THE BLOCKED AMINO-TERMINAL PEPTIDE OF FEATHER KERATIN*

By I. J. O'Donnell†

Proteins extracted from reduced and carboxymethylated feather keratins (SCM-keratins) have been studied by Harrap and Woods (1964*a*, 1964*b*, 1967). They have demonstrated the presence of electrophoretic heterogeneity amongst the proteins and have obtained a molecular weight of approximately 11,000 in agreement with earlier work of Woodin (1954). There was no indication of marked heterogeneity with respect to size. Using acid hydrolysis and determination of acetic acid produced they found an acetyl content of 1·30 moles/10^4 g in the rachis of fowl feathers. These were thought to be attached to primary amino groups since there were no *O*-acetyl groups. In the present paper the isolation and characterization of the predominant, and probably sole, amino-terminal tripeptide from goose feather calamus is described. Goose feather calamus was chosen because its extracted proteins had one of the simplest electrophoretic patterns of proteins from the feathers of a number of species (Harrap and Woods 1967).

Materials and Methods

The rachis and calamus from washed and defatted goose feathers (*Anser domesticus*) was kindly supplied by Mr. E. F. Woods. The reduced and carboxymethylated (SCM) proteins were prepared as described previously for wool (O'Donnell and Thompson 1964). In all, 63% of the original weight of calamus was obtained as soluble proteins.

Enzyme digestions were carried out for 7 hr at 37°C on 100 mg (10 μmoles) of SCM-calamus in 20 ml 0·1M ammonium acetate at pH 8·5 with 1 mg added enzyme. α-Chymotrypsin, Pronase, and Nagarse all gave the same acetyl peptide. Acetyl peptides were isolated from the enzyme digests by passage at 3–4°C (Ikenaka *et al.* 1966) through a column (20 by 0·9 cm diameter) of Dowex 50-X2 (50–100 mesh) in the hydrogen form (Narita 1958).

A partial acid hydrolysate was made from 1 μmole of the tripeptide by hydrolysis with 0·6 ml 6N HCl at 100°C for 30 min (Ambler 1963). The peptide mixture, after removal of acid, was fractionated by paper ionophoresis at pH 6·5. Mobilities, *M*, are expressed relative to aspartic acid, the position of the neutral amino acids being taken as zero.

Results

The acidic peptides isolated from the chymotryptic digest of SCM-keratin dissolved from goose feather calamus by passage through Dowex-50 were contained in a single peak when passed through Sephadex G-10, G-25, or G-50 in 0·01N ammonia. The material in this peak, which was insoluble at pH 3·5, gave a single moving

* Manuscript received August 5, 1970.

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

band on paper ionophoresis at pH 6·5. It was ninhydrin-negative but stained with chlorine–tolidine reagent. After elution from the paper the peptide was found to consist of equal amounts of serine, tyrosine, and *S*-carboxymethylcysteine, with less than 10% of any other amino acid. Hydrazinolysis showed *C*-terminal tyrosine while (tyr, SCMCySH) ($M = 0{\cdot}57$) and (ser, SCMCySH) ($M = 0{\cdot}73$) was isolated by paper ionophoresis from a partial acid hydrolysate of the tripeptide. In another preparation using iodo-[2-^{14}C]acetic acid the tripeptide was found to be radioactively labelled and had a mobility of 0·90. This shows that it has two charges (Offord 1966). Since Harrap and Woods (1964*a*) have shown the presence of acetyl groups in feather keratin it is highly probable that an acetyl group blocks the terminal amino group of this peptide. Its sequence may hence be written NAc-Ser-SCMCySH-Tyr. This tripeptide is probably the sole amino terminal peptide of goose feather calamus since an analysis (Table 1) of the acidic peptides passing through Dowex-50

TABLE 1

AMINO ACID COMPOSITION OF THE ACIDIC PEPTIDES IN A CHYMOTRYPTIC DIGEST OF REDUCED AND CARBOXYMETHYLATED GOOSE CALAMUS KERATIN

The whole Dowex-50 eluate was analysed.* Results are the average of three experiments and are expressed as moles of amino acid per mole of SCM-calamus (mol. wt. 10,980)

Amino Acid	No. of Moles	Amino Acid	No. of Moles
Aspartic acid	0·11	Valine	0·14
Threonine	0·12	Isoleucine	0·05
Serine	1·02	Leucine	0·12
Glutamic acid	0·12	Tyrosine	0·77
Proline	0·24	Phenylalanine	0·08
Glycine	0·20	*S*-Carboxymethyl-cysteine	0·88
Alanine	0·15		

* Hydrolyses were carried out *in vacuo* with constant boiling hydrochloric acid for 24 hr at 110°C. No correction factor has been applied for destruction of serine or threonine. 10 µl of 0·1M phenol or thioglycollic acid (Sanger and Thompson 1963) was added to minimize destruction of tyrosine.

would suggest that it contains the amino acids (ser, tyr, SCMCySH) corresponding to approximately 0·8–0·9 mole of tripeptide per mole of the *S*-carboxymethylated keratin of molecular weight 10,980 (Harrap and Woods 1964*b*). The same peptide was found in goose-feather rachis but not in seagull-feather rachis. This encouraging finding of approximate stoicheiometry of *N*-terminal peptide for this feather keratin is in contrast with the results for a low-sulphur fraction (component 8) of wool keratin. Here two acetylated peptides comprising only 50% of an *N*-terminus were found (O'Donnell and Thompson 1968), and there was also evidence for the presence of pyrrolid-2-one-5-carboxylic acid as an *N*-terminal group (O'Donnell 1968).

References

AMBLER, R. P. (1963).—*Biochem. J.* **89**, 349.
HARRAP, B. S., and WOODS, E. F. (1964*a*).—*Biochem. J.* **92**, 8.
HARRAP, B. S., and WOODS, E. F. (1964*b*).—*Biochem. J.* **92**, 19.
HARRAP, B. S., and WOODS, E. F. (1967).—*Comp. Biochem. Physiol.* **20**, 449.
IKENAKA, T., BAMMERLIN, H., KAUFMANN, H., and SCHMID, K. (1966).—*J. biol. Chem.* **241**, 5560.
NARITA, K. (1958).—*Biochim. biophys. Acta* **28**, 184.
O'DONNELL, I. J. (1968).—*Aust. J. biol. Sci.* **21**, 1327.
O'DONNELL, I. J., and THOMPSON, E. O. P. (1964).—*Aust. J. biol. Sci.* **17**, 973.
O'DONNELL, I. J., and THOMPSON, E. O. P. (1968).—*Aust. J. biol. Sci.* **21**, 385.
OFFORD, R. E. (1966).—*Nature, Lond.* **211**, 591.
SANGER, F., and THOMPSON, E. O. P. (1963).—*Biochim. biophys. Acta* **71**, 468.
WOODIN, A. M. (1954).—*Biochem. J.* **57**, 99.