residues is much smaller than the total number of free carboxyl groups. Evidently, most of the aspartyl and glutamyl residues behave like the α -aspartyl and α -glutamyl residues of the synthetic peptides; they do not undergo any rearrangement when heated with acetic anhydride and, accordingly, do not combine with thiocyanate. The figures recorded in Table I indicate, however, that some of the aspartyl and/or glutamyl residues are either present in the native proteins as β -aspartyl and γ -glutamyl residues or are present as α -residues and are converted into β - or γ -residues (reaction II) during the exposure to acetic anhydride and thiocyanate. This view is in agreement with the fact that fibroin, which is extremely poor in aminodicarboxylic acids, combines with a very small number of NCS residues only and that reduction of the free β - and γ -carboxyl groups by LiAlH_4 lowers considerably the number of incorporated NCS residues.

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