Carp Myogens of White and Red Muscles

PROPERTIES AND AMINO ACID COMPOSITION OF THE MAIN LOW-MOLECULAR-WEIGHT COMPONENTS OF WHITE MUSCLE

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1. The three main components of the 1.5–2 s ultracentrifugal peak of carp myogen (white muscle) have been isolated by ammonium sulphate fractionation and zone electrophoresis, and crystallized. 2. The molecular weights of these three proteins were determined by sedimentation and diffusion, by the Archibald method and by amino acid analysis, and found to lie between 9000 and 13000. 3. Their complete amino acid compositions were determined by column chromatography and by their ultraviolet spectra. Both methods revealed abnormal compositions, including the absence of tryptophan and methionine and the presence of large amounts of phenylalanine. At most 1 residue each of tyrosine, cysteine, proline, arginine and histidine was found/molecule. 4. The specific viscosity of component 3 was lower than that of other small globular proteins described so far, a fact that suggests that these proteins approximate more closely to the ideal case of the spherical protein molecule. Also, the presence of a single residue of several amino acids, the absence of disulphide bonds, and the apparent reversibility of denaturation by urea of component 3 suggest that the study of these molecules could provide new information on the structure of proteins.

The myogen from carp white muscle contains a group of proteins of high salting-out range and sedimenting at about 1.5 s; this group separates in the electrophoretic U-tube into three main components, 2, 3 and 5, which each correspond to roughly 10% of the myogen (Hamoir & Konosu, 1965). A fairly large amount of this slow-sedimenting fraction, though smaller than that in the carp, has also been found in frog (Deuticke, 1934), in plaice (Jebsen & Hamoir, 1958) and in cod myogens (Connell, 1958); it is absent from rabbit muscle (Deuticke, 1934) and present only in small amount, if any, in carp red muscle (cf. Hamoir & Konosu, 1965; Pechère & Focant, 1965). This group of proteins therefore appears to be specific for the white muscle of fishes and amphibians. Component 3 was isolated in crystalline state from carp muscle by Henrotte (1955) and called myogen II. It exhibited an abnormal ultraviolet spectrum with the three main absorption maxima of phenylalanine at 253, 259 and 265 m μ and a low absorption at 275 m μ . Subsequent improvement of the preparation (Hamoir, 1957) confirmed the absence of tyrosine and tryptophan and indicated a phenyl-

alanine content of approx. 15%. A similar protein also occurs in plaice myogen (Jebsen & Hamoir, 1958). The apparently restricted zoological distribution of this group of proteins, the absence of its main components in red muscle, their low molecular weights and their abnormal ultraviolet spectra induced us to examine the three main electrophoretic components of carp white muscle. A preliminary account of some of our results has already been given (Hamoir, 1961, 1962; Pechère, Hamoir, Konosu & Focant, 1964).

METHODS

The description of the starting material, the isolation of the myogen of white muscle, its fractionation with ammonium sulphate to isolate the slow-sedimenting fraction, the determination of the protein content of the solutions and their analysis by moving-boundary electrophoresis and ultracentrifugation carried out, unless specified, in the usual phosphate-NaCl buffer, pH 7.3 and *I* 0.075 (15.4 mM-Na₂HPO₄–3.9 mM-NaH₂PO₄–25 mM-NaCl), were as given by Hamoir & Konosu (1965).

Preparative zone electrophoresis on starch columns. In our experimental device, the electrophoretic cell in a moving-boundary electrophoresis apparatus was replaced by a U-tube having the form shown in Fig. 1. The left-hand part of the tube bore a sintered-glass plate covered with a

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Fig. 1. U-tube for zone electrophoresis connected to the rubber tubing (A) for the introduction of the sample into the starch column.

usual phosphate-NaCl buffer, pH 7.3 and 1.0-0.75, and in 0.1 N-NaOH were measured against the solvent in a 1 cm. quartz cell with the Beckman model DU universal spectrophotometer.

Specific refractive increment of component 3. Salt-free solutions of a concentration of about 3% (w/v) were prepared by dialysis against three changes of 2 l. of water for at least 72 hr. in a rocking system. The increment of refractive index was measured against the last dialysis fluid at $25 \pm 0.02^\circ$ and $589 m\mu$ (sodium lamp). The dry weight, expressed in g./100 ml. of solution, was determined after drying at 110° in air according to the procedure of Perlmann & Longworth (1948). A constant weight was obtained in 24 hr.

Viscosity of component 3. Measurements were made at $25 \pm 0.02^\circ$ in an Ostwald viscometer of 3 ml. capacity with a bulb of 0.414 ml., a capillary radius, r , of 0.01014 cm. and a capillary length of 10.2 cm. The average rate of shear, $\dot{\gamma}$, calculated from the formula $\dot{\gamma} = 8v/3\pi r^2 t$ (Kroepelin, 1929), where v is the volume of the bulb and t the flow time, varied from 410 to 430 sec^{-1} . The kinetic energy correction $\rho v/8\pi \eta t$ at the highest rate of shear employed was less than 0.03% and was neglected. The density correction was calculated by assuming additivity of the weights and volumes of the protein and the solvent; the density of component 3 was taken as 1.33, and the density increments given in Svedberg & Pedersen's (1940) Appendix III were used for the solvents.

Analytical starch-gel electrophoresis. The hydrolysed starch prepared by Connaught Medical Research Laboratories (Toronto, Canada) was used. The experiments were conducted at pH 5.0 and 1.0-0.5 (sodium acetate buffer) or pH 8.2 and 1.0-0.5 (sodium pyrophosphate-HCl buffer) with fourfold more concentrated solutions (1.0-2) as bridge buffer. After electrophoresis in an electric field of about 2 v/cm. of gel for 24 hr., the gel was stained for 10 min. with Amido Black 10B dissolved in methanol-water-acetic acid (5:5:1, by vol.) (Smithies, 1955).

Diffusion coefficients and the Archibald method. The rate of diffusion was measured on patterns obtained by centrifuging pure preparations in a synthetic-boundary cell at 59780 rev./min. at room temperature in the Spinco model E ultracentrifuge. A total enlargement of 6.75 was used. The area, A , of the peak was corrected for the radial dilution; the first picture, which was taken about 5 min. after maximum speed was reached, was assumed to correspond to the situation at zero time, as it was found that under our experimental conditions the correction to the true zero time was negligible. The diffusion coefficient was determined from a linear plot of $A^2/H^2 F^2$, where H is the maximum height and F the enlargement, against time, according to the formula $D = A^2/4tH^2 F^2$. The result was corrected to water and 20° in the usual way (Li, 1951). The molecular weights calculated according to Svedberg's equation from such values represent only maximal values, and may be largely in error in view of the self-sharpening effect that occurs for even a small variation of S with the concentration (Fujita, 1956; Baldwin, 1957). More accurate values were obtained with the Archibald method.

In this last procedure use was made of the relationship:

$$M = \frac{RT}{(1 - \bar{v}\rho) \omega^2} \cdot \frac{(dc/dx) x_0}{x_0 c_{x_0}}$$

where M is the molecular weight of the solute, R the gas

filter-paper disk before addition of the supporting medium. About 35 g. of potato starch (British Drug Houses Ltd., Poole, Dorset) was suspended in 150 ml. of sodium acetate buffer, pH 4.6 and 1.0-0.5, and the suspension was evacuated with a water pump. After the slurry had been poured, the starch column (2 cm. diam. $\times$ 17 cm.) was covered with a filter-paper disk and connected to the right-hand part of the U-tube and to the side vessels used for the moving-boundary experiments with the rubber stoppers described by Dubuisson, Distèche & Debot (1950). The apparatus was placed in the water bath at 1° used for free electrophoresis. By gentle suction through a rubber tubing connected to the top of the right-hand part of the U-tube, the sample was introduced into the column to a depth of 1-2 cm. A load of 300-400 mg. of the protein mixture, dissolved in 1.5-2 ml. of the acetate buffer, pH 4.6 and 1.0-0.5, just before the electrophoresis, was usually applied to the column. The amount of saturated CuSO_4 used for the positive copper electrode was 15 ml. and for the negative one 30 ml., and the ionic strength of the buffer in the positive side vessel was brought to 1.0-0.75 by the addition of solid NaCl to the acetate buffer. A current intensity of 11 or 26 ma for 16 hr. isolated component 2 or 5 respectively. The electrophoretic buffer was used for elution and examination of the fractions (0.5 ml.) at $260 m\mu$ in a quartz cell of 1 mm. light-path, and this showed that about 90% of the components was recovered in a pure state.

Ultraviolet absorption. The spectra of the pure protein samples dissolved at a concentration of 0.1% (w/v) in the

constant, T the absolute temperature, $\bar{v}$ the partial specific volume of the solute, ρ the density of the solution, $(dc/dx)x_0$ the concentration gradient at either end of the column of solution, c_x the corresponding concentrations of solute and x_0 the distance from the axis of rotation to the meniscus or to the bottom of the column of solution. About 0.1 ml. of purified CCl_4 was introduced into the cell before filling it with the solution to provide a dense transparent medium for the bottom. The ultracentrifuge was operated at room temperature at the speeds of 29 500, 33 450 and 35 600 rev./min. Pictures were taken 1 min. after reaching full speed and every 2 or 4 min. afterwards with a phase-plate angle, θ , of 45° . In the patterns, the line of the meniscus was narrower than the one corresponding to the boundary between the water and organic phases. The results obtained at the meniscus were therefore preferably used for the calculations. A total enlargement of 10 in the direction of sedimentation was used. The corresponding perpendicular enlargement was calculated according to the formula:

$$Y_{\text{enl.}} = \frac{K}{10 \lg \theta} Y_{\text{cell}}$$

(Dieu & Oth, 1954), in which $\lg \theta = 1$ and K is an optical constant of 12.020. The analysis of these patterns was made according to an unpublished method of J. Denoel. Under the conditions used, dc/dx varied linearly from the meniscus or the bottom till near the plateau region, and a linear equation could be fitted to the experimental points on the enlargement; for the lower part of the curve, a second linear equation corresponded satisfactorily to the experimental curve. These linear equations allowed the concentrations at the meniscus and at the bottom to be calculated with the formulae:

$$c_m = c_0 - \frac{1}{x_m^2} \int_{x_m}^x x^2 \frac{dc}{dx} dx \text{ and } c_b = c_0 + \frac{1}{x_b^2} \int_x^{x_b} x^2 \frac{dc}{dx} dx$$

(Klainer & Kegeles, 1955). The partial specific volume was not determined, but use has been made of the value given in Svedberg & Pedersen's (1940) Appendix II, and of the value calculated from the amino acid composition (McMeekin & Marshall, 1952; Schachman, 1957).

Amino acid analysis. (a) Microbiological method. This was carried out according to the procedure of Duchâteau & Florkin (1954). Hydrolysis in boiling 6N-HCl was carried out for 24 hr.

(b) Moore & Stein's method. For component 3, the protein was hydrolysed in boiling 6N-HCl for 22 hr. and the HCl was removed with the Craig evaporator (Craig, Gregory & Hausmann, 1950). The basic amino acids were separated on the 15 cm. column and the others on the 100 cm. column (Moore & Stein, 1951). The compositions of components 2 and 5, as well as that of the slow-moving impurity appearing in the preparations of component 3, were determined with the Spinco model 120B automatic amino acid analyser (Spackman, Stein & Moore, 1958) after various times of hydrolysis in 6N-HCl. Each analysis was carried out in duplicate. For the impurity in component 3, a single hydrolysis time of 36 hr. was adopted. The procedure was essentially as follows: To 0.5 ml. of a solution containing about 3–5 mg. of pure protein/ml., in a heavy-walled Pyrex tube, 0.5 ml. of conc. HCl (A.R.; Mallinckrodt, St Louis, Mo., U.S.A.) was added. The tube was sealed

in vacuo and placed in a bath of polyethylene glycol (Fluka A.-G., Buchs, Switzerland) at $107 \pm 1^\circ$ for 18, 36 or 72 hr. After removal from the bath, the contents of the tube were cooled rapidly and evaporated *in vacuo* over NaOH pellets. The resulting dry hydrolysate was redissolved and made up to exactly 5 ml. with sodium citrate buffer, pH 2.2, and 2 ml. of this last solution was used for each column. When needed, extrapolation to zero or infinite time of hydrolysis was carried out graphically. The final calculation of the results referring to each protein was then made by reference to the amount of initial sample hydrolysed. Tests showed virtually no losses in the hydrolysis step. Another portion of the same initial sample was used for protein content estimation by refractometry, and nitrogen content determination by the Kjeldahl procedure as described by Dubuison (1941), a standard of NH_4Cl being used.

RESULTS

Isolation of component 3 and of its slow-moving impurity

The myogen fraction precipitating between 82 and 100% saturation with ammonium sulphate was first redissolved in a small amount of water and brought to saturation at about 2° by the addition of solid ammonium sulphate containing 0.7% (w/w) of disodium hydrogen phosphate dodecahydrate to keep the pH neutral. The crystals appearing within a few hours were separated by filtration on a sintered-glass filter. Component 3 is precipitated nearly completely under these conditions (Fig. 2), but a small peak amounting to a few per cent

Fig. 2. Electrophoretic pattern of the supernatant obtained after crystallization of component 3 in phosphate-NaCl buffer, pH 7.3 and 10.075, after 307 min. at 5.44 v/cm. at a protein concentration of 1.5% (w/v).

and migrating with the mobility of component 5 is still present after two recrystallizations. In acetate buffer, pH 4.6, the main component still has an electrophoretic mobility of $-1 \times 10^{-5} \text{ cm}^2 \cdot \text{v}^{-1} \cdot \text{sec}^{-1}$, but the impurity is practically isoelectric. The components were separated by zone electrophoresis for 16 hr. at pH 4.6 and 1.05 (current intensity 23 mA). The purity of the fractions was checked by free electrophoresis, at pH 7.3; very occasionally a new small impurity was observed in front of component 3 at this pH that did not separate well in acetate buffer, pH 4.6.

Crystallization. The pure component crystallizes instantaneously under the conditions used for its isolation from the 82–100% saturated ammonium sulphate fraction (Fig. 3a) or by the addition of

solid dipotassium hydrogen phosphate and sodium dihydrogen phosphate in the molar ratio 9:1 until a slight turbidity appears (Fig. 3b).

Isolation of component 5

The supernatant obtained after elimination of component 3 by crystallization (cf. Fig. 2) was dialysed against water, freeze-dried and redissolved in acetate buffer, pH 4.6 and 1.05, immediately before zone electrophoresis. The elution profile at 260 m μ of the fractions isolated by this method usually showed the same components as did free electrophoresis at pH 7.3 or 4.6. A supplementary small peak was, however, sometimes observed after zone electrophoresis, partially separating from and running after the large slow peak corresponding to component 5, amounting to less than 1% of the mixture and too small to be observed in free electrophoresis; its ultraviolet spectrum differed from that of the main slow peak: the maxima of phenylalanine were scarcely visible and a much greater absorption was observed at 280 m μ . Therefore the tailing fractions of peak 5 were discarded. The purity of component 5 was checked by free electrophoresis at pH 7.3 and starch-gel electrophoresis at pH 8.2 and 5.0. A very small amount of impurity migrating at the speed of component 1 was observed at pH 8.2, but only when the amount of protein applied was high. It was considered to be negligible. Of the starting material 40% was isolated by zone electrophoresis as pure component 5.

Crystallization. An electrophoretically pure preparation at pH 7.3 and 1.075 was dialysed at 2° against 2 M-phosphate buffer (dipotassium hydrogen phosphate and sodium dihydrogen phosphate in the molar ratio 9:1); the solution, the concentration

(a)

(b)

Fig. 3. Crystals of component 3 obtained: (a) in the presence of ammonium sulphate (phase-contrast picture obtained by Henrotte, 1955); magnification 310 $\times$; (b) in the presence of phosphate; magnification 160 $\times$.

Fig. 4. Crystals of component 5 in the presence of phosphate; magnification 286 $\times$.

of which was much increased by this step, was clarified by centrifugation, and the same mixture of solid phosphates was added to bring the solution to saturation at about 2°. To obtain crystallization it was sometimes necessary to keep the saturated solution for several months in the cold room (Fig. 4).

Isolation of component 2

Component 2 was prepared from the first (0–82% saturated ammonium sulphate) fraction

Fig. 5. (a) Electrophoretic pattern of the fraction precipitating between 0 and 82% saturation with ammonium sulphate (Hamoir & Konosu, 1965) in phosphate-NaCl buffer, pH 7.3 and I 0.075, after 231 min. at 5.7 v/cm. at a protein concentration of 1.12% (w/v). (b) Elution curve of the same protein mixture after zone electrophoresis at pH 4.6 and I 0.05, showing the separation of component 2 and the appearance of a supplementary slow-moving peak behind component 5.

isolated by refractionation of the precipitate obtained between 77 and 100% saturation with ammonium sulphate (Hamoir & Konosu, 1965). The electrophoretic pattern of this starting material at pH 7.3 is given in Fig. 5(a) and the elution profile resulting from zone electrophoresis at pH 4.6 is shown in Fig. 5(b): components 2, 3 and 5 are clearly visible, whereas peaks 4, 7 and 8 are eliminated, as they move backwards at this pH and leave the column. The presence of the last small peak of Fig. 5(b) partially separated from component 5 has already been noticed in zone electrophoresis for the isolation of component 5. The amount of this component was apparently too small in this case to be observed in free electrophoresis at pH 7.3 or 4.6. The large fast-moving fraction that separates fairly well from the other components in front of the diagram has been examined by free electrophoresis at pH 7.3 and I 0.075 and by starch-gel electrophoresis at pH 8.2 and 5.0. A single peak migrating with the mobility of component 2 was observed, but a very small amount of impurity sometimes separated in front of this peak at the end of the run (Fig. 6). The amount of component 2 isolated corresponded to 8.4% of the protein added to the column.

Crystallization. The fractions containing component 2 dialysed against the usual buffer, pH 7.3 and I 0.075, were brought to saturation with solid ammonium sulphate containing 0.7% of disodium hydrogen phosphate dodecahydrate. The precipitate collected by centrifugation was redissolved in a minimum amount of water at 2°. The resulting solution was brought to saturation again with the

Fig. 6. Electrophoretic pattern in phosphate-NaCl buffer, pH 7.3 and I 0.075, after 295 min. at 5.63 v/cm. of a 0.75% (w/v) solution of component 2 isolated by zone electrophoresis.

Fig. 7. Crystals of component 2 in ammonium sulphate at neutral pH; magnification 133 $\times$.

Fig. 9. Intrinsic viscosity of component 3 (two preparations) at 10.075 (phosphate or acetate, 10.05, and 25 mM-NaCl). The arrow indicates the isoelectric point of the protein.

of the whole myogen (cf. Fig. 4 in Hamoir & Konosu, 1965). The slow-moving impurity tenaciously carried down with the crystals of component 3 seems therefore to be identical with component 5.

Specific refractive increment. The specific refraction of component 3 was measured on three preparations of 80–150 mg. for which the coefficient corresponding to a concentration of 1 g./100 ml. was found to be 178.15×10^{-5} , 178.0×10^{-5} and 177.9×10^{-5} . For component 3 the mean value of 178×10^{-5} was used, but 182×10^{-5} was used for the other proteins (cf. Hamoir & Konosu, 1965).

Viscosity. The outflow times of 1.1–1.2% (w/v) solutions of two preparations were measured in the usual phosphate-sodium chloride buffer, pH 7.3, and at pH 5.83 (4.2 mM-disodium hydrogen phosphate–37 mM-sodium dihydrogen phosphate–25 mM-sodium chloride) and pH 4.64 (50 mM-sodium hydroxide–100 mM-acetic acid–25 mM-sodium chloride) (Fig. 9). As determinations made at pH 7.3 at 2.2% and 1.1% (w/v) did not differ noticeably, the values used in Fig. 9 have not been extrapolated to zero concentration. At the isoelectric point of component 3 (4.2) the intrinsic viscosity was found to be only 2.3 ml./g. This value is lower than those obtained so far with globular proteins (Tanford, 1958) and surprisingly near to that (1.9 ml./g.) corresponding to a rigid unsolvated sphere of density 1.33. If the value 0.751 ml./g. at 25° is used for the partial specific volume, the viscosity increment $[\eta]/\bar{v}$ of this protein should be 3.06. If, from the amino acid composition (see below), we assume another value, 0.736 ml./g., a slightly higher result, 3.12, is obtained. According to the chart relating the axial ratio, the hydration and the viscosity coefficients of proteins (Onclay, 1941), the maximum hydration (spherical shape) compatible with these values is only 0.15 g. of water/g. of protein. As there is no reason to believe that the hydration of this protein is abnormally low, these results appear to indicate that we are dealing with particles of a spherical or nearly spherical shape.

Ultraviolet spectra. The spectra of several preparations of both components isolated by zone

Fig. 8. Descending mobilities of component 3 (x) and of its slow-moving impurity (●) in phosphate-NaCl or acetate-NaCl buffer, 10.075, as a function of pH.

same solid ammonium sulphate and kept at 2°. The crystals separated within a few days in the form of very thin hexagonal plates (Fig. 7).

Properties of component 3 and of its slow-moving impurity

pH-mobility curves. The electrophoretic behaviour of these components was examined in phosphate–25 mM-sodium chloride buffer, pH > 5.6 and 10.075, and acetate–25 mM-sodium chloride buffer, pH < 5.6 and 10.075 (Fig. 8). The change of buffer did not markedly influence the mobilities; the smallness of the variations observed in the pH range 7.3–5.7 and the low isoelectric points obtained (4.2 and 4.55) are in good agreement with the behaviour of components 3 and 5 respectively

Fig. 10. Ultraviolet absorption of component 3 in phosphate-NaCl buffer, pH 7.3 and *I* 0.075 (solid line), and in 0.1 *N*-NaOH (broken line).

Fig. 11. Ultraviolet absorption of the slow-moving impurity of component 3 in phosphate-NaCl buffer, pH 7.3 and *I* 0.075 (solid line), and in 0.1 *N*-NaOH (broken line).

electrophoresis, the purity of which had been checked by free electrophoresis at pH 7.3, were measured in the phosphate-sodium chloride buffer, pH 7.3 and *I* 0.075, and in 0.1 *N*-sodium hydroxide. The ultraviolet spectrum of component 3 (Fig. 10), with maxima at 253, 259 and 265 $m\mu$, is strikingly similar to that of phenylalanine, taking into account the usual shift of about 2 $m\mu$ towards the red that is observed when the aromatic amino acids are incorporated into proteins (Fromageot & Schneck, 1950). No significant difference was observed in acetate buffer, pH 4.6 and *I* 0.05. In 0.1 *N*-sodium hydroxide an increased absorption occurred, in agreement with the higher absorption of phenylalanine in alkali (Beaven & Holiday, 1952). Beyond 280 $m\mu$ the absorption was negligible at both pH values. Component 3 thus does not contain tyrosine or tryptophan. Its extinction coefficient, $E_{1\text{ cm.}}^{1\%}$, at 259 $m\mu$ and neutral pH is 1.80 (mean of three determinations). If the residual absorption at 300 $m\mu$ is subtracted from this, a mean value of 1.66 is found. The phenylalanine content, evaluated from this last value and from the molar extinction coefficient, 177, of phenylalanine in water corresponding to the maximum at

257 $m\mu$ (Fromageot & Schneck, 1950), is 13.8 g. of residue/100 g. of protein.

The spectra of several preparations of the slow-moving impurity (Fig. 11) did not differ significantly from that of component 3 at pH 7.3. The specific absorption, however, was slightly stronger, corresponding to about 14.2 g. of phenylalanine residue/100 g. of protein (mean of three preparations). In 0.1 *N*-sodium hydroxide a definitely stronger absorption than the one of component 3 was observed and the small maximum at 252 $m\mu$ was no longer visible. This larger increase of absorption observed in 0.1 *N*-sodium hydroxide might indicate that structural parameters are different; as the solutions were becoming slightly turbid during the measurements in alkali, this difference might also be due to an easier alteration of this component under these conditions.

Sedimentation. The results obtained did not differ in the presence of acetate at pH 4.6 and 5.35 or in the presence of phosphate at pH 7.3; the rate of sedimentation extrapolated consistently to approx. 1.6 s at zero concentration (Fig. 12). The slow-moving impurity gave corrected values of

1.38 and 1.55 s at 1% and 0.5% (w/v) respectively, not differing significantly from those of component 3.

Molecular weight from diffusion and sedimentation results. Two preparations of component 3 at 1% and 0.5% (w/v) concentration in sodium acetate buffers, pH 4.6 and 10.05, and pH 5.35 and 10.1, respectively, were ultracentrifuged. Linear

Fig. 12. Sedimentation-concentration curve of component 3: ●, acetate buffer, pH 4.6 and 10.05; ×, acetate buffer, pH 5.35 and 10.1; △, phosphate-NaCl buffer, pH 7.3 and 10.075. Two values, 1.39 and 1.43 s at 1% and 0.5% (w/v) respectively in the presence of phosphate-NaCl buffer, pH 7.3 and 10.075, have been omitted for clarity.

extrapolation (Fig. 13) gave a diffusion coefficient of $12.0 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$. With a partial specific volume at 20° of 0.749 ml./g. and a corrected rate of sedimentation of 1.63 s, the molecular weight was found to be 13100. Another similar preparation dissolved in the usual buffer, pH 7.1 and 10.075, was ultracentrifuged under the conditions described for the Archibald procedure at a concentration of 1.02% (w/v). The mean value from two determinations and from eight

Fig. 13. Diffusion coefficient of component 3 in acetate buffer, pH 4.6 and 10.05 (● and ○), and pH 5.36 and 10.1 (×), as a function of the concentration.

Table 1. Amino acid composition of component 3 as determined by the microbiological method after 24 hr. hydrolysis

Component 3 preparations (a) and (b) were homogeneous by free electrophoresis in acetate buffer, pH 4.6 and 10.05.

Amino acid	Amino acid found (μmoles/mg. of protein)			Amino acid residue (mg./mg. of protein)*	Nitrogen (mg./100 mg. of protein)†	Minimum mol. wt.‡
	Prep. (a)	Prep. (b)	Mean			
Gly	1.212	0.759	—	—	—	—
Ala	1.515	1.392	1.454	0.103	2.04	688
Val	0.376	0.393	0.385	0.038	0.54	2597
Leu	0.641	0.732	0.687	0.078	0.96	1456
Ile	0.473	0.526	0.500	0.056	0.70	2000
Ser	—	—	—	—	—	—
Thr	0.487	0.462	0.475	0.048	0.67	2105
Met	0	0	0	0	0	—
Cys (half)	—	—	—	—	—	—
Pro	0	0	0	0	0	—
Phe	0.733	0.842	0.788	0.116	1.10	1269
Tyr	0	0	0	0	0	—
Trp	—	—	—	—	—	—
Asp	1.209	1.247	1.228	0.141	1.72	814
Glu	0.530	0.578	0.554	0.072	0.78	1805
NH ₃	—	—	—	—	—	—
Lys	0.931	0.992	0.962	0.123	2.69	1040
His	0.071	0.077	0.074	0.010	0.31	13514
Arg	0.075	0.069	0.072	0.011	0.40	13889
Total				0.796	11.91	

* (mean μmoles of amino acid/mg. of protein) × residue mol. wt. × 10⁻³.

† (mean μmoles of amino acid/mg. of protein) × *n* × 1.4, where *n* is the number of N atoms in the considered amino acid.

‡ 1000/(mean μmoles of amino acid/mg. of protein).

Table 2. *Amino acid composition of component 3 as determined by the Moore & Stein method after 22 hr. hydrolysis*

Component 3 preparation (c) was homogeneous by free electrophoresis in phosphate-NaCl buffer, pH 7.3 and 10.075; preparation (d) was homogeneous by free electrophoresis in acetate buffer, pH 4.6 and 10.05, where the resolution is lower (see p. 101).

Amino acid	Preparation (c)										Preparation (d)									
	Amino acid found (μmoles/mg. of protein)	Amino acid residue (mg./mg. of protein)*	Nitrogen of protein†	Minimum mol. wt.‡	No. of residues/molecule		Amino acid found (μmoles/mg. of protein)	Amino acid residue (mg./mg. of protein)*	Nitrogen of protein†	Minimum mol. wt.‡	No. of residues/molecule		Calc. mol. wt.	Calc. contribution	Assumed	Calc. mol. wt.	Calc. contribution	Assumed	Calc. mol. wt.	Calc. contribution
					Calc. §	Assumed					Calc. §	Assumed								
Gly	0.665	0.088	0.93	1504	6.93	7	0.637	0.036	0.89	1570	7.44	7	399	7.44	7	399	7.44	7	399	7.44
Ala	1.663	0.118	2.33	600	17.38	17	1.601	0.114	2.24	625	18.70	19	1350	18.70	19	1350	18.70	19	1350	18.70
Val	0.399	0.039	0.56	2506	4.16	4	0.379	0.037	0.53	2638	4.43	4	396	4.43	4	396	4.43	4	396	4.43
Ileu	0.742	0.084	1.04	1348	7.73	8	0.735	0.083	1.03	1361	8.59	9	905	8.59	9	1018	8.59	9	1018	8.59
Ile	0.303	0.034	0.42	3300	3.16	3	0.292	0.033	0.41	3425	3.41	3	339	3.41	3	339	3.41	3	339	3.41
Ser	0.412	0.039	0.58	2427	4.80	4	0.393	0.034	0.55	2545	4.59	5	348	4.59	5	435	4.59	5	435	4.59
Thr	0.410	0.041	0.57	2439	4.27	4	0.434	0.044	0.61	2304	5.07	5	404	5.07	5	505	5.07	5	505	5.07
Met	0	0	0	—	0	0	0	0	0	—	0	0	0	0	0	0	0	0	0	0
Cys (half)	—	—	—	—	—	—	0.055¶	0.006	0.08	—	—	—	—	—	—	—	—	—	—	—
Pro	0	0	0	—	0	0	0	0	0	—	—	—	0	0	0	0	0	0	0	0
Phe	0.814	0.120	1.14	1228	8.49	8	0.784	0.115	1.10	1276	9.16	9	1177	9.16	9	1324	9.16	9	1324	9.16
Tyr	0	0	0	—	0	0	0	0	0	—	—	—	0	0	0	0	0	0	0	0
Trp	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Asp	1.395	0.161	1.95	717	14.54	15	1.330	0.153	1.86	752	15.53	16	1726	15.53	16	1841	15.53	16	1841	15.53
Glu	0.675	0.087	0.94	1481	7.04	7	0.663	0.086	0.93	1508	7.75	8	903	7.75	8	1032	7.75	8	1032	7.75
NH ₃	0.727	0.012**	1.02	1375	7.58	8**	0.589	0.010**	0.82	1698	6.88	7**	—	6.88	7**	—	6.88	7**	—	6.88
Lys	0.937	0.120	2.62	1067	9.77	10	0.903	0.116	2.53	1107	10.55	11	1281	10.55	11	1409	10.55	11	1409	10.55
His	0.104	0.014	0.44	9615	1.08	1	0.089	0.012	0.37	11236	1.04	1	137	1.04	1	137	1.04	1	137	1.04
Arg	0.089	0.014	0.50	11236	0.93	1	0.083	0.013	0.47	12048	0.97	1	156	0.97	1	156	0.97	1	156	0.97
		H ₂ O						H ₂ O					H ₂ O			H ₂ O			H ₂ O	
Total		0.910	15.07			89		0.883	14.42			99								
Recovery		91.0%	107.4%					88.3%	101.6%											

* (μmoles of amino acid/mg. of protein) × residue mol. wt. × 10⁻³.

† (μmoles of amino acid/mg. of protein) × n × 1.4, where n is the number of N atoms in the considered amino acid.

‡ 1000/(μmoles of amino acid/mg. of protein).

§ (μmoles of amino acid/mg. of protein) × assumed minimum mol. wt. The values 10 425 and 11 642 were used for preparations (c) and (d) respectively, as being the means of 9615 and 11 236, and of 11 236 and 12 048 (see preceding columns).

|| Assuming that the protein is made of a single peptide chain.

** Not included in total.

†† Estimated from the ultraviolet absorption of the protein.

pictures, by using the partial specific volume given by Svedberg & Pedersen (1940), was 9830 ± 200 . With the slightly lower partial specific volume calculated from the amino acid composition ($0.736 \text{ ml./g. at } 20^\circ$), the molecular weight was 9340.

Amino acid composition. Four preparations of component 3 have been analysed, two by the microbiological method (Table 1) and two by the chromatographic procedure of Moore & Stein (Table 2). This protein is devoid, not only of tyrosine and tryptophan as shown by its ultra-violet absorption, but also of methionine and proline. The molecule obviously contains only 1 residue of histidine and 1 residue of arginine, as well as apparently 1 residue of half-cystine. The phenylalanine content (11.8g. of residue/100g. of protein) agrees fairly well with the estimation from spectroscopic data. The minimum molecular weight, 11034, from the amounts of histidine and arginine as determined by the Moore & Stein method, agrees fairly well with the one given by the Archibald procedure. It is much lower than the one, 14000, assumed previously on the basis of the results obtained by the microbiological method (Table 1) and on sedimentation and diffusion data (Hamoir, 1962).

To check whether the slow-moving impurity corresponds to denatured component 3 or to component 5, three zone electrophoreses were carried out on the recrystallized preparation of component 3. The impurity protein was recovered and dialysed exhaustively against water in the cold and freeze-dried. About 12mg. of the recovered powder was dissolved in 2.2ml. of the usual buffer, pH 7.3 and 10.075, giving a clear solution containing 5.4mg. of protein/ml. by refractometry. No nitrogen determination was carried out in this case. Two 0.5ml. samples were submitted to hydrolysis in 6N-hydrochloric acid for 36hr. (Table 3). The absence of half-cystine clearly shows that we are not dealing with a denatured form of component 3.

Properties of component 5

Ultraviolet spectrum. A preparation was examined at pH 7.3 and 10.075 and behaved exactly like the slow-moving impurity of component 3. Component 5 therefore does not contain tyrosine or tryptophan either, and also has a content of phenylalanine of about 14.2g. of residue/100g. of protein.

Sedimentation. The sedimentation-concentration curve obtained with two preparations in the usual phosphate buffer, pH 7.3 and 10.075, was extrapolated to give a value of about 1.7s (Fig. 14). In view of the spreading of the experimental results, this value does not appear to differ significantly

Table 3. *Amino acid composition of the slow-moving impurity as determined by the Moore & Stein method after 36 hr. hydrolysis*

The slow-moving impurity was homogeneous by free electrophoresis in phosphate-NaCl buffer, pH 7.3 and 10.075.

Amino acid	Amino acid found ($\mu\text{moles/mg. of protein}$)		
	Sample 1	Sample 2	Mean
Gly	0.736	0.737	0.737
Ala	1.752	1.731	1.742
Val	0.438	0.432	0.435
Leu	0.797	0.797	0.797
Ile	0.378	0.375	0.377
Ser	0.384	0.382	0.383
Thr	0.527	0.521	0.524
Met	0	0	0
Cys (half)	0	0	0
Pro	0	0	0
Phe	—	0.880	0.880
Tyr	0	0	0
Trp	—	—	—
Asp	1.487	1.487	1.487
Glu	0.732	0.728	0.730
NH ₃	0.515	0.517	0.516
Lys	1.308	1.318	1.313
His	0.091	0.092	0.092
Arg	0.086	—	0.086

Fig. 14. Sedimentation-concentration curve of component 5 in phosphate-NaCl buffer, pH 7.3 and 10.075 (two preparations). Two values, 1.38 and 1.55s, corresponding to a third preparation at 1% and 0.5% (w/v) respectively, have been omitted for clarity.

from that of 1.6s found for component 3. The two values obtained for the slow-moving impurity of component 3 fit this curve as well as they do that of Fig. 13.

Molecular weight from diffusion and sedimentation data. One preparation was ultracentrifuged at concentrations of 0.25, 0.5 and 1% (w/v) at pH 7.3 and 10.075. The diffusion coefficients obtained were extrapolated to give a value of about $15 \times 10^{-7} \text{ cm.}^2 \text{ sec.}^{-1}$, giving a molecular weight of 11130 (Fig. 15). A 1% (w/v) solution of the same

Fig. 15. Diffusion coefficient of component 5 in phosphate-NaCl buffer, pH 7.3 and 1.0-0.75, as a function of the concentration. The value obtained at 0.25% (w/v) represents the mean of two practically identical independent determinations.

preparation was examined by the Archibald procedure at 33450 rev./min. at 0, 4, 8, 12 and 16 min. The values of the molecular weight obtained from data at the meniscus varied from 10950 to 11715 with an average of 11350.

Amino acid composition. A sample of pure component 5 solution was dialysed exhaustively against water in the cold; its protein concentration was then found to be 5.27 mg./ml. by refractometry, and its nitrogen content (mean of triplicate determinations) 15.21%. Table 4 shows that the content of aromatic amino acids agrees very well with the estimation from the spectroscopic data. The agreement between the molecular weight calculated from the amino acid composition and that from the physicochemical data is also remarkably good. Both estimations of the total recovery from the chromatographic analysis are nevertheless somewhat high, and it is possible that a small systematic error was introduced during the determination either of the protein content or of the nitrogen content of the initial sample.

Properties of component 2

Ultraviolet spectrum. It can be deduced from a comparison of the electrophoretic patterns at pH 7.3 of two preparations and of their absorption that the minute amounts of impurity visible in front of the main peak in Fig. 6 increase the ultraviolet absorption by about 15%. The spectrum of the purer preparation (Fig. 16) is very different from those of components 3 and 5. A new maximum at 276 mμ is observed at pH 7.3, and this is shifted to 295 mμ in 0.1N-sodium hydroxide. A small amount of tyrosine is thus present, together with the usual large percentage of phenylalanine. If the absorption due to phenylalanine in 0.1N-sodium hydroxide at 295 mμ is neglected, and assuming that the correction for the residual absorption at this wavelength is 0.25, the absorption

Fig. 16. Ultraviolet absorption of component 2 in phosphate-NaCl buffer, pH 7.3 and 1.0-0.75 (solid line), and in 0.1N-NaOH (broken line).

of tyrosine at 295 mμ for a 1% (w/v) solution is $2.11 - 0.25 = 1.86$. The molar extinction coefficient of tyrosine at the maximum (293 mμ) is 2.340 (Fromageot & Schneck, 1950), and the tyrosine content is thus 1.30 g. of residue/100 g. of protein. The ratio of the extinction, at neutral pH, of tyrosine at 259 mμ to that at 274.5 mμ is 0.378, and the phenylalanine content thus evaluated is 13.1 g. of residue/100 g. of protein.

Sedimentation. The corrected rates of sedimentation of two preparations at pH 7.3 and 1.0-0.75 were 1.58 and 1.47 s at 0.4 and 0.7% (w/v) respectively. These values are similar to those of components 3 and 5, and were also extrapolated to give a value of about 1.7 s.

Amino acid composition. About 24 mg. of the purified component 2 was dissolved in 7.5 ml. of water, giving a clear solution containing 2.91 mg. of protein/ml. by refractometry and 13.67% of nitrogen by Kjeldahl analysis (mean of triplicate determinations). Table 5 shows that the content of aromatic amino acids is in good agreement with the results from the ultraviolet spectrum.

There was a fairly large discrepancy between the two estimations of the recovery from the chromatographic columns, probably owing to some error in the value adopted for the protein content of the preparation that gave a higher

Table 4. Amino acid composition of component 5 as determined by the Moore & Stein method after various times of hydrolysis

Amino acid	Amino acid found (μmoles/mg. of protein)										Amino acid residue of 100mg. of protein)*	Nitrogen (mg./100mg. of protein)†	Minimum mol. wt.‡	No. of residues/molecule		Calc. mol. wt. contribution
	18 hr. hydrolysis			36 hr. hydrolysis			72 hr. hydrolysis			Averaged or extra-polated				Calc. §	Assumed	
	Sample 1	Sample 2	Mean	Sample 1	Sample 2	Mean	Sample 1	Sample 2	Mean							
Gly	0.826	0.815	0.821	0.803	0.815	0.809	0.813	0.814	0.814	0.815	0.046	1.14	1227	8.72	9	513
Ala	1.670	1.728	1.699	1.705	1.740	1.723	1.714	1.668	1.691	1.704	0.121	2.39	587	18.24	18	1278
Val	0.435	0.429	0.432	0.446	0.461	0.454	0.458	0.465	0.462	0.463**	0.046	0.65	2160	4.96	5	495
Leu	0.843	0.820	0.827	0.816	0.828	0.822	0.821	0.829	0.825	0.825	0.093	1.16	1212	8.83	9	1017
Ile	0.351	0.345	0.348	0.378	0.387	0.383	0.424	0.430	0.427	0.435**	0.052	0.64	2183	4.90	5	565
Ser	0.439	0.420	0.430	0.400	0.400	0.400	0.354	0.302	0.328	0.430¶	0.042	0.67	2083	5.14	5	435
Thr	0.637	0.604	0.621	0.591	0.597	0.594	0.568	0.564	0.566	0.643¶	0.065	0.90	1555	6.88	7	707
Met	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0	0
Cys (half)	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0	0
Pro	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0	0
Phe	0.898	0.910	0.904	0.904	0.918	0.911	0.907	0.908	0.908	0.908	0.133	1.27	1101	9.72	10	1470
Tyr	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0	0
Trp	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0††
Asp	1.558	1.537	1.548	1.509	1.410	1.460	1.529	1.524	1.527	1.512	0.174	2.12	661	16.18	16	1840
Glu	0.780	0.774	0.777	0.761	0.774	0.768	0.766	0.767	0.767	0.771	0.099	1.08	1297	8.25	8	1032
NH ₂	0.574	0.579	0.577	0.528	0.560	0.544	0.473	0.436	0.455	0.538¶	0.009††	0.75	1859	5.76	6††	—6
Lys	1.161	1.354	1.258	1.244	1.203	1.224	1.219	1.287	1.253	1.245	0.159	3.49	803	13.33	13	1664
His	0.101	0.104	0.103	0.090	0.092	0.091	0.092	0.093	0.093	0.096	0.013	0.40	10417	1.03	1	137
Arg	0.088	0.089	0.089	0.093	0.092	0.093	0.088	0.091	0.090	0.091	0.014	0.51	10989	0.97	1	156
Total										H ₂ O 0.001	1.058	17.17			107	11321
Recovery											105.8%	112.9%				

* (mean μ moles of amino acid/mg. of protein) $\times$ residue mol. wt. $\times 10^{-3}$.† (mean μ moles of amino acid/mg. of protein) $\times n \times 1.4$, where n is the number of N atoms in the considered amino acid.‡ 1000/(mean μ moles of amino acid/mg. of protein).§ (mean μ moles of amino acid/mg. of protein) $\times$ assumed minimum mol. wt. The value 10703 was used, as being the mean of 10417 and 10989 (see preceding column).

|| Assuming that the protein is made of a single peptide chain.

¶ Extrapolated to zero time of hydrolysis.

** Extrapolated to infinite time of hydrolysis.

†† Not included in the total.

‡‡ Estimated from the ultraviolet absorption of the protein

Table 5. Amino acid composition of component 2 as determined by the Moore & Stein method after various times of hydrolysis

Amino acid	Amino acid found (μ moles/mg. of protein)										Amino acid residue (mg./mg. of protein)*	Nitrogen (mg./100 mg. of protein)†	Minimum mol. wt.‡	No. of residues/molecule		Calc. mol. wt. contribution
	18 hr. hydrolysis			36 hr. hydrolysis			72 hr. hydrolysis			Averaged or extrapolated				Calc. §	Assumed	
	Sample 1	Sample 2	Mean	Sample 1	Sample 2	Mean	Sample 1	Sample 2	Mean							
Gly	0.660	0.765	0.713	0.649	0.783	0.716	0.653	0.766	0.710	0.713	0.041	1.00	1403	9.40	9	513
Ala	1.517	1.766	1.642	1.498	1.761	1.620	1.502	1.756	1.629	1.634	0.116	2.29	612	21.53	22	1562
Val	0.247	0.302	0.275	0.270	0.323	0.297	0.296	0.342	0.319	0.327**	0.032	0.46	3058	4.31	4	396
Leu	0.660	0.771	0.716	0.668	0.770	0.719	0.675	0.783	0.729	0.729**	0.082	1.02	1372	9.61	10	1130
Ile	0.366	0.454	0.410	0.414	0.473	0.444	0.431	0.502	0.467	0.467**	0.053	0.65	2141	6.15	6	678
Ser	0.421	0.478	0.450	0.387	0.467	0.427	0.342	0.399	0.371	0.489¶	0.043	0.68	2045	6.44	6	522
Thr	0.257	0.328	0.308	0.287	0.332	0.310	0.261	0.313	0.287	0.334¶	0.034	0.47	2994	4.40	4	404
Met	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0	0
Cys (half)	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0	0
Pro	0.072	0.088	0.080	0.048	0.074	0.061	0.072	0.077	0.075	0.072	0.007	0.10	13889	0.95	1	97
Phe	0.727	0.857	0.792	0.741	0.861	0.801	0.723	0.875	0.799	0.797	0.117	1.12	1255	10.50	11	1617
Tyr	0.081	0.074	0.078	0.081	0.084	0.083	0.069	0.069	0.069	0.077	0.013	0.11	12987	1.01	1	163
Trp	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0††
Asp	1.191	1.369	1.280	1.040	1.361	1.201	1.201	1.368	1.285	1.255	0.144	1.76	797	16.54	17	1955
Glu	0.730	0.826	0.778	0.716	0.778	0.747	0.725	0.825	0.775	0.767	0.099	1.07	1304	10.11	10	1290
NH ₃	0.509	0.627	0.568	0.519	0.634	0.577	0.610	0.749	0.680	0.536¶	0.009††	0.75	1886	7.06	7††	—
Lys	0.787	0.930	0.859	0.804	0.967	0.886	0.789	0.976	0.883	0.876	0.112	2.45	1142	11.54	12	1536
His	0	0	0	0	0	0	0	0	0	0	0	0	—	0	0	0
Arg	0.072	0.079	0.076	0.072	0.095	0.084	0.069	0.086	0.078	0.079	0.012	0.44	12658	1.04	1	156
Total											H ₂ O 0.001	—	—	—	—	18
											0.906	14.37	—	—	114	12030
											90.6%	105.1%				

* (mean μ moles of amino acid/mg. of protein) $\times$ residue mol. wt. $\times 10^{-3}$.† (mean μ moles of amino acid/mg. of protein) $\times n \times 1.4$, where n is the number of N atoms in the considered amino acid.‡ 1000/(mean μ moles of amino acid/mg. of protein).§ (mean μ moles of amino acid/mg. of protein) $\times$ assumed minimum mol. wt. The value 13178 was used, as being the mean of 13889, 12987 and 12658 (see preceding column).

|| Assuming that the protein is made of a single peptide chain.

¶ Extrapolated to zero time of hydrolysis.

** Extrapolated to infinite time of hydrolysis.

†† Not included in the total.

‡‡ Estimated from the ultraviolet absorption of the protein.

value by the measurement of the refractive index increment than would have been expected from its nitrogen content. The apparent low weight recovery may be the source of the difference between the values for the mean minimum molecular weight and of the sum of the calculated molecular weight contributions (Table 5).

Comparison of the slow-moving impurity with components 3 and 5

The composition of component 5 does not differ significantly from that of the slow-moving impurity of component 3 (Table 3). The small differences found are within the limits of error, except perhaps for glycine, threonine and lysine. The amino acid analyses therefore substantiate the identity suggested by the pH-mobility curves of Fig. 8, the ultraviolet absorption and the sedimentation data.

DISCUSSION

The main properties of the three crystalline muscle proteins isolated are summarized in Table 6. These data confirm that these proteins are closely related, having in common a low molecular weight (9000–13 000), a high salting-out range in ammo-

nium sulphate (70–100% saturation) and a very unbalanced amino acid composition. They are devoid of tryptophan and methionine, contain $12.5 \pm 1\%$ (w/w) of phenylalanine and either no or at most a single residue each of tyrosine, half-cystine, proline, arginine and histidine/molecule.

They differ from other low-molecular-weight protein molecules, such as cytochrome *c*, lysozyme and ribonuclease, by their acidic character. The presence of a single half-cystine residue/molecule (component 3) or the absence of sulphur-containing amino acids (components 2 and 5) also excludes the occurrence of disulphide bonds, as in lysozyme and ribonuclease. A similar absence of disulphide bonds has been observed for several bacterial extracellular enzymes (Pollock & Richmond, 1962). These authors have suggested that such molecules could unfold more easily, pass the extracellular wall and refold reversibly. Preliminary results showing that the viscosity and the crystallization of component 3 are unchanged after treatment for 4 hr. in 8M-urea at room temperature support this suggestion. However, such a process seems not to occur in the carp under normal conditions, as these small proteins are not observed on ultracentrifugation of carp serum (G. Hamoir, unpublished work). Also, as the optical

Table 6. *Main properties of components 2, 3 and 5*

	Component 2	Component 3	Component 5
Isoelectric point	—	4.2	4.55
$S_{20,w}^0$ (s)	~1.7	1.6	~1.7
$D_{20,w}^0$ (cm. ² sec. ⁻¹)	—	12.0×10^{-7}	15.0×10^{-7}
Molecular weight			
(a) <i>S</i> and <i>D</i> ($\bar{v}_{200} = 0.749$)	—	13100	11130
(b) Archibald method with			
$\bar{v}_{200} = 0.749$	—	9830 ± 200	11350
$\bar{v}_{200} = 0.736$	—	9340	—
(c) Amino acid analysis	12030	9389, 10454*	11321
Phenylalanine content (g. of residue/100 g. of protein)			
(a) amino acid analysis	11.7	12.0, 11.5*	13.3
(b) u.v. absorption	13.1	13.8	14.2
Content (residues/molecule) (based on the analytical mol. wt.)			
phenylalanine	11	8, 9*	10
tryptophan	0	0	0
tyrosine	1	0	0
methionine	0	0	0
cysteine	0	1	0
proline	1	0	0
histidine	0	1	1
arginine	1	1	1
Acid groups/molecule	20	14, 17*	18
Base groups/molecule	13	12, 13*	15
Excess of acid groups/molecule at pH 7	7	2.5, 4.5*†	2.5†

* First value for prep. (c), second value for prep. (d); see Table 2.

† Assuming that half of the histidine group is charged.

properties of phenylalanine residues in proteins are normally obscured by the presence of other aromatic residues, a unique opportunity is afforded from this point of view by the crystalline components 3 and 5. The occurrence of a single residue of tyrosine (component 2), of half-cystine (component 3), of proline (component 2), of histidine (components 3 and 5) and of arginine also permits investigation of the influence of protein structure on these residues. The macromolecular properties of these proteins similarly appear worthy of further investigation. According to viscometric determinations relating to component 3, the specific increment of these molecules is lower than that of other small globular proteins described so far (Tanford, 1958), a fact that suggests that they approximate more closely to the ideal case of the spherical protein molecule.

From the nutritional point of view, some consideration should be given to the occurrence in fish myogen of a notable protein fraction that is devoid of tryptophan and methionine. The observation by Konosu, Katori, Ota, Eguchi & Mori (1956) that the muscle of the common carp has a slightly lower content of these two amino acids than the muscle of many species of fish may thus be due to the presence of a larger proportion of this particular protein fraction. However, as carp myogen only represents 2.7%, on a weight basis, of the total fresh muscle (Hamoir, 1955), and as the protein content of this tissue is about 16% (Konosu *et al.* 1956), the quantity of myogen that lacks the two essential amino acids corresponds to only 5% of the total muscle protein of the carp. The amino acid composition of the structural proteins of the muscle, on the other hand, does not seem to vary much according to origin (Connell & Howgate, 1959; Kubo, 1957). Also, in mammals, there is more connective tissue devoid of tryptophan and poor in methionine (about 17% of the muscle proteins) than in fishes (about 3% in teleost fishes and 10% in elasmobranch fishes) (Jebesen, 1962). The tryptophan and methionine content of whole fish meat for these reasons reaches almost the same level as that of beef, pork or chicken meat (Konosu & Matsuura, 1960). As a source of essential amino acids, the muscle proteins of the fish compare favourably with those of domestic animals (Konosu *et al.* 1956), in spite of the existence of this protein fraction. But its easy isolation affords an opportunity to prepare new dietary mixtures.

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