Soluble Proteins from Fowl Feather Keratin

I. Fractionation and Properties

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A method is described for the fractionation of reduced and alkylated proteins of fowl feather. Fowl feather extracts were chromatographed on a Sephadex G-75 column in 4 M urea containing 1 M NaCl and separated into four fractions, GF-1, 2, 3, and 4. The elution patterns were used to compare the components of different feather parts, barbs, rachis+medulla, and calamus. In all cases, GF-3 was the main fraction and the percentages with respect to the total peak area found for barbs, rachis+medulla, and calamus were about 65%, 74%, and 93%, respectively. Each of the fractions was examined by polyacrylamide disc gel electrophoresis and all were heterogeneous. The slowly moving bands mainly corresponded to fraction GF-1, intermediate bands to GF-2 and 3, and faster bands to GF-4. Many other polypeptide chains, which have not been found previously, were newly separated from three minor fractions of fowl body feather. The molecular weights of fractions GF-2 and 3 were estimated by calibrated gel filtration to be 33,000 and 10,500, respectively. Marked differences were found in the amino acid compositions of various fractions from fowl feather. The GF-1 fraction and insoluble residue had very similar compositions; in both cases the contents of serine, glycine, and proline were lower and those of helix-favoring amino acids, namely, lysine, tyrosine, and methionine, were higher than those found in other fractions.

The proteins found in feather are insoluble in normal protein solvents. This property is largely due to cross-linking by cystine residues and hydrogen bonds. It is possible to dissolve feather proteins by treating them with reagents which can break these cross-links without peptide bond scission \((1-3)\). Soluble proteins from feathers have usually been prepared by reduction and alkylation of the disulfide bonds \((4)\).

Although soluble derivatives of feather keratins from various birds have been shown to be heterogeneous by electrophoresis and by column chromatography, they have been reported to be relatively homogeneous with respect to molecular weight \((5-7)\). It is known from these studies that the feather extracts are a mixture of several kinds of polypeptide chain. To determine the primary structures of feather keratins, each of these chains must be isolated in a pure state and the amino acid sequence in each chain determined. Purification of soluble keratin derivatives is a complex problem.

In recent years, O'Donnell \((8)\) has found that the proteins extracted from the calamus or rachis of emu feather give the simplest electrophoretic pattern. The major bands in acrylamide gel
electrophoresis of the extract were separated in an almost pure state by chromatography on DEAE-cellulose and the amino acid sequence of one of the bands was completely determined (9). O'Donnell and Inglis (10) also determined the amino acid sequence of a major component of the silver gull feather calamus and compared it with that of emu feather.

In the present work, soluble S-carboxymethyl (SCM) proteins were prepared from fowl body feather. Gel filtration was used for the isolation and examination of individual protein species in various SCM-extracts, and optimal conditions were examined for the fractionation of extracted feather proteins. Significant differences were found in elution patterns among morphological feather parts, namely, barbs, rachis-medulla, and calamus. Differences in electrophoretic patterns, amino acid compositions, and molecular weights of fractionated SCM-proteins were also reported.

MATERIALS AND METHODS

Preparation of Feather Samples—White body feathers from meat-type fowl (White Cornish × White Plymouth Rock), 70 days old, were used. The feathers were scoured with a dilute non-ionic detergent solution, rinsed in tap water and then in distilled water, air-dried, and degreased repeatedly by soaking in fresh dry acetone and then air-drying again. The dry feathers were separated into morphologically distinct parts according to the method described by Schroeder et al. (11). In the case of body feathers, the rachis and medulla were not separated. The proportions of the three parts expressed as percentages of the total weight were barbs 72%, rachis-medulla 24%, and calamus 4%.

Extraction of Fowl Feather Proteins—To 1 g of dry feathers, 25 ml of 0.05 M Na₂B₄O₇-0.1 M KH₂PO₄ buffer solution (pH 8.6) containing 8 M urea, 0.4 ml of 5 % EDTA (disodium salt), and 0.67 ml of 2-mercaptoethanol purified by distillation were added, and this mixture was shaken for 4 h at 40°. In order to prevent reoxidation of thiol groups produced by reduction, the reduced proteins were treated with either monooiodoacetic acid or ethyleneimine.

Carboxymethylation: To the reduced mixture, 1.8 g of monooiodoacetic acid was added and the pH was adjusted to 8.6 with 6 M NaOH.

Aminoethylation: Three 1-ml portions of ethyleneimine were added to the mixture at 10-min intervals (12). The above two mixtures were allowed to stand for 30 min, then insoluble materials were collected by filtration, washed with several changes of 8 M urea and finally with distilled water, and air-dried. The amount of proteins dissolved was expressed as a percentage of the total weight of dried feathers used. In all cases, more than 85 % of the feather proteins dissolved. In order to remove various salts from the protein solution, 25 ml of the filtrate was loaded on a Sephadex G-75 column (3 × 30 cm) equilibrated with 4 M urea. The column was eluted with the same solution. The protein concentration in the effluent was monitored by measurement of the absorption at 280 nm. When necessary, the protein solution was concentrated by ultrafiltration (Bio Engineering, Tokyo).

Gel Filtration—Sephadex G-75 and G-150, which had a particle range of 40–120 μm (supplied by Pharmacia Fine Chemicals AB) were used. Dry powder was suspended in 4 M urea and allowed to swell for 24–48 h at room temperature, with occasional shaking. The supernatant was decanted to remove the small grain-size fraction. The gel suspension was degassed at 40–50° in a vacuum flask and poured into a 2.5 × 100 cm column in the conventional manner. After the column had been equilibrated with 500 ml, corresponding to the bed volume of the column, of eluant, 5 ml of 1–2% protein solution was injected into the bottom of the gel column and ascending chromatography was carried out. The flow rate of the column was maintained at 20 ml/h with a peristaltic pump and the effluent was collected in 5-g portions with a balance-operated fraction collector (Toyo Seisakusho, Tokyo). The absorbance of the effluent was measured at 280 nm and the void volume of the columns was determined by applying Blue Dextran 2,000. The peak area of the elution curve was defined as the total value of the absorbance of each of the fractions corresponding to the peak and was expressed as a percentage to the total area for all peaks.

Polyacrylamide Disc Gel Electrophoresis—Polyacrylamide disc gel electrophoresis was carried out essentially as described by Ornstein (13) and Davis (14) except that gels containing 8 M urea were used. Running gels containing 7.5%
acrylamide were 5.0 cm long and the spacer gels containing 2.5% acrylamide were 0.5 cm long. The running pH was 8.3. About 50–200 μg of the SCM-protein samples was applied. Electrophoresis was initially carried out at a constant current of 2 mA/tube. As soon as the tracking dye reached the top of the running gel, the current was increased to 4 mA/tube. After electrophoresis, the gels were removed from the tubes, treated with 10% trichloroacetic acid for 1 h, stained overnight with 0.025% Coomassie Brilliant Blue R-250 in water–methanol–acetic acid (8:1:1, v/v), and finally destained with the same solution. These gels were photographed and scanned at 600 nm, using a recording spectrodensitometer, model FD-AIV (Fujiriken, Tokyo).

Estimation of Molecular Weight—Gel filtration was used to estimate the molecular weights of SCM-proteins. A column (1.7 × 80 cm) of Sephadex G-75 was similarly calibrated with SCM-proteins of known molecular weight. The following SCM-proteins, which are known to be single polypeptide chains, were used: cytochrome c (horse) 12,400, α-lactoalbumin 15,500, myoglobin (horse skeletal muscle) 17,200, chymotrypsinogen-A (bovine) 25,700, ovalbumin 43,000, bovine serum albumin 67,000. These marker proteins were purchased from Miles-Seravac, England. The protein solution was applied to a gel column equilibrated with 4 M urea containing 1 M NaCl. The column was eluted with the same solution at a flow rate of 20 ml/h, collecting 2-g samples. The absorbance of the effluent was measured at 280 nm. Test tubes were weighed before and after collection of eluted solvent to determine the elution weight accurately, as described by Fish et al. (15). Based on the weight determinations, the elution positions were expressed in terms of relative elution volume, $V_e/V_o$, defined as the ratio of elution volume, $V_e$, to void volume, $V_o$. The relative elution volumes were measured for all the standard SCM-proteins in duplicate. When the relative elution volume was plotted against the logarithm of molecular weights for a series of standard proteins, a linear plot was obtained and the molecular weights of SCM-feather proteins were estimated from this calibration curve.

Amino Acid Analysis—Samples for amino acid analysis were dialyzed against tap water and distilled water to remove urea and other salts, then freeze-dried. The samples (10 mg) were hydrolyzed with 1 ml of constant-boiling HCl in evacuated, sealed tubes for 24 h at 110°. After hydrolysis, HCl was removed by vacuum evaporation at low temperature and the hydrolyzate was analyzed with a JEOL-JLC 5AH amino acid analyzer.

RESULTS

Gel Filtration—Gel filtration of the soluble SCM-proteins from whole fowl feathers was carried out on a Sephadex G-75 column as a preliminary experiment to select the optimum experimental conditions. Figure 1 shows an elution profile obtained when the whole feather extract was eluted from the Sephadex G-75 column with 4 M urea. This chromatogram gave two peaks, one of which appeared near the void volume as a symmetric peak and the other as a highly asymmetric peak, which extended over about 40 tubes (tube numbers 60 to 100). This asymmetric peak seems to be due to the presence of various components. On elution from the Sephadex G-75 column with 4 M urea containing 1 M NaCl, SCM-feather extracts were separated into four fractions, as shown in Fig. 2 (solid line). These were termed GF-1, 2, 3, and 4 in order of elution from the gel filtration (GF) column.

Each of three fractions, $f_1$, $f_2$, and $f_3$, taken from Fig. 1 was rechromatographed on the same column in 4 M urea containing 1 M NaCl. The
Fig. 2. Gel filtration of SCM-proteins from whole fowl feathers and of three fractions from Fig. 1 on Sephadex G-75, eluting with 4 M urea-1 M NaCl. The experiments were carried out in the presence of 1 M NaCl and other conditions were as in Fig. 1. –, SCM-proteins from whole feathers; -----, f₁ from Fig. 1; ----, f₂ from Fig. 1; ——, f₃ from Fig. 1.

Results are shown as a dotted line (f₁), a dashed line (f₂), and a dashed and dotted line (f₃) in Fig. 2. Fractions f₁ and f₂ each gave a single peak corresponding to fractions GF-1 and 3, respectively; while f₃ gave two peaks, one of which corresponded to GF-3 and the other to GF-4. These data show that the presence of NaCl permits better separation of the components in the SCM-extracts. Various urea solutions containing different concentrations of NaCl were also used as eluants. Elution with 8 M urea containing 1 M NaCl did not give good resolution between fractions GF-3 and 4, and elution with 4 M urea containing 0.1 M to 0.5 M NaCl tended to increase the skew of peaks. Consequently, 4 M urea containing 1 M NaCl was found to be the most suitable eluant. Figure 3 shows a chromatogram obtained when whole feather extract was eluted from a Sephadex G-150 column with the same eluant as in Fig. 2. Two small peaks corresponding to fractions GF-2 and 4 in Fig. 2 were indistinct. From these results, Sephadex G-75 was found to be more effective than G-150 for separation of the soluble SCM-proteins from fowl body feathers. In a separate experiment, chromatography of S-β-aminoethylated (SAE) proteins prepared from whole feathers was performed under the same conditions as in Fig. 2. The chromatogram obtained was identical with that for the SCM-form (not shown). This indicates that there are no differences between SCM- and SAE-forms of the soluble derivatives of feather keratins on gel filtration.

Table I. Proportions of various fractions obtained from different feather parts. Results expressed as percentages with respect to total peak area.

<table>
<thead>
<tr>
<th>Feather part</th>
<th>GF-1</th>
<th>GF-2</th>
<th>GF-3</th>
<th>GF-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole feather</td>
<td>9.5</td>
<td>11.2</td>
<td>68.8</td>
<td>10.5</td>
</tr>
<tr>
<td>Rachis-medulla</td>
<td>3.6</td>
<td>10.9</td>
<td>74.1</td>
<td>11.4</td>
</tr>
<tr>
<td>Barbs</td>
<td>9.3</td>
<td>13.6</td>
<td>65.2</td>
<td>11.9</td>
</tr>
<tr>
<td>Calamus</td>
<td>2.4</td>
<td>5.0</td>
<td>92.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Polyacrylamide Disc Gel Electrophoresis—Figure 4 shows a densitometric tracing of the SCM-proteins from whole fowl feathers, Fig. 5 shows those of the three parts of fowl feathers, and...
Fig. 4. Densitometric tracing of the disc gel electrophoresis pattern of SCM-proteins prepared from whole fowl feathers. The direction of migration is indicated by the horizontal arrow and migration positions of the proteins by vertical arrows. Concentration in arbitrary units. For details, see "MATERIALS AND METHODS." ○, origin; +, anode.

Fig. 5. Densitometric tracings of the disc gel electrophoretic patterns of SCM-proteins from the three parts of fowl feathers. Details as in Fig. 4.

Fig. 6. Densitometric tracings of the disc gel electrophoretic patterns of GF-1, 2, 3, and 4. Details as in Fig. 4.

Fig. 6 shows those of the four fractions (GF-1 to 4) taken from Fig. 2. Electrophoretic bands were divided according to their migration distance into three groups, the slow-moving group (Group S), intermediate group (Group I), and fast-moving group (Group F). The protein bands separated by electrophoresis were termed 1, 2, 3, and so on in increasing order of mobility, as shown in Figs. 4, 5, and 6. Six protein bands, that is, peak 6 of calamus (Fig. 5a), peaks 2 and 3 of rachis-medulla (Fig. 5b), peak 5 of barbs (Fig. 5c), and peak 4 of GF-3 (Fig. 6c), appeared as asymmetric peaks. Each of these peaks seemed to contain at least two components, a and b. The second (I) group contained the major proteins of the three morphological parts, although the distribution of these varied greatly from tissue to tissue. It was found that the first (S) group contained the minor proteins of calamus and the third (F) group those of barbs; electrophoretic analysis of rachis-medulla gave the simplest pattern, being free from both groups. These electrophoretic results were essentially identical with those of Kemp and Rogers.
In the case of GF-1 to 4, the electrophoretic mobility of the protein bands tended to increase in the order GF-1 < GF-2 < GF-3 < GF-4 (Fig. 6). The densitometric tracing of GF-1 showed 4 peaks, three of which corresponded to the slow-moving bands shown in Fig. 4. The slowest moving band was a trace component which has not previously been observed (Fig. 6a). The main fraction, GF-3, showed 9 peaks, four of which, designated as peaks 2 to 5, were predominant (Fig. 6c). Eight of these, peaks 2 to 9, were identical with peaks 4 to 11 of the whole feathers shown in Fig. 4.

Finally, GF-4 showed 8 peaks, four of which, peaks 1 to 4, were classified as Group I and the rest as Group F (Fig. 6d). As shown in Figs. 2c and 2d, overlap of two electrophoretic patterns was observed over the range of migration distances 2.0–3.3 cm, but the 8 peaks of GF-4 were minor components which had different mobilities from those of GF-3. Generally, judging from

![Graph](image)

**Fig. 7.** Calibration curve of relative elution volume \((V_e/V_0)\) against logarithm of molecular weight for a series of standard proteins chromatographed on Sephadex G-75 (1.7 x 80 cm) in 4 M urea containing 1 M NaCl. Protein concentrations were 0.3–1%. \(V_e/V_0\) values for the separated peaks GF-2 and 3 are indicated by arrows.

**TABLE II.** Amino acid analyses of fowl body feather keratins and their fractions. Results uncorrected for destruction during hydrolysis; tryptophan not determined.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Whole feather</th>
<th>Insoluble residue</th>
<th>Whole extract</th>
<th>Fraction GF-1</th>
<th>Fraction GF-2</th>
<th>Fraction GF-3</th>
<th>Fraction GF-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>1.2</td>
<td>5.3</td>
<td>0.4</td>
<td>3.3</td>
<td>0.8</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.3</td>
<td>1.4</td>
<td>trace</td>
<td>1.0</td>
<td>trace</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.7</td>
<td>4.2</td>
<td>4.8</td>
<td>4.5</td>
<td>3.6</td>
<td>4.3</td>
<td>4.1</td>
</tr>
<tr>
<td>SCM-Cysteine</td>
<td>0</td>
<td>8.7</td>
<td>8.3</td>
<td>6.8</td>
<td>5.9</td>
<td>8.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>6.3</td>
<td>8.5</td>
<td>5.0</td>
<td>7.1</td>
<td>9.2</td>
<td>4.7</td>
<td>6.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.3</td>
<td>6.2</td>
<td>4.5</td>
<td>5.2</td>
<td>4.9</td>
<td>6.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Serine</td>
<td>15.7</td>
<td>8.1</td>
<td>14.6</td>
<td>9.8</td>
<td>13.4</td>
<td>16.4</td>
<td>16.4</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>8.6</td>
<td>11.3</td>
<td>7.7</td>
<td>11.3</td>
<td>8.2</td>
<td>7.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Proline</td>
<td>11.7</td>
<td>7.5</td>
<td>11.8</td>
<td>8.7</td>
<td>12.8</td>
<td>11.4</td>
<td>11.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>11.5</td>
<td>8.5</td>
<td>11.9</td>
<td>9.8</td>
<td>10.3</td>
<td>11.3</td>
<td>11.2</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.6</td>
<td>5.8</td>
<td>5.3</td>
<td>6.3</td>
<td>5.8</td>
<td>5.2</td>
<td>4.7</td>
</tr>
<tr>
<td>Cystine</td>
<td>4.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Valine</td>
<td>7.7</td>
<td>7.3</td>
<td>9.1</td>
<td>7.4</td>
<td>9.7</td>
<td>9.3</td>
<td>9.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.3</td>
<td>1.2</td>
<td>trace</td>
<td>0.7</td>
<td>trace</td>
<td>0</td>
<td>trace</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.3</td>
<td>4.0</td>
<td>4.9</td>
<td>4.4</td>
<td>4.7</td>
<td>5.0</td>
<td>4.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.4</td>
<td>6.8</td>
<td>7.0</td>
<td>8.3</td>
<td>6.7</td>
<td>7.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.6</td>
<td>2.8</td>
<td>1.2</td>
<td>2.5</td>
<td>1.0</td>
<td>1.0</td>
<td>2.5</td>
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<tr>
<td>Phenylalanine</td>
<td>3.6</td>
<td>2.4</td>
<td>3.5</td>
<td>2.9</td>
<td>3.0</td>
<td>3.4</td>
<td>3.5</td>
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</table>

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these four electrophoretic patterns, the whole extract of fowl body feathers contained at least 4 major and 17 minor components.

**Estimation of Molecular Weight**—Figure 7 shows a calibration curve of relative elution volume against log molecular weight for a series of standard proteins. From this graph, the molecular weights of fractions GF-2 and 3 were estimated to be 33,000 and 10,500, respectively. Those of fractions GF-1 and 4 were estimated to be above 70,000 and below 10,000, respectively, as they fell outside the linear section of the calibration plot.

**Amino Acid Analyses**—Table II shows the results of amino acid analyses of various samples prepared from fowl body feathers. The composition of whole feathers was essentially identical with that given by Ward et al. (17). Differences in composition were found between the soluble fraction and insoluble residue. These results show that during the extraction procedure serine-, proline-, and glycine-rich components were concentrated in the soluble fraction and aspartic and glutamic acid-, lysine-, histidine-, tyrosine-, and methionine-rich ones remained in the insoluble materials. Marked differences were also found in the contents of many amino acid residues among the soluble fractions. The content of serine was very high in GF-3 and 4, and low in GF-1, and that of proline was high in GF-2 and low in GF-1. The relative contents of glutamic acid, lysine, tyrosine, and histidine in GF-1 were higher than those in other fractions. The distribution of aspartic acid was also uneven; the content in GF-2 was 9.2 per 100 residues while that in GF-3 was 4.7 per 100 residues. GF-1 and the insoluble residue had a similar composition, with low contents of proline, serine, and glycine, and high contents of the helix-favoring amino acids, namely, lysine, tyrosine, and methionine.

**DISCUSSION**

The soluble keratin derivatives from whole fowl feathers were fractionated into four components, GF-1, 2, 3, and 4, by elution with 4 M urea containing 1 M NaCl from a Sephadex G-75 column. Of these four fractions, GF-3 comprised approximately 69% of the total, while the remaining 31% consisted of three minor fractions. SCM-proteins from different parts of fowl feather were also chromatographed under the same experimental conditions.

The protein components extracted from calamus were simpler than those from barbs or rachis-medulla. SCM-proteins from calamus were predominant in GF-3 but absent in GF-4 (Table I). The ratio of the main fraction GF-3 to the three minor fractions (GF-1, 2, and 4) was 2 : 1 and 3 : 1 in the barbs and rachis-medulla extracts, respectively, but that for the calamus extract was roughly 13 : 1.

The molecular weights of major proteins prepared from various feathers have been reported to be in the range of 10,000 to 11,000 by several workers (3, 5, 16). O'Donnell (9) studied an electrophoretically homogeneous component (Band 3) isolated from the calamus or rachis of emu feathers and obtained a molecular weight of 10,459 in the SCM-form. The main fraction, GF-3, had a molecular weight coinciding with the values in the literature (3, 5, 16), but fractions GF-1 and 4 were found to give different values. Harrap and Woods (5) and Jeffrey (16) have estimated the molecular weights of aggregates to be 40,000 and 37,500, respectively. The molecular weight of GF-2 was found to correspond to that of these aggregates.

Harrap and Woods (5) found that 90% of the rachis extract of fowl feathers was in a monomeric form and estimated the molecular weight to be 10,400. In the present experiment, GF-3 and 4 are assumed to be present in a monomeric form on the basis of their molecular weights. Consequently, 93% of the calamus extract, 86% of the rachis-medulla extract, 77% of the barbs extract appeared to be present in monomeric form.

O'Donnell (8) found that the calamus of emu feathers was richer in uniform chains than the rachis, medulla, or barbs. This agrees with the chromatographic results obtained in the present experiments on fowl feathers. The migration distances of the protein bands in the electrophoretic patterns of GF-1, 2, 3, and 4 showed a tendency to increase in this order (Fig. 6). This indicates that their electrophoretic mobilities depend greatly on the difference in molecular size. The slow-moving bands, which are concentrated in fraction GF-1, had a molecular weight above 70,000 and the intermediate band of GF-2, one of 33,000. These high-molecular proteins may be present in a
polymeric form.

In a separate experiment to investigate the possibility that these proteins are aggregated, sodium dodecyl sulfate (SDS) was used as a dispersion agent. SDS-electrophoretic analysis of GF-1 gave two broad bands, one of which disintegrated into smaller subunits and the other of which could not penetrate 10% polyacrylamide gel. In the case of GF-2, a zonal band appeared at a migration distance identical with that of GF-4 (not shown).

The amino acid composition of GF-2 was similar to those of GF-3 and 4. There was a significant difference in the compositions of GF-1 and 3. Fractions GF-1 and 4 had similar compositions in the following respects; in both cases the contents of glutamic acid, lysine, tyrosine, and histidine were higher than those in GF-3.

These results suggest that GF-2 contains various aggregates, but that some components of GF-1 are high-molecular proteins, different from GF-3 or 4. If GF-1 and 2 contain aggregates, their monomer units may resemble the polypeptide chain of GF-4 rather than that of GF-3. Kemp and Rogers have pointed out that each of the main bands contains at least two components (18). In the present experiments some of the strong bands were assumed to be heterogeneous, based on the asymmetry of their peaks. All of the strong bands may consist of at least two components, but there is no evidence for a multi-component system.

When the extract from whole feathers was subjected to polyacrylamide gel electrophoresis, bands corresponding to peak 1 of GF-1 and fractions GF-2 and 4 were not observed. This is presumably due to the low proportions of these components in the whole extract. The presence of a number of minor components renders the soluble feather proteins more difficult to isolate in a pure state. The major proteins of barbs have been fractionated by a combination of Sephadex G-75 and DEAE-cellulose chromatographies, and some of them have been found to be homogeneous electrophoretically. A method for isolating the homogeneous proteins will be described in a subsequent paper.

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REFERENCES