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The Proteins of the Keratin Component of Bird's Beaks

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Abstract

Birds' beaks have an outer shell of hard keratin which consists almost entirely of proteins which are very rich in glycine [about 30 residues per 100 residues (residues %)], contain moderate levels of tyrosine and serine (each about 8 residues %), and which have relatively low contents of cystine (about 2·5 residues %), lysine, histidine, isoleucine and methionine.

Major protein fractions in the *S*-carboxymethyl form isolated from the beaks of six different orders of birds have similar amino acid compositions, isoelectric points (pH 4·2–4·9) and molecular weights (13 000–14 500). Detailed chromatographic electrophoretic and compositional studies of the proteins of kookaburra beak reveal them to be a family of closely related proteins with only limited heterogeneity, in contrast to mammalian keratin systems.

The major kookaburra beak fraction is similar in overall composition and molecular weight to fowl epidermal scale, kookaburra claw and turtle scute proteins and shows some resemblance to reptile claw protein. Beaks also contain small amounts of protein which are distinctly different from the major fraction but which resemble feather keratin proteins in composition and size.

Introduction

Compared with studies of mammalian keratins, there have been relatively few studies made on the keratins of birds and reptiles. Those which have been made are mostly confined to feather and its constituent proteins (reviewed by Fraser *et al.* 1972). In terms of amino acid composition, sequence, molecular size and structure, feather proteins show very little resemblance to mammalian proteins (Woodin 1954; Harrap and Woods 1964*a*, 1964*b*, 1967; Woods 1971; O'Donnell 1973*a*, 1973*b*). Furthermore, earlier work by Rudall (1947) showed that on the basis of X-ray studies a pleated sheet structure very similar to that found in feather could be observed for the hard portions of beak and lizard claw; this structure is not found in native mammalian keratins. These data suggest that mammalian and non-mammalian keratins do not share common structural components.

An examination of amino acid composition data for beak (Block and Bolling 1951) and lizard claw (Fraser *et al.* 1972) indicates that these keratins are distinctively different from feather, but their relatively high contents of glycine and tyrosine suggest that they may bear some structural relationship to the glycine–tyrosine-rich proteins of mammalian keratins (Gillespie 1972; Gillespie and Frenkel 1974*a*, 1974*b*). Furthermore, it has been suggested (Gillespie 1974; Gillespie and Frenkel 1974*b*) that homology between the mammalian and non-mammalian keratin proteins could provide some evidence that the former proteins are components of primitive keratins.

It is thus of some interest to investigate the proteins of non-mammalian keratins and their relationship to feather and mammalian keratins. This paper presents a

study of the amino acid composition, molecular size and extent of heterogeneity of the beak proteins from a wide range of Australian birds and a comparison of these data with that available for feather, other non-mammalian and certain mammalian keratin proteins.

Materials and Methods

Origin and Preparation of Keratin Samples

Sources of the keratins are given at the end of this paper. Beaks from 12 species of Australian birds were used in these studies although only the results for the following six species, each belonging to a different order, are presented here: barn owl (*Tyto alba*), kookaburra (*Dacelo novaeguinae*), brown hawk (*Falco berigora*), pallid cuckoo (*Cuculus pallidus*), magpie (*Gymnorhina tibicen*) and glossy black cockatoo (*Calyptorhynchus lathama*). For a description and comprehensive classification of these birds, see Hill (1967).

Each tissue was carefully dissected to separate the hard horny keratin layer from underlying soft or bony material. The keratin was ground to pass 50 mesh in a micro-Wiley mill, thoroughly extracted with 0·15 M sodium chloride solution to remove any soluble non-keratinous constituents, washed with water, dried with ethanol and then defatted with light petroleum.

Preparation of Soluble Proteins

The keratins were dissolved by extraction with 0·2 M potassium thioglycollate in 6 M urea at pH 11. Generally 85–90% of the keratin was solubilized by this technique. For electrophoresis in the reduced form, the proteins were dialysed against and stored in 0·1% β-mercaptoethanol. For all other experiments the proteins were alkylated with iodoacetate, dialysed against deionized water and then fractionated by precipitation of the major constituent proteins with zinc acetate (0·02 M) at pH 6 (Gillespie and Frenkel 1974*a*). The precipitate, representing 95–98% by weight of the total protein extracted, was then dissolved by stirring with 0·02 M sodium citrate solution, dialysed against running deionized water and freeze-dried. The supernatant was made 0·02 M with respect to citrate, dialysed and freeze-dried.

Electrophoresis

Charge heterogeneity of the proteins was studied by electrophoresis on cellulose acetate in urea at pH 8·9 (Blagrove *et al.* 1975) and in urea (6 M), citric acid (0·2 M) and sodium chloride (0·03 M) at pH 2·9.

Electrophoresis of the *S*-carboxymethyl proteins in sodium dodecyl sulphate (SDS)–polyacrylamide gels was carried out by the method of Weber and Osborn (1969). For proteins in the reduced form, 0·1% β-mercaptoethanol was included in the electrode buffer and sample solution. α-Chymotrypsinogen A, myoglobin and fowl feather rachis protein were used as calibrating proteins.

Isoelectric focusing in polyacrylamide slabs in the pH range 2·3–5 (all components are isoelectric in this range) was performed using a modification of the method described by Karlsson *et al.* (1973) in a modified Beckman Microzone apparatus (Marshall and Gillespie 1976). The gel contained urea (4 M), acrylamide (5%), *N,N'*-methylenebisacrylamide (0·025%), *N,N,N',N'*-tetramethylethylenediamine (2%), pH 2·5–4 and pH 4–6 ampholines (2% each), pH 5–8 ampholines (1%), glycerol (5%) and ammonium persulphate (0·03%) (all percentages are w/v). The electrode buffers used were phosphoric acid (1 M) for the anode, and urea (4 M) and pH 5–8 ampholines (0·5%) for the cathode. Following pre-electrophoresis for 20 min at 400 V (4 mA), samples (100 μg protein dissolved in 5 μl cathode buffer containing 5% glycerol) were loaded and electrophoresis continued for 4·5 h at 800 V (6–2 mA). The pH gradient was determined after transverse sections of gel cut from the edge of the slab had equilibrated in degassed water for 1 h. The remaining gel was washed thoroughly with 10% trichloroacetic acid and the protein bands stained as described by Weber and Osborn (1969).

Gel Filtration over Controlled-pore Glass

Molecular weight determinations of *S*-carboxymethyl keratin proteins were carried out by gel filtration over controlled-pore glass (mean pore diameter 12·3 nm) in a buffer containing urea (6 M),

SDS (0·5%) and sodium phosphate (0·05 M) at pH 7 as described by Frenkel and Blagrove (1975). The column was calibrated with myoglobin and the *S*-carboxymethyl derivatives of insulin-B chain, wool high-tyrosine protein 0·62 (Frenkel *et al.* 1973), and cytochrome C.

Ion-exchange Chromatography

The zinc-precipitable proteins of kookaburra beak were fractionated by chromatography on DEAE-cellulose in a buffer containing glycylglycine (0·05 M), NaOH (0·025 M) and urea (6 M), pH 8·3. The linear salt gradients used are shown on the appropriate figures.

Amino Acid Analysis

Beak protein and keratin were hydrolysed for 22 h *in vacuo* at 108°C with 6 M HCl containing 2 mM phenol and the amino acids were determined with a Beckman–Spinco 120C amino acid analyser.

Table 1. Amino acid composition (as residues %) of zinc-precipitable proteins isolated from six bird beaks

For comparison, the composition of kookaburra beak keratin is included

Amino acid	Kookaburra beak keratin	Beak proteins					
		Barn owl	Kookaburra	Brown hawk	Pallid cuckoo	Magpie	Glossy black cockatoo
Lys	0·6	1·6	0·2	0·9	1·5	0·5	0·4
His	1·1	1·0	1·1	1·1	1·2	0·4	0·9
Arg	3·5	4·1	3·3	3·8	4·1	3·6	3·4
Cys(Cm)	–	4·7	4·6	5·2	4·7	5·2	5·0
Asp	4·2	5·7	3·7	5·1	5·3	3·8	3·2
Thr	3·6	3·7	3·6	3·9	3·5	2·7	3·0
Ser	6·7	8·7	6·3	7·0	9·6	9·8	10·2
Glu	4·2	6·2	3·2	5·3	6·2	4·5	4·6
Pro	9·6	6·7	9·6	7·8	7·7	7·6	8·0
Gly	31·3	27·1	33·9	29·7	25·2	31·9	28·9
Ala	6·3	5·9	6·1	4·4	6·6	5·9	5·3
½Cys	3·8	–	–	–	–	–	–
Val	4·5	4·9	4·0	5·1	6·0	4·8	5·5
Met	0·8	0·7	0·3	0·3	0·5	0·1	0·1
Ile	1·7	2·6	1·5	2·4	2·2	1·6	2·1
Leu	7·3	5·0	5·4	5·2	6·4	4·9	5·2
Tyr	8·6	8·1	9·4	9·4	7·2	9·6	9·5
Phe	3·5	3·1	3·7	3·4	2·4	3·1	4·3

Results

Amino Acid Composition of Soluble Beak Proteins

Amino acid analyses were first performed on the dissected horny parts from the tip of the beaks but extremely large variations in composition were found between different species. Barn owl beak, for example, contained 4·3 residues tyrosine per 100 residues (residues %) whilst cockatoo beak contained 9·4 residues %. Much of this variation was found to be due to the presence of variable amounts of a protein which contains hydroxyproline. Very careful dissection could eliminate this impurity and the analysis of a hydroxyproline-free kookaburra beak is shown in Table 1. It was found that this contaminating protein was not solubilized during the alkaline-thioglycollate extraction of the keratin protein and subsequent analyses were made on the soluble proteins. The unextracted residue from the barn owl was hydrolysed and analysed and was found to have an amino acid composition typical of collagen.

The composition of zinc-precipitable beak proteins from birds of six different orders are compared in Table 1. In contrast to the variability found for whole beak, these proteins show an overall similarity in composition. This is particularly evident in their contents of glycine (about 30 residues %), tyrosine, serine and proline which together account for 55–60% of the total residues. Their content of half-cystine, determined as *S*-carboxymethyl cysteine, is low when compared to mammalian keratins (Gillespie and Frenkel 1974*a*). The levels of methionine, isoleucine, lysine and histidine are relatively low for each of these beak proteins: methionine and for three species, lysine also, are present at considerably less than one residue per mole suggesting that a substantial proportion of the proteins do not contain these amino acids. Although there is a considerable variation in the content of many amino acids when the beak proteins are compared (Table 1), this does not detract from the idea that there is a great overall similarity in composition between the members of this group of proteins.

Table 2. Amino acid composition (as residues %) of proteins isolated from beak, feather, fowl epidermal scale, turtle scute and lizard claw

Amino acid	Kookaburra beak		Feather[A]	Bird claw[B]	Fowl epidermal scale[C]	Turtle scute[D]	Lizard claw[E]
	Zinc-precip.	Zinc-soluble					
Lys	0·2	0·3	0·9	0·3	0·9	0·3	0·6
His	1·1	0·1	0·3	1·1	0·7	1·3	1·9
Arg	3·3	2·8	4·3	3·4	4·3	4·5	3·1
Cys(Cm)	4·6	7·7	7·8	4·9	5·4	5·5	11·3
Asp	3·7	5·2	5·9	4·0	4·2	2·3	5·5
Thr	3·6	5·0	4·4	3·8	3·9	1·6	3·0
Ser	6·3	13·6	14·0	7·0	9·4	5·6	6·0
Glu	3·2	5·9	7·9	3·8	5·8	4·0	3·6
Pro	9·6	13·3	10·0	9·4	6·9	10·1	9·8
Gly	33·9	16·3	13·0	30·8	26·0	30·3	29·4
Ala	6·1	6·6	6·6	6·1	6·5	3·0	4·7
Val	4·0	7·3	7·8	4·7	6·5	6·7	4·0
Met	0·3	0·2	0·2	0·5	0·7	0·1	0·6
Ile	1·5	4·2	3·7	1·7	2·7	1·8	2·7
Leu	5·4	7·0	7·6	5·8	6·3	8·1	2·2
Tyr	9·4	1·7	2·0	9·0	6·6	12·7	4·6
Phe	3·7	2·6	3·3	3·6	3·0	2·3	3·1

[A] Mean of analytical data for proteins isolated from fowl and seagull rachis, fowl and emu calamus, and fowl and turkey barbs (Harrap and Woods 1967).
[B] Zinc-precipitable protein isolated from kookaburra claw.
[C] Proteins isolated from fowl epidermal scale (Kemp and Rogers 1972).
[D] Zinc-precipitable protein isolated from turtle scute (*Chelonia mydas*).
[E] Zinc-precipitable protein isolated from lizard claw (*Varanus varanus*).

As the zinc-precipitable proteins comprise at least 80% by weight of beak keratin and a comparison of the amino acid analyses of these proteins with the parent keratin (e.g. kookaburra, Table 1) shows them to have few significant differences, the zinc-precipitable proteins can be regarded as representative of the bulk of beak protein. However, it should not be overlooked that beak contains small amounts of other proteins—for example there is evidence for the presence of a fraction moderately rich in methionine as well as a zinc-soluble protein fraction.

All beaks have been found to contain a protein fraction (up to 5%) which is soluble in zinc acetate at pH 6, and this fraction from kookaburra beak has been examined in detail. Its amino acid composition differs greatly from that of the kookaburra zinc-precipitable fraction (Table 2) as it contains considerably more *S*-carboxymethyl cysteine, aspartic acid, threonine, serine, glutamic acid, proline, valine, isoleucine, and leucine but lesser amounts of histidine, glycine, tyrosine and phenylalanine.

Heterogeneity of Zinc-precipitable Beak Proteins

The extent of charge heterogeneity of the zinc-precipitable beak proteins was examined by electrophoresis on cellulose acetate strips at pH 8·9 in urea–barbitone buffer and at pH 2·9 in urea–citric acid buffer. At each pH, 5–8 protein bands were usually obtained with the bulk of the protein contained in 2–3 bands. The patterns obtained after electrophoresis at pH 8·9 are shown in Fig. 1.

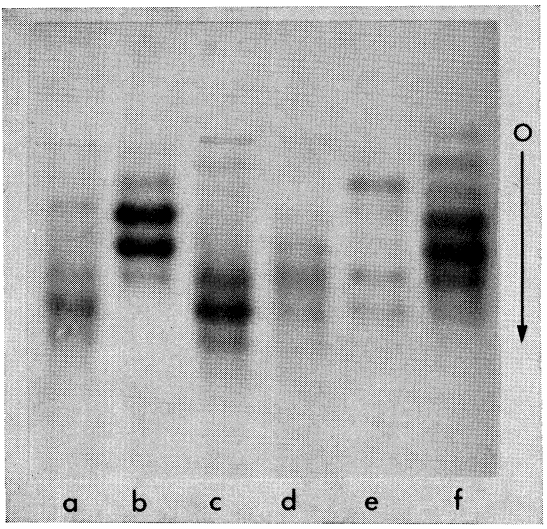

Fig. 1. Electrophoresis on cellulose acetate at pH 8·9 of zinc-precipitable protein from six bird beaks. (*a*) Barn owl. (*b*) Kookaburra. (*c*) Brown hawk. (*d*) Pallid cuckoo. (*e*) Magpie. (*f*) Glossy black cockatoo.

Charge heterogeneity was also examined by isoelectric focusing in polyacrylamide gels. It can be seen (Fig. 2) that for each beak, all protein components had isoelectric points between pH 4·2 and pH 5·1, with apparently all but one component isoelectric below pH 4·9. Only one component, that with an isoelectric point at pH 4·9, appears to be shared by each sample. The patterns obtained in Fig. 2 also reveal a considerable variation in the number and proportion of components, ranging from kookaburra (Fig. 2*b*) with two major, four relatively minor and two trace bands, to the cockatoo (Fig. 2*f*) which shows a total of at least 16 bands. The isoelectric focusing patterns thus resolve up to twice the components that can be obtained by electrophoresis on cellulose acetate at either acid or alkaline pH (Fig. 1). This suggests that the components resolved by cellulose acetate electrophoresis at each pH are largely different and that the full extent of heterogeneity is more nearly revealed by the sum of the components resolved at the two pH values.

Molecular Size of Beak Proteins

The molecular weights of zinc-precipitable beak proteins were determined by gel filtration, SDS–polyacrylamide gel electrophoresis and, for the kookaburra protein only, in the ultracentrifuge (Woods 1976).

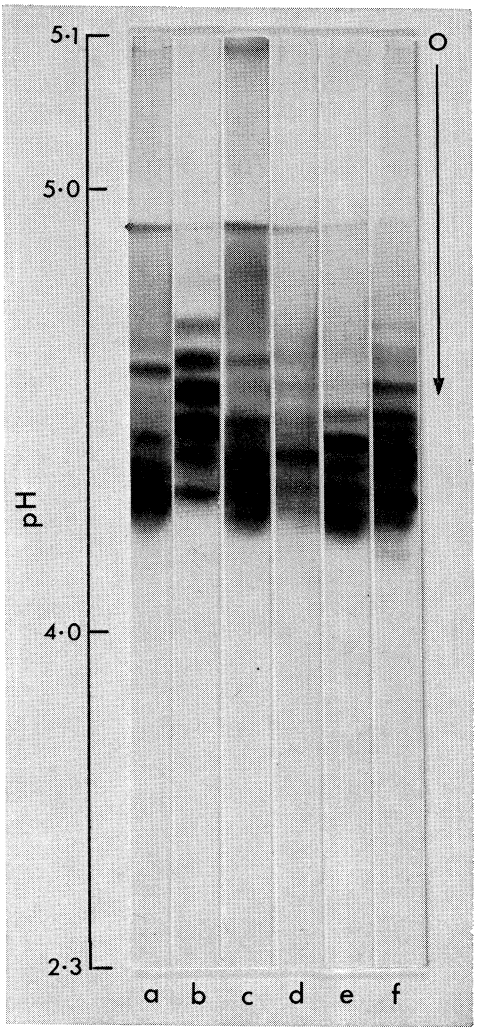

Fig. 2. Isoelectric focusing in a polyacrylamide slab of zinc-precipitable protein from six bird beaks. (*a*) Barn owl. (*b*) Kookaburra. (*c*) Brown hawk. (*d*) Pallid cuckoo. (*e*) Magpie. (*f*) Glossy black cockatoo.

Gel filtration over controlled-pore glass with proteins in the *S*-carboxymethyl form indicated that the zinc-precipitable beak proteins (isolated from the six beaks examined in the electrophoretic studies) had indistinguishable molecular weights of the order of 13 000. With this system, feather protein gave a value of about 10 800 which is comparable with the sequence molecular weight of 10 500 (O'Donnell 1973*b*).

Electrophoresis of the *S*-carboxymethyl derivative of beak proteins in SDS–polyacrylamide gels gave apparent molecular weights in the range 19 000–21 000,

which are considerably higher than those found by gel filtration or in the ultracentrifuge. Fowl feather protein under these conditions also gave an anomalously high value of about 14 000. However, E. F. Woods (unpublished data) has found that if the reduced protein is electrophoresed in the presence of SDS and β-mercaptoethanol then the correct molecular weight is obtained. Similarly, electrophoresis of reduced beak proteins in SDS–β-mercaptoethanol gave lower values for apparent molecular weight which are comparable with those obtained on the *S*-carboxymethyl protein by chromatography on porous glass or in the ultracentrifuge (Woods 1976). The molecular weights obtained are not identical for each species of beak although the range of values for the major component of each is small (13 000–14 500). Both the hawk (Fig. 3*d*) and magpie (Fig. 3*f*) contain two major molecular

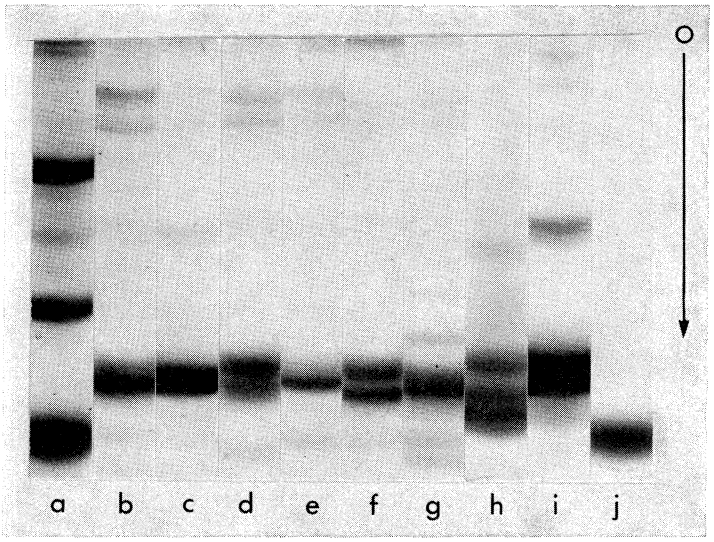

Fig. 3. Electrophoresis in SDS–polyacrylamide–β-mercaptoethanol gels of proteins from six bird beaks compared with proteins from bird claw, feather and lizard claw. All proteins are in the reduced form. (*a*) Standard proteins (α-chymotrypsinogen A, apomyoglobin, fowl feather rachis). (*b*) Barn owl. (*c*) Kookaburra. (*d*) Brown hawk. (*e*) Pallid cuckoo. (*f*) Magpie. (*g*) Glossy black cockatoo. (*h*) Goanna claw. (*i*) Kookaburra claw. (*j*) Kookaburra feather.

weight species, and each beak shows a varying number and proportion of minor components of higher and lower molecular weights. Fig. 3 (*i*, *j*) also shows the electrophoretic patterns obtained with the reduced proteins of kookaburra claw and feather. The feather protein is essentially a single component with molecular weight about 11 000, whereas the claw provides a very similar pattern to that of kookaburra beak with a single major protein of molecular weight about 14 000.

Whole extracts of beak run in the -SH form in SDS–polyacrylamide–β-mercaptoethanol show the presence of minor components (for kookaburra traces only) having molecular weights in the range 10 500–11 000, which are very similar to the value for feather protein (Fig. 3). As the zinc-soluble *S*-carboxymethyl protein fraction has already been shown to have an amino acid composition similar to feather (see

p. 470), it seemed likely that this fraction would also be similar in size to that of feather and be responsible for the minor gel bands referred to above. Because beak proteins in the -SH form cannot be fractionated with zinc acetate it was possible to carry out these studies only indirectly—after they had been alkylated.

The molecular size of the zinc-soluble fraction from kookaburra beak in the *S*-carboxymethyl form was compared with the zinc-precipitable fraction and with feather proteins by SDS–polyacrylamide gel electrophoresis (Fig. 4). It can be seen that the zinc-soluble proteins are considerably smaller than the zinc-insoluble proteins and very similar, but not identical in size, to feather protein. A molecular weight determination of the zinc-soluble fraction of kookaburra beak was made by gel filtration over controlled-pore glass which had already given apparently correct values for the beak and fowl feather proteins in the *S*-carboxymethyl form. Using this system, a value of 10 500 was obtained for the zinc-soluble fraction which is virtually identical with feather protein.

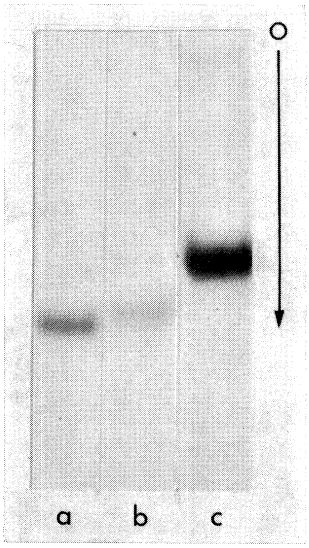

Fig. 4. Electrophoresis in an SDS–polyacrylamide slab of the *S*-carboxymethyl derivative of (*a*) fowl feather rachis protein, (*b*) zinc-soluble kookaburra beak protein and (*c*) zinc-precipitable kookaburra beak protein.

Molecular Basis of Charge Heterogeneity

In order to investigate the basis of the charge heterogeneity shown by all the beak preparations, the zinc-precipitable proteins of kookaburra beak, which appeared to be at least heterogeneous from electrophoretic studies, were subjected to chromatographic fractionation on DEAE-cellulose at pH 8·3. It can be seen (Fig. 5*a*) that there are two major and about six minor peaks indicating a comparable degree of resolution to that shown by cellulose acetate electrophoresis at a similar pH (Fig. 1*b*). Fractions were collected as indicated (Fig. 5*a*) and compared with the unfractionated protein by cellulose acetate electrophoresis (Fig. 6*a*). It can be seen that a fractionation of the components has taken place and that although there is some overlap between successive fractions, the two major components of the unfractionated protein have been largely separated and concentrated into fractions 3 and 4. Electrophoresis of these fractions on cellulose acetate at pH 2·9 generally indicated only minor

increases in the number of resolved components, giving a total heterogeneity comparable with that found by isoelectric focusing. This is in marked contrast to the great heterogeneity revealed by chromatography of mammalian keratin proteins (Gillespie and Frenkel 1974*b*; Marshall and Gillespie 1976). It is of interest that fraction 3 (Fig. 6*a*) gave two major components on cellulose acetate at pH 2·9 whilst fraction 4 did not show any significant increase in heterogeneity under acid conditions.

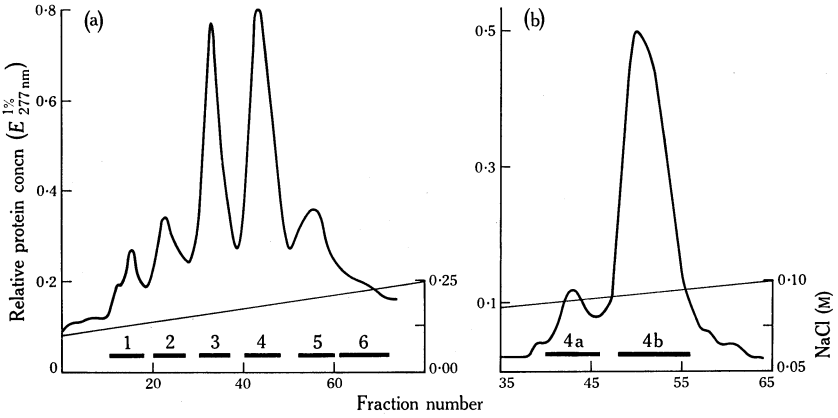

Fig. 5. (*a*) Elution profile obtained by chromatography of zinc-precipitable kookaburra beak protein on DEAE-cellulose at pH 8·3. A linear salt gradient of 0–0·25 M NaCl was applied, and fractions were pooled as indicated on the profile. (*b*) Elution profile obtained by chromatography on DEAE-cellulose at pH 8·3 of fraction 4 obtained by the initial separation. A linear salt gradient of 0·05–0·10 M NaCl was applied and fractions were pooled as indicated on the profile.

Rechromatography of fraction 4 on DEAE-cellulose at pH 8·3 using a shallower salt gradient than in the initial separation resolves the protein into two peaks (Fig. 5*b*). At high loading, electrophoresis of these fractions (Fig. 6*b*) indicates that the major peak, 4b, contains only traces of contaminating protein. This fraction was subsequently used for molecular weight studies in the ultracentrifuge (Woods 1976).

Amino acid compositions for fractions from the initial chromatographic separation (fractions 2, 3 and 5) and for the purified component (fraction 4b) are compared in Table 3. It can be seen that the fractions have similar compositions, suggesting that the zinc-precipitable fraction of beak constitutes a single family of closely related proteins. The composition of the major fractions of the zinc-precipitable fraction, 3 and 4b (fraction 3 containing relatively small amounts of other proteins), differ by 10% or more only in their levels of arginine, aspartic acid, glutamic acid, valine, leucine and phenylalanine. These differences would be equivalent to no more than one residue per mole in each case. It should be noted that when the composition of fraction 4b is recalculated on the basis of residue per mole, many amino acids are not present in integral levels, suggesting further heterogeneity.

Unfortunately not all components of the zinc-precipitable fraction (e.g. peaks 1 and 6 of Fig. 5*a*) were recovered in amounts sufficient for amino acid analysis. However, an examination of the data in Table 3 indicates that these minor fractions contain less glycine and valine and more *S*-carboxymethyl cysteine, aspartic acid,

serine, proline and tyrosine than the starting material and thus must be considerably different in composition from the major components.

Comparison of Beak Proteins with Other Non-mammalian Keratin Proteins

On the basis primarily of glycine content and to a lesser extent serine, tyrosine and carboxymethyl cysteine contents (Table 2), the proteins of the avian–reptilian keratins can be divided into two distinct groups. The first, which contains a moderate level of glycine, comprises feather and the zinc-soluble beak protein, and the second, which contains almost twice as much glycine, contains fowl scale, turtle scute, reptile claw and the zinc-precipitable proteins of avian claw and beak. Within the second group, beak and claw, with almost identical composition, have an overall similarity to the other members although there are significant differences in composition.

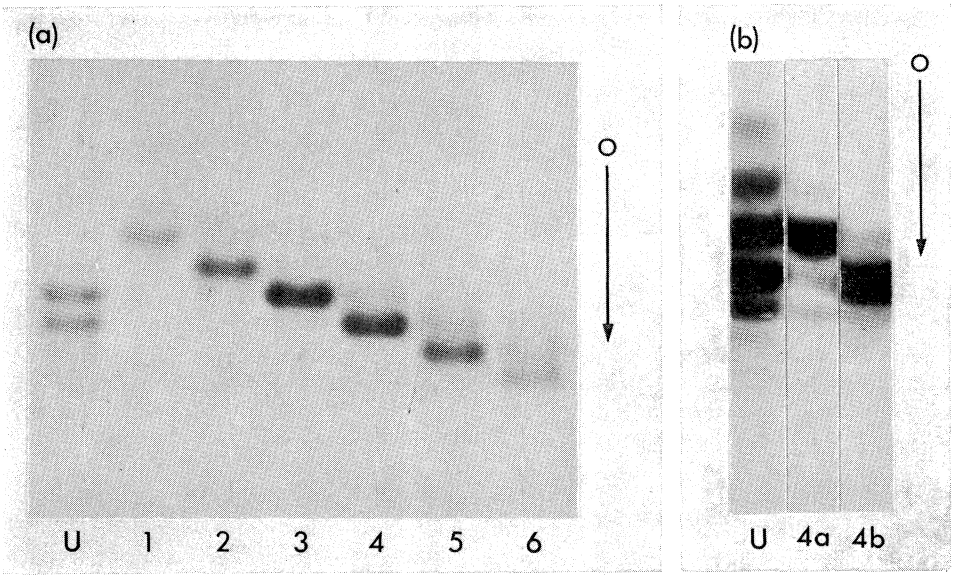

Fig. 6. (*a*) Electrophoresis on cellulose acetate at pH 8·9 of fractions obtained from chromatography on DEAE-cellulose (Fig. 5*a*). Zinc-precipitable kookaburra beak protein is included for comparison (U). (*b*) Electrophoresis on cellulose acetate at pH 8·9 of fractions obtained from rechromatography on DEAE-cellulose of fraction 4 (Fig. 5*b*). Zinc-precipitable kookaburra beak protein is included for comparison (U).

The molecular weight of the scale proteins has been found to be about 14 500 by Walker and Bridgen (1976), whilst gel filtration over controlled-pore glass of the turtle scute protein gave a value of about 13 000 which is similar to that of the zinc-precipitable beak proteins.

By contrast with feather protein (Table 2), the zinc-precipitable fractions of kookaburra beak and lizard claw show some similarities to each other, particularly in their exceptionally high content of glycine and in the overall distribution of many amino acids. Nevertheless, lizard claw contains about twice as much *S*-carboxymethyl cysteine and isoleucine, and half as much tyrosine and leucine as does the beak fraction. Other differences between them show up if the lizard claw protein (in the reduced form) is electrophoresed in polyacrylamide gels in the presence of SDS and

β-mercaptoethanol, for three major bands are obtained—a major one having a molecular weight of about 11 500 and the other two having values of 13 500 and 15 000 (Figs 3*b–h*). Further fractionation of these proteins would be of interest in order to determine whether the 11 500 molecular weight subunit is a feather-like protein and the other components beak-like in their composition.

Table 3. Amino acid composition (as residues %) of fractions obtained from DEAE-cellulose chromatography of zinc-precipitable kookaburra beak protein

The composition of the starting material is included for comparison

Amino acid	Zinc-precip. protein	Fractions			
		2	3	4b[A]	5
Lys	0·2	Trace	Trace	Trace	Trace
His	1·1	0·9	1·1	1·1	1·0
Arg	3·3	3·0	3·3	2·8	2·7
Cys(Cm)	4·6	3·8	4·4	4·1	3·5
Asp	3·7	3·4	3·6	3·2	3·5
Thr	3·6	3·6	3·7	3·5	3·6
Ser	6·3	5·9	6·0	5·7	6·1
Glu	3·2	3·2	3·3	2·9	3·2
Pro	9·6	9·3	9·4	9·2	9·3
Gly	33·9	36·6	33·9	37·8	36·8
Ala	6·1	6·1	6·4	5·9	6·3
Val	4·0	4·6	4·9	4·4	4·5
Met	0·3	0·4	0·4	0·3	0·4
Ile	1·5	1·5	1·5	1·5	1·5
Leu	5·4	5·3	5·5	4·9	5·1
Tyr	9·4	8·3	8·7	9·1	9·0
Phe	3·7	4·1	4·1	3·7	3·6

[A] This protein was obtained after rechromatography of fraction 4 on DEAE-cellulose (see Figs 5*a* and *b*).

Discussion

The data presented in this paper indicate that the hard keratin of birds' beaks is almost entirely composed of a relatively small number of proteins of about 14 000 molecular weight. These proteins characteristically have about 30% of their amino acid residues as glycine and are also moderately rich in tyrosine. They contain relatively low levels of *S*-carboxymethyl cysteine when compared with mammalian keratin proteins. The full extent of heterogeneity of these beak proteins has not been investigated but it is clearly limited in comparison with mammalian keratins—comparable to the situation found in feather (Harrap and Woods 1967)—and as with feather, no evidence has been found for the presence of different major protein classes equivalent to those of the mammalian keratins.

The major beak proteins from birds of six different orders showed overall similarities in composition and molecular weights, but in both of these parameters they differed quite distinctly from feather protein. The beak proteins appear to be closely related in these properties to the epidermal scale (isolated from fowl) and show some resemblance to reptile claw protein.

In addition to the major proteins, most beaks contain minor protein components (the zinc-soluble fraction) which are similar to feather protein in amino acid composition and size. In spite of this apparent similarity, feather proteins are zinc-precipitable, suggesting that there may be differences in structure between them. It should not be overlooked, however, that during the fractionation of the beak proteins the feather-like components are present to less than 0·2 mg/ml and the apparent solubility of their zinc complex may be due to this fact. Whatever the case, it would be of interest to know whether the rather poor feather-type X-ray diffraction pattern obtained with beak (R. D. B. Fraser, personal communication) actually originates in this minor beak fraction. The presence of a feather-like protein in beak leads to the question of whether beak-like proteins occur in feather. It is possible that emu feather calamus at least does contain a beak-like component. The amino acid composition of emu calamus is unusual in that it has higher levels of glycine and tyrosine and lower levels of half cystine and valine than comparable parts of other feathers (Harrap and Woods 1967). Furthermore, an examination of the composition of chromatographically prepared fractions of solubilized emu calamus (O'Donnell 1973*a*) suggests the presence in calamus of a minor component relatively rich in glycine and tyrosine and poor in *S*-carboxymethyl cysteine and valine, which may be similar in composition to beak protein. Unfortunately, no molecular weight data are available on which further identification of this protein can be made. However, it is possible to speculate that the separate tissues concerned with the synthesis of beak and feather are capable of synthesizing both feather and beak type proteins. Since these proteins would be expected to confer quite different mechanical and physical properties on the keratin, the exact proportions synthesized would depend on the structure–function relations required of the particular keratin. An analogous situation exists in mammals where the different forms of hard keratin produced by a single animal have been found to contain the same three groups of structural proteins in widely different proportions (Gillespie 1968).

From the details of amino acid composition, isoelectric point and molecular weight of the major proteins from a variety of beaks, and the composition of chromatographic fractions obtained from a single beak, it is apparent that these proteins form a very closely related group. When compared to the three main classes of mammalian keratin protein, they most resemble the high-tyrosine proteins (Gillespie 1974; Gillespie and Frenkel 1974*b*). The levels of most amino acids in the major beak protein lie within the ranges found for a wide variety of mammalian high-tyrosine proteins (Gillespie 1972) although the beak protein generally contains less tyrosine, phenylalanine and *S*-carboxymethyl cysteine and more alanine and valine than the mammalian proteins. These two protein types also differ in their molecular weight ranges, beak proteins (13 000–14 500) being significantly larger than the majority of mammalian high-tyrosine proteins (6 000–9 000) (Gillespie and Frenkel 1976). Despite these differences, it is still tempting to look upon mammalian high-tyrosine proteins as vestigial remnants of the glycine-rich keratins of birds and reptiles. However, any relationship between them on present knowledge appears to be restricted to certain features in their unusual composition and the observation that the highest proportions of high-tyrosine proteins in mammalian keratins are associated with tough, pointed structures such as claws and quills (Gillespie and Frenkel 1974*a*) which superficially at least might be expected to require similar mechanical properties

to avian beaks and claws. It is hoped that future amino acid sequence studies with purified beak proteins will provide further information on which relations between these two groups of proteins can be further assessed.

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