A SEARCH FOR A SIMPLE KERATIN—FRACTIONATION AND PEPTIDE MAPPING OF PROTEINS FROM FEATHER KERATINS

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Abstract

S-Carboxymethylated (SCM) proteins have been prepared from the various morphological parts of the feathers (a natural β-keratin) from one species of bird and also from the calami of feathers from several species of birds. The complexity of the extracts has been examined by acrylamide-gel electrophoresis and size–charge peptide maps of radioactively labelled SCM-peptides in enzyme digests of the extracts. The results suggest that (1) the protein mixture from the natural β-keratins such as feather is simpler than that from α-keratins such as wool and prekeratin proteins of epidermis; (2) the proteins extracted from the emu calamus (or rachis) and silver gull calamus are simpler in their acrylamide-gel pattern than those from fowl or goose feather calamus, (3) the peptide maps of radioactively labelled SCM-peptides of the feather extracts studied (emu, silver gull, and goose calamus) are no more complex than those from the homogeneous protein SCM-lysozyme which has approximately the same number of *S*-carboxymethylcysteine residues per molecule, and this indicates that the protein components corresponding to the bands in the acrylamide-gel electrophoresis patterns of the feather extracts could be homogeneous; (4) the rachis and calamus proteins, which are probably identical, form one class of proteins while the barbs and medullary proteins form another class but are not identical.

The proteins corresponding to the bands seen in acrylamide-gel patterns of SCM-proteins extracted from emu feather calamus and rachis have been separated by chromatography on columns of DEAE-cellulose. It is necessary to use buffers containing 8M urea. The proteins have very similar amino acid compositions but a difference can be seen in peptide maps of protein band 2 and protein band 3.

I. INTRODUCTION

Previous work has shown that proteins of α-keratin such as those from wool and the precursor keratins from cow's lip epidermis are not homogeneous but consist of many families of proteins which differ markedly in composition (Thompson and O'Donnell 1967; O'Donnell 1971*b*; Gillespie 1972; see also Fraser *et al.* 1972). In this paper attention has been turned to the other major group of keratins, the natural β-keratins, typified by birds' feathers and beak, reptile claw, and toad skin.

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The aim was to establish whether any of them are simpler than α-keratins in containing a limited number of homogeneous proteins. A preliminary appraisal, using acrylamide-gel electrophoresis, of the *S*-carboxymethylated (SCM) proteins extracted from lizard claw (*Varanus varius*), toad skin (*Bufo marinas*), and feathers suggested that the feather proteins were the simplest and so attention was concentrated on them.

The results of Harrap and Woods (1964*a*, 1967), using moving-boundary electrophoresis and acrylamide-gel electrophoresis, had suggested a reasonably simple situation for SCM-proteins extracted from feather and the finding of a unique amino-terminal peptide NAc-Ser-SCMCys-Tyr in approximately molar amount in SCM-proteins from goose calamus (O'Donnell 1971*a*) was encouraging. Furthermore, the small molecular weight of approximately 10,500–10,800 (Harrap and Woods 1964*b*; Jeffrey 1970) for SCM-proteins from feather gave hope of their ready separation. However, comparative studies using DEAE-cellulose chromatography of SCM-proteins from feathers of various birds led Woods (1971) not to be so optimistic. On the basis of these fractionation studies, monitored by acrylamide-gel electrophoresis, he suggested that the proteins had a high degree of heterogeneity.

As before, in studies on the precursor keratins of α-keratin, acrylamide-gel electrophoretic patterns and the complexity of size–charge peptide map radioautographs of labelled SCM-peptides have been used to assess the extent of heterogeneity (O'Donnell 1971*b*).

II. Materials and Methods

(*a*) *Preparation and Extraction of SCM-Proteins from Feathers*

The feathers used were from the domestic fowl (*Gallus domesticus*), duck (*Anas platyrhynchos*), goose (*Anser domesticus*), emu (*Dromaius novae-hollandiae*), turkey (*Meleagris gallopavo*), sulphur-crested cockatoo (*Cacatua galerita*), and silver gull (*Larus novae-hollandiae*). The emu beak was from the same bird as were the feathers. All of these were kindly given to me by Dr. E. F. Woods. One of the component parts of feather, i.e. calamus, rachis, barbs, or medulla, was used in each extraction. Two procedures were used.

(i) *Extraction and Cold Alkylation.*—The keratin (55 mg, containing approximately 20 μmoles cystine) was extracted for 16–24 hr at 3–4°C under nitrogen with 5 ml of urea buffer (8M urea, 0·01M Tris, 0·001M Versene at pH 7·4), 200 μl 5N potassium hydroxide, and 50 μl mercaptoethanol (700 μmoles) adjusted to pH 10·5. The pH was checked periodically and kept constant. The mixture was then homogenized in a Potter–Elvejhem tissue homogenizer and to this was added 0·75 ml 3M Tris buffer (pH 8·5) and 250 μl (750 μmoles) of 3M iodoacetic acid. After 15 min excess mercaptoethanol (100 μl) was added and the insoluble material centrifuged off. The extract was dialysed free of urea, using 18/32 Visking dialysis tubing, against dilute ammonia (*c.* 0·05M) solution. The extract was either freeze-dried by itself (if it was to be used for enzyme digestion) or, alternatively, in the presence of 3 ml of the urea buffer described above (if it was to be stored). After drying the latter, 1·5 ml water was added and it was stored in the deep-freeze in this condition.

(ii) *Extraction and Alkylation with Labelled Iodoacetic Acid.*—The keratin (55 mg) was first reduced with 5 ml urea buffer containing 45 μmoles of dithiothreitol at pH 10·5 as described in the previous paragraph. After the homogenization 50 or 100 μl (0·05 or 0·1 mCi) of radioactive [2-^{14}C]iodoacetic acid (Amersham) was added and, after 15 min, the mixture was subjected to mercaptoethanol reduction and alkylation with non-radioactive iodoacetic acid as described in the previous procedure. This latter reduction was to ensure complete reduction of all the disulphide bonds in the keratin.

(b) Acrylamide-gel Electrophoresis

Slab-gel electrophoresis was carried out in the vertical EC 474 and EC 494 apparatuses (E.C. Apparatus Corporation, Philadelphia, Pa.) with a gel buffer containing 8M urea and a 6 or 7½% acrylamide gel (3 or 6 mm thick). The system used was that described by Sparrow and Crewther (1973) and is similar to the "Discontinuous" system in Section 1E of Technical Bulletin 128 of the manufacturers of the apparatus except that no stacking gel was used. 100-μl aliquots, containing 100–300 μg protein in buffer of 8M urea–0·01M Tris–0·001M Versene at pH 7·4, together with a trace of bromphenol blue as marker dye, were loaded into each slot. The gels were stained by one of three methods:

(1) With amido black in the standard manner.

(2) With a mixture of Kiton Rhodamine B (C.I. 45100) and Coomassie violet R (C.I. 42650) (Frater 1970). The gel from the EC 474 apparatus was rocked for ½ hr with 100 ml 5% trichloroacetic acid and then with 500 ml water for a similar period. It was then rocked with 250 ml of a solution of 0·0125% Coomassie violet R and 0·0025% Kiton Rhodamine B, this being renewed after ½ hr and the staining allowed to continue overnight. The gel was destained in 5% acetic acid.

(3) With 10% trichloroacetic acid containing 0·1M potassium chloride. This was renewed after ½ hr and the staining allowed to continue overnight.

(c) Fractionation of SCM-Proteins extracted from Feathers

Three methods were used:

(i) *Acrylamide Gel Stained with Amido Black.*—One-sixth (*c.* 5–10 mg in 500 μl of urea buffer) of the SCM-proteins extracted from 55 mg of emu calamus (or rachis) or silver gull calamus was loaded across the full width (12 cm) of a 6 or 7½% acrylamide gel of 6 mm thickness. After staining with amido black and destaining in the normal manner the bands were cut from the gel and extracted with 50% formic acid using a Potter–Elvejhem tissue homogenizer. The gel was centrifuged off and the dye removed from the protein bands by passing the solution through a small column of Dowex-1 resin (formate form) in 50% formic acid (Wada and Snell 1972). The protein fractions were dialysed against water and then dilute ammonia solution to keep them soluble, and then either freeze-dried or dried in a vacuum desiccator over sodium hydroxide and phosphorous pentoxide. The fractions were then digested with trypsin and chymotrypsin for peptide mapping.

(ii) *Acrylamide Gel and Trichloroacetic Acid.*—The procedure was as outlined above but the bands were revealed with 5 or 10% trichloroacetic acid containing 0·1N potassium chloride. The bands were cut out and extracted with formic acid solution as described above and prepared for digestion with trypsin plus chymotrypsin.

(iii) *Fractionation on DEAE-cellulose.*—The SCM-proteins extracted from 55 mg of emu feather rachis or calamus were diluted to 10 ml with starting buffer of 8M urea–0·01M Tris–0·001M Versene at pH 7·4. It was loaded on a column of DEAE-cellulose (Whatman DE 52) also equilibrated with starting buffer and operated at 25°C. After the passage of approximately 60 ml of buffer a salt gradient was applied. Details are given in the legend of Figure 6. After radioactive counting, and monitoring with acrylamide-gel electrophoresis, the required fractions were bulked, dialysed against dilute ammonia, and freeze-dried in the presence of 3 ml urea buffer. 1·5 ml of water was then added and the solutions stored in a deep-freeze.

(d) Enzyme Digestions of Proteins and Preparation of Size–Charge Peptide Maps

The radioactively labelled proteins or protein fractions were digested for 8–24 hr at 37°C with trypsin and chymotrypsin (1%) in 0·5 ml or 1 ml of 2% ammonium bicarbonate at pH 8·4. The digests were then either stored frozen or fractionated on a column (130 by 0·9 cm) of Sephadex G50 in 0·05M ammonium bicarbonate. An aliquot of each fraction was then subjected to paper electrophoresis and size–charge peptide maps were thus produced as described previously (O'Donnell 1971*b*).

(*e*) *Other Methods*

The reduction, carboxymethylation, and radioactive labelling of lysozyme was done as described previously, as also was liquid scintillation counting, radioautography, amino acid analysis, and high voltage paper electrophoresis (O'Donnell 1971*b*).

III. Results

(*a*) *Extraction of Feathers*

The precentage of proteins extracted from the component parts of feathers is listed in Table 1. Where they overlap with the data of Harrap and Woods (1967) the agreement is good. A notable feature, also observed by Harrap and Woods, is that emu rachis is soluble to an extent of 90–95% whereas the value for calamus is only 50–60%.

TABLE 1

SOLUBILITY OF FEATHER PARTS IN 8M UREA–DITHIOTHREITOL AT pH 10·5

Species	Percentage of protein dissolved			
	Calamus	Rachis	Barbs	Medulla
Duck	95			
Cockatoo	94			
Goose	94	84	80	82
Fowl	89			
Emu	50–60	90–95		
Turkey	95			
Silver gull	95			

(*b*) *Storage of Proteins*

The proteins could be stored indefinitely in 8M urea buffers at pH 9–10 and −4°C. In the absence of urea they formed gels after a period of time and it was not always possible to get them completely into solution again even in buffers containing 8M urea. Thus at least some of the aggregation was irreversible.

(*c*) *Acrylamide-gel Electrophoresis*

The patterns of bands on the gel after electrophoresis of SCM-proteins extracted from feather were substantially the same whether shown up by staining with amido black, Coomassie violet R and Kiton Rhodamine B, or precipitated with trichloroacetic acid in the presence of potassium chloride. The last-named method was found to be the most convenient.

A comparison of the acrylamide-gel electrophoretic patterns of the extracted proteins from the calami of the feathers of several birds is shown in Figure 1. The patterns are more revealing than those obtained by Harrap and Woods (1967) in buffers not containing 8M urea. Harrap and Woods concluded that the proteins from silver gull rachis gave the simplest pattern but, in the present work, it was concluded that the pattern from the emu feather proteins was somewhat simpler. In some experiments

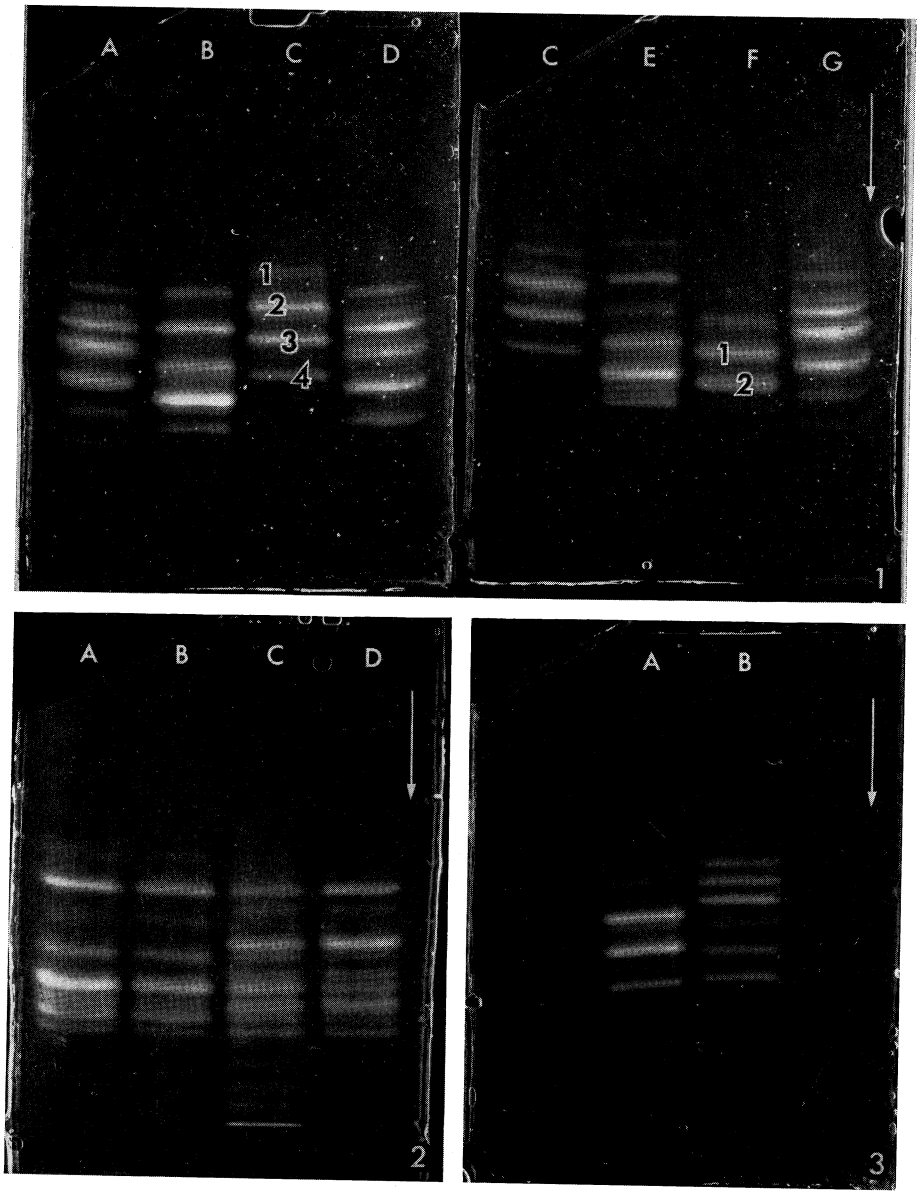


Fig. 1.—Acrylamide-gel electrophoresis patterns of SCM-proteins extracted from the calami of feathers of a variety of birds: *A*, cockatoo; *B*, duck; *C*, emu; *D*, domestic fowl; *E*, goose; *F*, silver gull; *G*, turkey. The numbers 1 and 2 refer to major bands, and 3 and 4 to minor bands.

Fig. 2.—Acrylamide-gel electrophoresis patterns of SCM-proteins extracted from the morphological parts of a goose feather: *A*, calamus; *B*, rachis; *C*, barbs; *D*, medulla.

Fig. 3.—Acrylamide-gel electrophoresis patterns of SCM-proteins extracted from emu rachis (*A*) and emu beak (*B*).

the fastest band in the silver gull calamus extract (and also the goose calamus extract) gave evidence of splitting into two bands. The pattern of the SCM-proteins from the

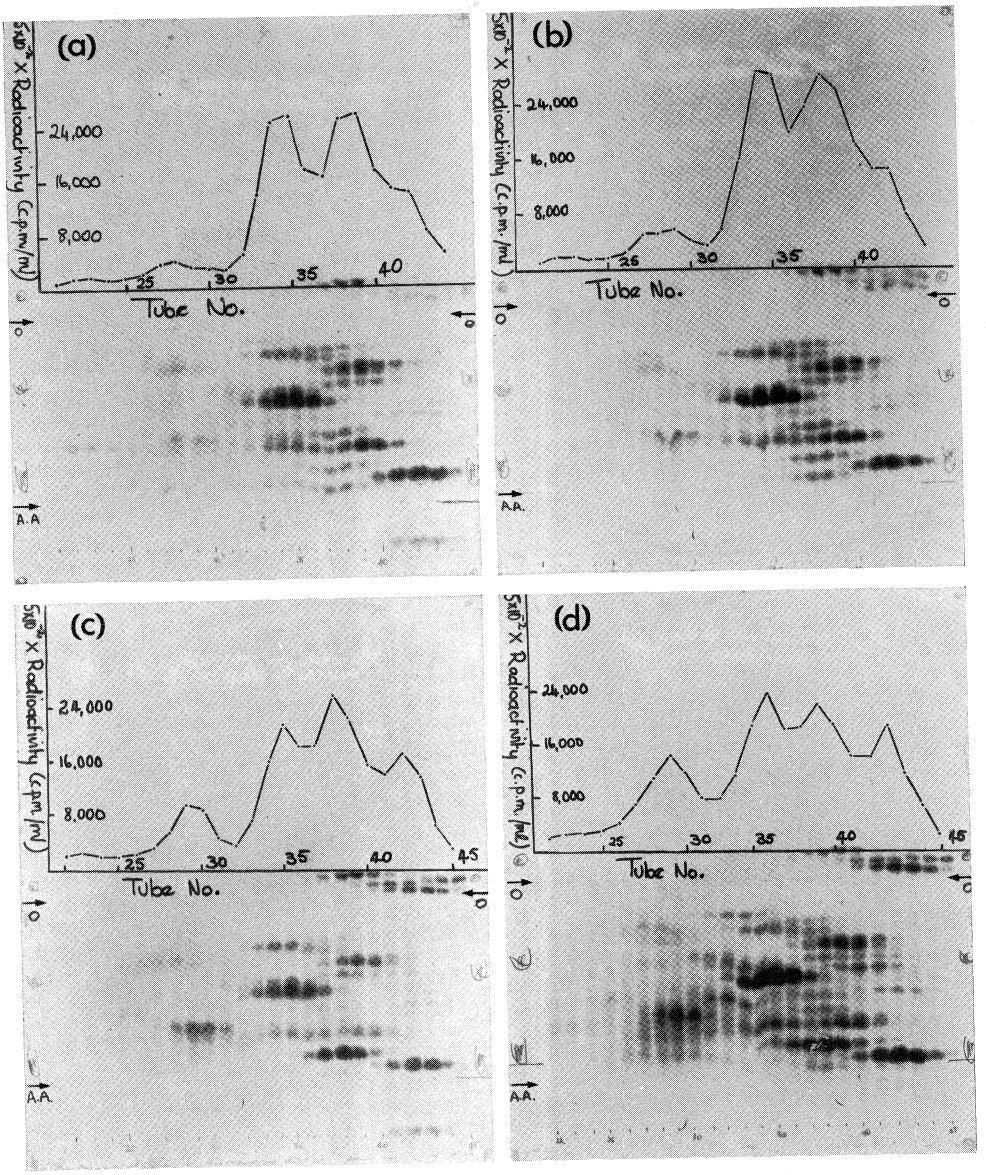


Fig. 4.—Size–charge peptide map radioautographs of the SCMCys-containing peptides in tryptic–chymotryptic digests of SCM-proteins extracted from (*a*) rachis, (*b*) calamus, (*c*) medulla, and (*d*) barbs of goose feathers. Upper elution patterns in each case are from columns (130 by 0·9 cm diameter) of Sephadex G50 in 0·05M ammonium bicarbonate at pH 8·5. Fraction size approximately 2·5 ml. O, origin. A.A., aspartic acid.

calamus of emu feather has two major and two minor bands (and some fainter ones as well). These have been numbered 1–4. It can be seen that the extracted proteins

from goose feather rachis and calamus have indistinguishable acrylamide-gel patterns (Fig. 2) and peptide maps (Fig. 4).

The patterns of the SCM-proteins from the various parts (rachis, calamus, barbs, medulla) of feathers from one bird, the goose, are shown in Figure 2. It is seen that, as was also the case of the proteins from the feathers of emu, the rachis and calamus give indistinguishable patterns. Those from the barbs and medulla though different from rachis are somewhat similar to each other. However, there are some components of equal mobility in extracts of all four tissues.

Figure 3 shows that the SCM-proteins from the emu beak give a step-ladder pattern on acrylamide-gel electrophoresis, with more bands than in the patterns from the rachis or calamus of emu feather. Two bands appear to be more predominant than the rest.

(*d*) *Peptide Maps of Feather Proteins*

The peptide map radioautographs in Figures 4 and 5 feature only the radioactively labelled peptides containing *S*-carboxymethylcysteine in tryptic–chymotryptic digests of either whole extracts, or of various bands visible in the acrylamide-gel patterns of the proteins obtained from goose, silver gull, and emu feathers. Four conclusions emerge and these are discussed below:

(1) In the proteins from the feather of one species of bird the sections of peptide chain around the SCMCys residues are identical in positions on peptide maps of proteins derived from calamus and rachis but there are some differences (and also some similarities) on maps from medulla and barb extracts (Fig. 4). This is in agreement with the conclusions from comparison of the acrylamide-gel patterns of proteins extracted from the four morphological parts of goose feather.

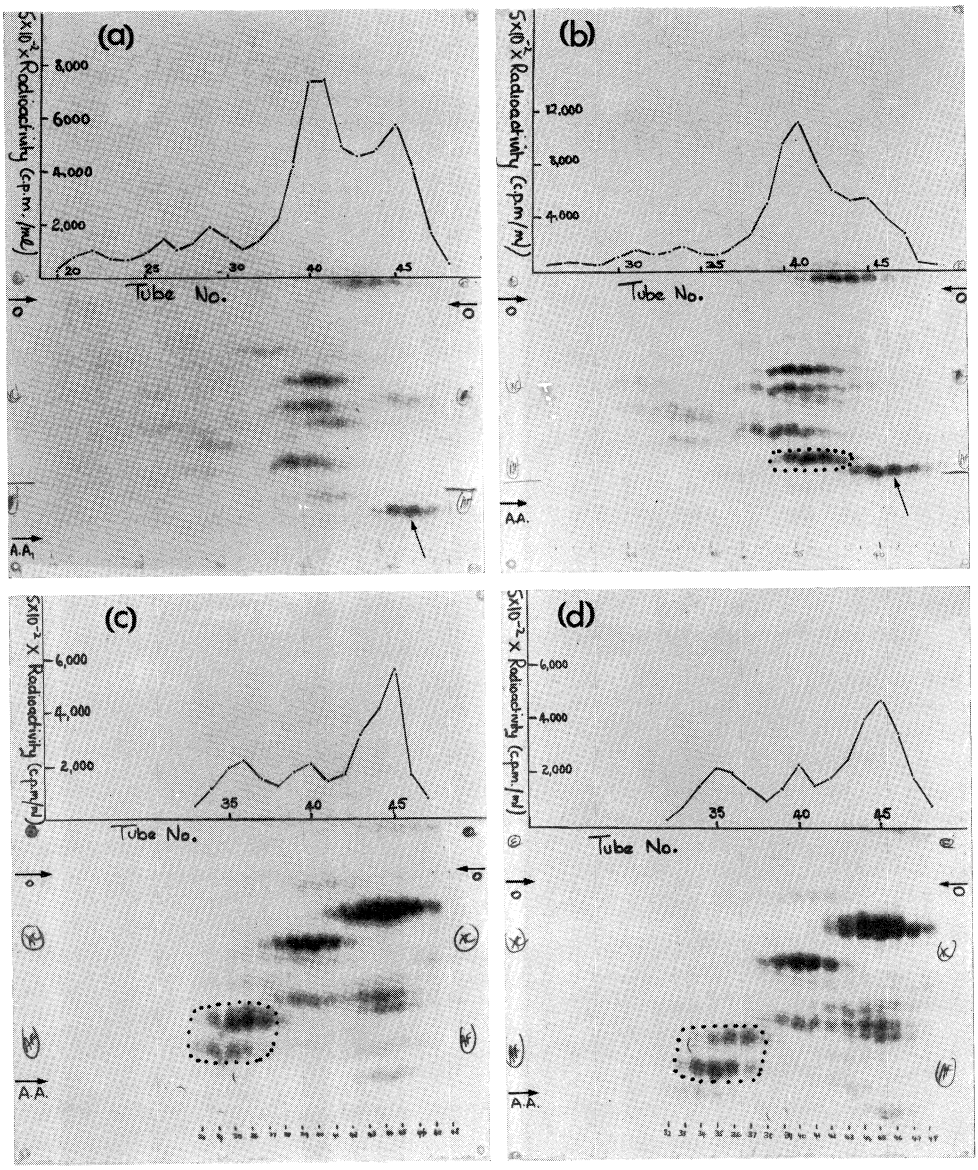
(2) There are differences in the peptide maps of proteins extracted from the corresponding parts of feathers from different species (Fig. 5). Again this is in accord with the results from acrylamide-gel electrophoresis. The peptide indicated by an arrow [in Figs. 5(*a*), 5(*b*), and 5(*e*)] in peptide maps of proteins derived from the calamus of emu and goose feather, and identified as NAc-Ser-SCMCys-Tyr (O'Donnell 1971*a*, 1973) is not present in the corresponding map of the feathers of the silver gull.

(3) The major protein bands visible in the acrylamide-gel pattern of extracts from emu or silver gull feather calamus show at least one difference in their peptide maps (Fig. 5). The protein bands prepared by extracting the proteins from acrylamide gels after electrophoresis are not completely free of each other, but it can be seen that the peptide surrounded by dots in Figure 5 is unique to band 2 protein from emu feather calamus. A similar difference can be seen in the dotted area of the peptide maps corresponding to band 1 protein and band 2 protein of calamus from the feather of the silver gull.

(4) The complexity of the peptide maps for the proteins extracted from feathers (with approximately 8 SCMCys residues per molecule for emu calamus extract) is no greater than that derived from the protein SCM-lysozyme, a single chain containing 8 SCMCys residues per molecule.

(e) Separation on DEAE-Cellulose of Component Bands of SCM-proteins Extracted from the Rachis or Calamus of Emu Feather

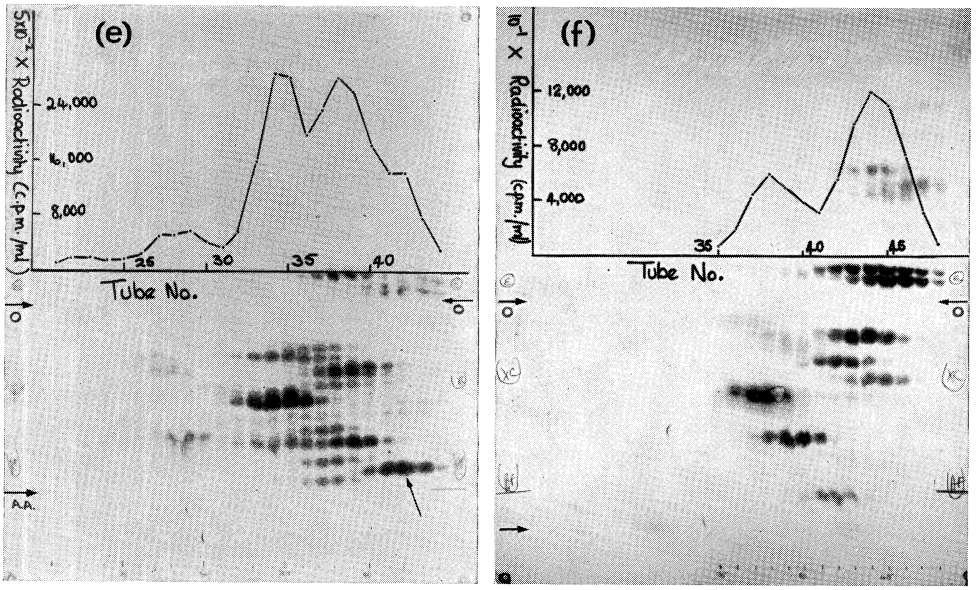
It was found to be necessary to carry out the fractionation of SCM-proteins extracted from feathers in buffers containing 8M urea. In the absence of urea results



Figs. 5(*a*)–5(*d*)

were irreproducible, recoveries of proteins were small and variable, and much protein remained adsorbed to the DEAE-cellulose and could only be removed with 0·1N NaOH. In the presence of buffers containing 8M urea separation of the four predominant bands present in the pattern of SCM-proteins from emu calamus or rachis was

straightforward (Fig. 6). After bulking the required tubes the fractions were dialysed against dilute ammonia and freeze-dried in the presence of urea as described in Section II. The separation achieved on acrylamide gels of the tubes bulked in Figure 6 is shown in Figure 7, whilst that achieved when proteins from 12 similar preparations from emu feather rachis were mixed is shown in Figure 8. In the latter case only protein band 1 needs to be re-run on DEAE-cellulose to make it pure. The relative percentages finally recovered in protein bands 1–4 from fractionation on DEAE-cellulose were approximately 18, 38, 29, and 15, respectively.



Figs. 5(*e*)–5(*f*)

Fig. 5.—(*a*)–(*f*) Size–charge peptide map radioautographs of the SCMCys-containing peptides in tryptic–chymotryptic digests of SCM-proteins extracted from the feathers of various birds. (*a*) Band 2 protein from emu feather calamus; (*b*) band 3 protein from emu feather calamus (Fig. 1); (*c*) band 1 protein from silver gull calamus; (*d*) band 2 protein from silver gull calamus; (*e*) whole protein extract from goose feather calamus. A similarly obtained peptide map for chicken SCM-lysozyme (*f*), with 8 SCMCys residues per molecule, is shown for comparison with that of the extracted feather proteins. Band 3 protein from emu feather calamus has 8 SCMCys residues per molecule (O'Donnell 1973). The sloping arrows point to the amino terminal peptide NAc-Ser-SCMCys-Tyr. The dotted areas show differences in isolated protein bands from extracts of feathers of a particular bird. The upper elution patterns were obtained as in Figure 4. O, origin; A. A., aspartic acid.

(*f*) *Amino Acid Composition of Protein Bands* 1–4 *from Emu Feather Rachis or Calamus*

A comparison of the amino acid compositions (Table 2) of protein bands 1–4 (Fig. 8) shows that they are very similar. There are small differences in several residues. The decrease in arginine and an increase in SCMCys between protein band 1 and protein band 4 is consistent with the higher mobility of band 4.

Comparison of the compositions of protein bands 1–4 with the extract of whole calamus shows that the small amounts of lysine histidine, and methionine must be present in minor protein bands which have not been yet isolated on the acrylamide gel.

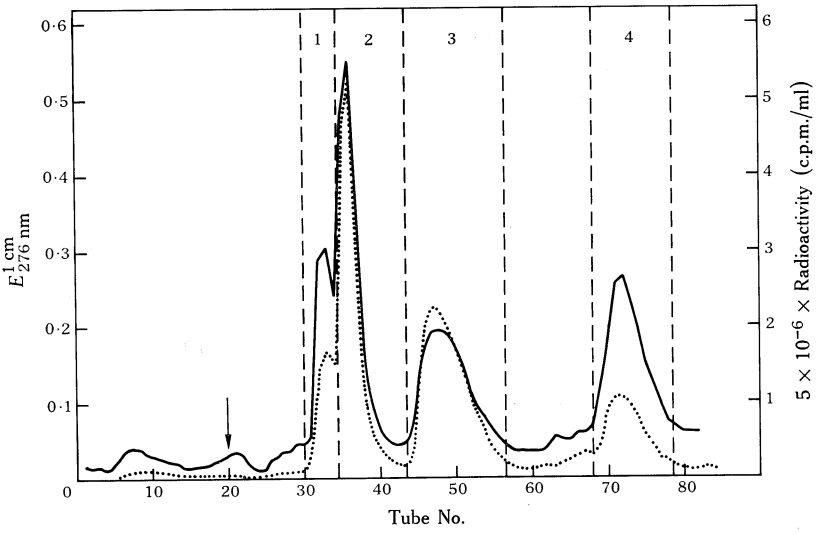
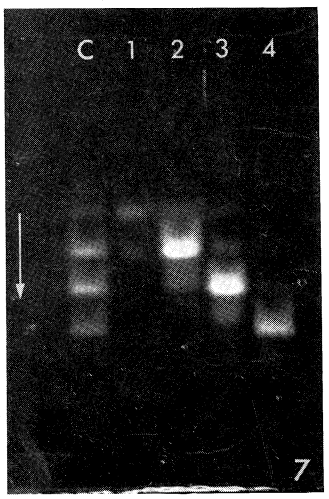


Fig. 6.—Fractionation at 25°C on DEAE-cellulose (DE 52) of SCM-proteins (*c* 52 mg) extracted from the rachis (55 mg) of emu feathers. Column dimensions were approximately 20 by 0·9 cm diameter. Fraction size was 3 ml and flow rate was 12–20 ml hr. The buffer was 8M urea–0·01M Tris–0·001M Versene at pH 8·4. A linear salt gradient was applied at tube 20 (↓) consisting of 100 ml starting buffer and 100 ml starting buffer+0·075M potassium chloride. 50 μl of each fraction was taken for radioactive counting with 0·45 ml water and 5 ml scintillation mixture. 50 μl of selected fractions were run on acrylamide gel before tubes were bulked for dialysis and freeze-drying. ——— Absorption at 276 nm. · · · · Radioactivity.



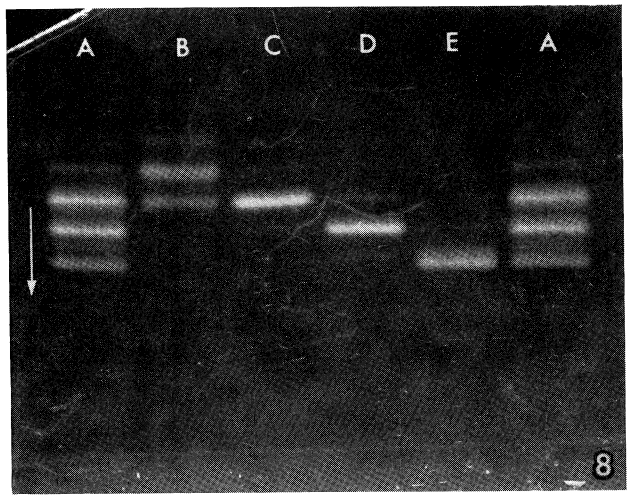


Fig. 7.—Acrylamide-gel patterns of the bulked tubes 1, 2, 3, and 4 and of unfractionated material (C, control) indicated in Figure 6.

Fig. 8.—Acrylamide-gel electrophoresis patterns of bulked protein fractions from 12 preparations of SCM-proteins from emu feather rachis separated on DEAE-cellulose. *A*, unfractionated material; *B*, band 1 protein; *C*, band 2 protein; *D*, band 3 protein; *E*, band 4 protein. Amino acid analyses of preparations B, C, D, and E are given in Table 2. Amino acid sequence of this preparation of band 3 protein is given in O'Donnell (1973).

The analysis of the whole calamus extract agrees well with that of Harrap and Woods (1967). Table 2 also shows that calamus and rachis have different amino acid compositions despite the fact that the SCM-proteins extracted from them (*c.* 95% of the rachis was dissolved and *c.* 60% of the calamus) appear to have identical acrylamide-gel patterns and peptide maps. The values showing the main differences are *italicized* in Table 2.

TABLE 2

AMINO ACID ANALYSES OF EMU FEATHER CALAMUS AND RACHIS, OF WHOLE EXTRACT OF SCM-PROTEINS FROM CALAMUS, AND OF PROTEINS OCCURRING IN BANDS 1, 2, 3, 4 (FIG. 1) OF ACRYLAMIDE-GEL PATTERNS OF SCM-PROTEINS EXTRACTED FROM RACHIS OR CALAMUS

These proteins were separated on DEAE-cellulose (Fig. 6). The values are given as residues of amino acids per 100 residues. All hydrolyses were carried out *in vacuo* in the presence of thioglycollic acid to minimize destruction of SCM-cysteine and tyrosine (Sanger and Thompson 1963). n.d., not determined

Amino acid	Calamus*	Rachis*	Whole extract of calamus†	Bands*			
				1	2	3	4
Lysine	2·1	0·8	1·1	0	0	0	0
Histidine	0·6	0·3	0·2	0	0	0	0
Arginine	4·6	4·1	4·4	4·4	4·2	4·1	3·6
SCM-cysteine	*(4·6)*‡	*(6·6)*‡	6·1	5·7	6·2	7·4	7·5
Aspartic acid	6·3	6·3	6·1	6·2	6·2	6·4	6·2
Threonine	4·1	5·2	4·8	5·0	5·3	5·0	5·2
Serine	14·9	15·8	15·6	17·3	17·3	17·8	17·4
Glutamic acid	*9·0*	*6·1*	7·7	6·1	6·1	6·1	5·8
Proline	*8·9*	*12·0*	11·6	12·4	12·8	13·5	12·7
Glycine	*13·2*	*9·4*	12·8	11·0	9·8	10·5	10·5
Alanine	4·7	4·5	4·8	4·6	4·4	4·7	5·1
Valine	*7·8*	*9·6*	5·9	9·1	9·7	9·7	8·1
Methionine	0·8	0	0·3	0	0	0	0
Isoleucine	4·2	4·7	3·7	4·0	4·2	4·6	3·9
Leucine	7·9	8·1	8·5	8·6	8·4	9·5	9·6
Tyrosine	3·4	3·2	3·0	2·4	2·2	1·9	1·9
Phenylalanine	2·8	2·9	2·6	3·2	3·0	2·9	2·4
Tryptophan§	n.d.	n.d.	0·6	n.d.	n.d.	0	n.d.

* Analyses on single hydrolysates (calamus, rachis) or duplicate hydrolysates (bands) were carried out for 24, 48, and 72 hr and values were averaged except in the cases of serine, threonine, valine, and isoleucine which were extrapolated either for destruction or incomplete release during hydrolysis.

† Average of values for two preparations—single 24 hr hydrolyses only.

‡ Determined as cysteic acid in separate experiments by the method of Moore (1963).

§ Determined in separate experiments using the method of Liu and Chang (1971).

IV. DISCUSSION

The acrylamide-gel electrophoretic patterns of SCM-proteins extracted from the natural β-keratins, bird's feather and beak, are simpler than those (or the corresponding starch-gel patterns) of the proteins isolated from the α-keratin, wool (O'Donnell and Thompson 1964; Darskus and Gillespie 1971; Sparrow and Crewther 1972; Gillespie 1973), and the precursor keratin proteins from cow's lip

epidermis (O'Donnell 1971*b*). The natural β-keratins do not consist of highly heterogeneous families of proteins as do the α-keratins. The acrylamide-gel patterns of SCM-proteins extracted from feathers from a number of species of birds are different, and that for emu feather calamus (or rachis) seems to be the simplest. In general the results agree with those of Harrap and Woods (1967) and Woods (1971). These authors also found that the proteins were homogeneous with regard to molecular size and had a molecular weight of approximately 10,800 (Harrap and Woods 1964*b*; cf. Jeffrey 1971). These authors found that the proteins tended to aggregate and readily form gels. The acrylamide-gel electrophoretic patterns presented in the present work are more detailed than previous ones because of the use of buffers containing 8M urea which probably eliminates these interactions. Kemp and Rogers (1972) in their studies on SCM-proteins from adult and embryonic feathers and scales used buffers at pH 9·5 containing 5M urea and also got good resolution.

The differences in the proteins extracted from the feathers of various birds seen in the size–charge peptide map radioautographs (Fig. 5) of peptides containing radioactively labelled SCMCys residues show that there are some amino acid sequence variations in these regions. However, there are also similarities as shown by the existence of an amino-terminal tripeptide, NAc-Ser-SCMCys-Tyr, in the SCM-proteins extracted from both emu calamus (O'Donnell 1973) and goose calamus (O'Donnell 1971*a*). This peptide is marked by arrows in Figure 5. It does not exist in the corresponding proteins from silver gull feather calamus.

The great similarity in the size–charge peptide maps and gel-electrophoresis patterns of the SCM-proteins extracted from the rachis and calamus from the feathers of a single species of bird (Fig. 2) suggests that identical proteins are being extracted from the two tissues. However, the whole rachis and calamus have different morphology and are not identical in amino acid composition (Table 2) nor in their degree of solubility in solutions of urea-reducing agent (Table 1). The differences in the peptide maps (Fig. 2) and acrylamide-gel patterns of proteins extracted from the barbs, medulla, and rachis or calamus or both are in accord with the results of Harrap and Woods (1967), and Kemp and Rogers (1972). The differences in solubility of the emu rachis and calamus may be related to the presence of γ-glutamyl links as have been found in other keratins (Harding and Rogers 1971).

The ready fractionation of the SCM-proteins extracted from emu feather calamus or rachis in buffers containing 8M urea (cf. Woods 1971) and the difficulty and poor recoveries in the absence of urea is doubtless due to the tendencies of these proteins to aggregate. This aggregation phenomena was previously reflected in the tendencies of solutions of these proteins to form gels on standing in dilute borate buffer at 2–4°C (Harrap and Woods 1967). Since the amino terminus of feather proteins is acetylated (O'Donnell 1971*a*) and the purified bands from emu SCM-keratin from feather contain no lysine, no precautions are needed to prevent carbamylation of amino groups in urea solutions.

The four predominant components in the SCM-protein extracted from emu feather rachis and calamus have very similar amino acid analyses. Though there appears to be a decrease in arginine content and an increase in SCMCys content between protein band 1 and protein band 4 (Table 2), the separations of some of the bands during acrylamide-gel electrophoresis must be due to amide differences

since their molecular weights are very similar. Jeffrey (1970), using acrylamide-gel of varying porosities, found that six of the bands present in the acrylamide-gel patterns of SCM-keratins extracted from duck feather rachis had values for molecular weight in the range 10,500±1,500 and Harrap and Woods (1964*b*) found the SCM-proteins extracted from fowl feather rachis to be homogeneous with regard to molecular weight. But, despite these similarities, there are also at least some amino acid sequence differences between the protein bands, e.g. between protein band 2 and protein band 3 of SCM-proteins from emu feather calamus and protein bands 1 and 2 from silver gull feather rachis (dotted areas in Fig. 5).

Finally, the answer to the question "Is there a major homogeneous protein present in the SCM-proteins extracted from emu feather rachis or calamus?" is "probably yes". The facts which support this belief are (1) the single-bandedness of the acrylamide-gel electrophoretic pattern; (2) the equality of degree of complexity of the size–charge peptide maps of SCMCyS-containing peptides of protein band 2 and protein band 3 with that from SCM-lysozyme containing the same number of SCMCys residues per molecule; and (3) the finding of a unique amino terminal tripeptide in almost molar amount in a similar keratin (goose calamus). The proteins extracted from the calamus or rachis have the simplest acrylamide-gel pattern. Protein band 3, comprising approximately 29% of the extracted proteins, has been chosen initially for determination of its amino acid sequence and this is reported in a separate paper (O'Donnell 1973).

V. References

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