METHIONYL SEQUENCES IN LOW-SULPHUR PROTEINS OF WOOL*

By R. Hosken,† B. A. Moss,† I. J. O'Donnell,‡ and E. O. P. Thompson†

In the protein fractions extracted from reduced and carboxymethylated wool methionine occurs mainly in the low-sulphur protein fraction (*S*-carboxymethylkerateine, SCMKA) (Crewther *et al.* 1965). There are two major components (components 7 and 8) in this fraction and evidence for three methionyl sequences, namely -Met-Glu(NH_2)-, -Met-Ala-, and -Met-Asp-, was obtained by estimations of *N*-terminal groups following cyanogen bromide cleavage (Thompson and O'Donnell 1967).

In a recent communication Corfield, Fletcher, and Robson (1967) have detailed a large number of amino acid sequences in proteins dissolved from wool oxidized with performic acid. These include two methionyl sequences deduced from the structure of peptides isolated from tryptic digests, namely Ala-Lys-Glu-Asp-Met-Ala-Leu-CySO_3H-Leu-Lys- and Glu-Thr-Met-(Leu, Phe)-Asp-Asp-Arg.

It was of interest to determine whether the findings in the separate studies could be checked and brought into agreement. Cyanogen bromide cleavage of isolated component 8 yielded only two main fractions. The *N*-terminal fraction was found to have a *C*-terminal sequence of Glu-Thr-HSL, [homoserine lactone (HSL) is formed from methionine during reaction with cyanogen bromide] corresponding to the second sequence of Corfield, Fletcher, and Robson (1967) but the new *N*-terminal residue of the second fraction was glutamine, suggesting the sequence Glu-Thr-Met-Glu(NH_2)- in component 8. Further investigation of the sequence of the second fraction (CNBrl of Thompson and O'Donnell 1967) by stepwise degradation by the Edman procedure has given evidence for a sequence following the glutamine in agreement with that obtained by Corfield, Fletcher, and Robson (1967) if it is assumed that the glutaminyl residue had not been detected by them. The corrected sequence according to our data is Glu-Thr-Met-Glu(NH_2)-Phe-Leu-Asp-Asp-Arg.

Experimental

The protein fraction CNBrl (70 mg) was dissolved in 2 ml water containing 0·3 ml *N*-ethylmorpholine. Pyridine (3 ml) and phenyl isothiocyanate (0·1 ml) were added and reacted at 40°C for 1 hr. Extraction with benzene, cyclization, extraction of the thiazolinone, and isolation of the phenylthiohydantoin formed on subsequent acid treatment followed the procedure of Blombäck *et al.* (1966).

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† School of Biological Sciences, University of New South Wales, Kensington, N.S.W. 2033.

‡ Division of Protein Chemistry, CSIRO Wool Research Laboratories, Parkville, Vic. 3052.

Results and Discussion

The yield of phenylthiohydantoin (PTH) estimated by ultraviolet absorption was unreliable since the ratio of the minimum to the maximum extinction was considerably higher than for pure phenylthiohydantoins (Sjöquist 1957). The phenylthiohydantoins were identified by thin-layer chromatography (Cherbuliez, Baehler, and Rabinowitz 1964) and by regeneration to amino acids, usually by acid hydrolysis (Van Orden and Carpenter 1964), which were quantitatively estimated on a Beckman model 120C amino acid analyser.

TABLE 1

STEPWISE PHENYLISOTHIOCYANATE DEGRADATION OF CNBrl

Step No.	Phenylthiohydantoin*	Amino Acid Regenerated†
1	Glutamine	Glutamic acid; small amounts of alanine, aspartic acid, glycine (*c.*10% of glutamic acid)
2	Phenylalanine	56% phenylalanine; small amounts of glutamic acid, and glycine (*c.* 15% of phenylalanine)
3	Leucine	37% leucine; small amounts phenylalanine and glycine (*c.* 20% of leucine)
4	Aspartic acid	19% aspartic acid. Glycine (*c.* 65% of aspartic acid)
5	Aspartic acid	68% aspartic acid; glycine also present in equal amount
6	Arginine or histidine	After hydrolysis with NaOH, an amino acid running at a rate more similar to lysine (presumably ornithine by decomposition of arginine) was obtained.

* Identified by thin-layer chromatography.

† Yields (uncorrected for losses) expressed as percentage of previous residue.

In successive steps of the degradation, the residual protein was difficult to dissolve and the yield of PTH and regenerated amino acid fell considerably. However, there was no problem in identifying the major PTH formed in each step and the results were clear despite the poor overall yield. These results are collected in Table 1.

The low yield in step 1 (see Thompson and O'Donnell 1967) is presumed to be due to previous losses during formation and isolation of CNBrl, when *N*-terminal glutamine residues can cyclize to pyroglutamic acid residues. The yields in all subsequent steps are not good and can only be accepted as good evidence for the sequence in conjunction with those of Corfield, Fletcher, and Robson (1967).

Attempts were made to confirm the sequence by isolation of acidic *N*-terminal peptides from CNBrl after enzymic digestion of the succinylated protein (Yaoi, Titani, and Narita 1964), or after boiling an aqueous solution to cause cyclization of the *N*-terminal glutaminyl residue to a pyroglutamyl residue followed by enzymic

digestion. Although acidic fractions unadsorbed on sulphonated polystyrene were obtained following Pronase or Nagarse digestion, the predominant amino acid in hydrolysates was glutamic acid and only small amounts of phenylalanine, the second residue, were present. This suggested that rapid hydrolysis by the enzymes of the bond following the succinylglutamine or pyroglutamic acid residue occurred.

With the correction reported in this communication we are in agreement with the two sequences reported by Corfield, Fletcher, and Robson (1967). The absence of less than 10% of the amino acids aspartic acid or alanine in step 1 of the degradation compared with the glutamic acid recovered suggests that component 8 is substantially free of the other component (component 7) of the low-sulphur protein fraction of wool from which the other two methionyl sequences originate. This and previous evidence for differences in amino acid sequences in components 7 and 8 (Thompson and O'Donnell 1967), which both have molecular weights of about 45,000 and *N*-terminal acetylated residues, cannot be reconciled with suggestions by Corfield (1963), Blackburn and Lee (1966), and Robson (1966) that wool may contain a single protein with high-sulphur and low-sulphur moieties as structural features and of molecular weight of approximately 75,000.

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