Studies on the Proteins of Fish Skeletal Muscle

3. COD MYOSIN AND COD ACTIN

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(Received 1 May 1954)

The myofibrillar protein components of fish skeletal muscle have, so far, not been resolved in the same way as have those of mammalian muscle. The only fish myofibrillar proteins which appear to have been isolated and characterized at all fully are carp tropomyosin and carp nucleotropomyosin (Hamoir, 1951*a, b*). Of the major components, it seems that the proteins hitherto called fish myosins more nearly resemble mammalian actomyosin than mammalian myosin, and fish actin has not been described. Roth (1947), using carp, was unable to observe L-myosin in short-duration extracts which normally furnish solutions rich in this protein in the case of rabbit. A suggested explanation postulated differences in the adenosine triphosphate (ATP) balance of the muscle between fish and rabbit. Earlier, Guba (1943) had noticed that the apparent extraction of actin concomitant with that of myosin proceeds more rapidly with fish than with rabbit or frog. However, Hamoir (1949, 1951*c*) observed electrophoretic components in carp extracts corresponding to α - and β -myosins (identifiable with actomyosin and myosin, respectively (Dubuisson, 1946*a*; Weber & Portzehl, 1952)), and was able to separate the β -component. This component although possessing some of the properties of pure rabbit myosin did not behave comparably in the ultracentrifuge. On the other hand, addition of ATP to a fish 'actomyosin' solution in the presence of calcium and magnesium ions (Hamoir, 1951*c*) resulted in the appearance of a boundary which sedimented at very nearly the same rate as rabbit L-myosin. If it is at all valid to extrapolate from the known properties of the corresponding preparations of mammalian muscle, then these observations are obviously conflicting. This paper represents an attempt to clarify the position with regard to cod at least, and also describes some characteristics of cod actin.

The protein from carp muscle briefly studied by Suyama (1950, 1951) obviously corresponds to actomyosin, as do the fish 'myosins' isolated by Subba Rao (1948).

EXPERIMENTAL

Materials

The experimental material used throughout has been cod (*Gadus callarius* L.). The fish were never more than about 40 cm. in length, and were always alive before the experiments.

Buffers. Unless otherwise stated, buffers contained the following: pH 3.5: 0.2N Na acetate, 0.185N-HCl, 0.015M-KCl, $I=0.4$ (e.g. Fig. 3). pH 4.0: 0.2N Na acetate, 0.160N-HCl, 0.04M-KCl, $I=0.4$ (e.g. Fig. 3). pH 4.6: 0.2N Na acetate-acetic acid mixture, 0.2M-KCl, $I=0.4$ (e.g. Fig. 3). pH 6.0: 0.009M- Na_2HPO_4 , 0.072M- KH_2PO_4 , 0.3M-KCl, $I=0.4$ (e.g. Fig. 3). pH 7.4: 0.0312M- Na_2HPO_4 , 0.007M- KH_2PO_4 ($I=0.1$), KCl to make up to $I=0.3$ (e.g. Fig. 1*b*), 0.4 (e.g. Figs. 1*a*, 3, 4, 5*a-c*) or 0.5 (e.g. Fig. 2).

ATP. This was prepared from cod muscle. The fish were killed by decapitation, with as little struggling as possible, quickly skinned and filleted, and the fillets transferred immediately to a cold room at 0° where they were minced. The procedure of LePage (1949) was then followed using 500–600 g. mince. The yield of dry dibarium salt was about 2 g./kg. mince, which is smaller than can be obtained from rabbit muscle, but the availability and expendability of the fish made its use profitable in this case. The preparations were not analysed. Tarr (1949) estimates the amount of ATP and ADP together as 25–200 mg./100 g. in the skeletal muscle of resting marine fish.

Methods

Electrophoresis. The Tiselius apparatus with Philpot-Svensson optical system was used for electrophoresis. The bath temperature was +0.5°, and the tall-form cell with flow-through modification (Kekwick, Lyttleton, Brewer & Dreblow, 1951) was usually used. Isoelectric points were determined by the moving boundary method.

Diffusion. Diffusion experiments were conducted in the same apparatus as was used for electrophoresis. The diffusion of the myosin and actin preparations was conducted at +0.5°, but in one experiment on actin a temperature of 25° was used. The flow-through cell proved very useful as a boundary sharpening device, particularly with the more concentrated viscous solutions of myosin, since compensation of shear-formed boundaries resulted in deformation. Use of a diagonal edge, slit or wire diaphragm gave results within 5% of one another. Diffusion coefficients were com-

puted by the height-area method, but sometimes the method of moments and shape-analysis was carried out. The quoted diffusion coefficients are the mean of the results obtained from both limbs of the cell. A control experiment on a 2% solution of crystalline bovine plasma albumin (Armour, Chicago) in 0.1N acetate buffer pH 4.7 at 0.5° gave a value of $2.85 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$, which is in reasonable agreement with the corrected diffusion constant for this protein.

Ultracentrifuging. One sample each of a myosin preparation and a partially polymerized actin sample were examined in the Svedberg oil-turbine ultracentrifuge through the kindness of Dr R. A. Kekwick.

Viscosity. Ostwald viscometers held at 0° and 25° were used. In some cases the rate of shear was increased to 2000–10 000 sec^{-1} by applying controlled but variable amounts of positive pressure to the viscometer; typically the time of outflow of about 6.6 ml. of water at 100 cm. water pressure was about 46 sec.; diameter of capillary 0.054 cm.

Double refraction of flow (D.R.F.). D.R.F. was observed by swirling the solution contained in a small beaker between crossed polaroids.

Osmotic pressure. Determinations at 0° were made in Adair (1949) toluene osmometers using collodion membranes.

Hydration. Hydration was estimated from the equilibrium amounts of water absorbed at 20° by salt-free, freeze-dried material placed over a saturated aqueous solution of K_2SO_4 (cf. Adair & Robinson, 1931).

Chemical analyses. The acidic and basic groups of the protein preparations were determined at pH 11.5 and 2.2 respectively, using the dye-binding method of Fraenkel-Conrat & Cooper (1944). The tryptophan and tyrosine contents of cod actin were determined by Block & Bolling's (1945) adaptation of the Millon-Folin method.

RESULTS

As far as nomenclature is concerned, it has become customary to regard pure myosin from skeletal muscle as a monodisperse preparation of sedimentation constant about 7 Svedberg units which has zero ATP-sensitivity, i.e. L-myosin (Weber & Portzehl, 1952); there are some dissentients from this view (Morales & Botts, 1953; Blum & Morales, 1953). Since the protein to be described does not satisfy the ultracentrifugal criterion, but does exhibit other important characteristics of myosin and certainly differs from actomyosin, the difficulty of naming it arises. It has been thought best to retain the word myosin for the fish protein rather than introduce further complications of nomenclature.

Preparation of cod myosin

Unless otherwise stated, all operations were carried out at 0°.

Extraction of cod muscle for periods of 20 hr. with solutions which normally extract actomyosin from rabbit muscle ($>0.5 \text{ M-KCl}$, pH 7–8), followed by precipitation at an ionic strength of 0.05 and re-solution of the precipitate, furnishes typical

actomyosin solutions, i.e. having high viscosity (cf. Fig. 2), pronounced opalescence, and with ATP-sensitivities (Portzehl, Schramm & Weber, 1950) greater than 100. Electrophoresis of such solutions gives very asymmetric boundaries (cf. Dubuisson, 1946b) corresponding to the so-called α -myosin with sometimes also what is possibly β -myosin (Fig. 1a). The average mobility of the former in several experiments was $-3.05 \times 10^{-5} \text{ cm}^2 \text{ v}^{-1} \text{ sec}^{-1}$.

On the other hand, attempts to prepare myosin by the method of Guba & Straub (1943), which with rabbit gives myosins of low ATP-sensitivities, resulted in preparations of ATP-sensitivity 20–30. Addition of pyrophosphate and reducing the pH to 6.5 as in Hasselbach & Schneider's (1951) method, still gave preparations of fairly high ATP-sensitivity. For example, extraction of coarsely minced cod muscle with the pyrophosphate-containing buffer, pH 6.5, $I=0.35$ shown below, for periods of 90 and 300 min. resulted in extracts having ATP-sensitivities of 14 and 21, respectively. However, by reducing the time of extraction to 5 min., solutions having ATP-sensitivities of 0–5 were obtained which still retained most of the properties of myosin and which are regarded as being the cod-muscle equivalent of rabbit-muscle myosin. The method of preparation was to extract coarsely minced cod muscle (50 g.) by slow stirring (5 min.) with 150 ml. of the following:

(total $I=0.35$) adjusted to pH 6.5 with a small amount of HCl. The brei was centrifuged at 4000 g for 30 min., and the supernatant dialysed against 7 times its volume of distilled water. The precipitated gel was separated by centrifuging, washed with phosphate buffer pH 7.5, $I=0.05$, redissolved in 0.5 M-KCl and again precipitated by dialysis until the ionic strength fell to 0.05. The final centrifuged gel was usually dissolved in

$0.3 \text{ M-KCl} + 0.0312 \text{ M-Na}_2\text{HPO}_4 + 0.007 \text{ M-KH}_2\text{PO}_4$,
pH 7.4, $I=0.4$, and dialysed against the same buffer.

Properties of cod myosin

Preparations made in the above manner usually had ATP-sensitivities of 0–5; some which had slightly higher values were not improved by an intermediate dialysis at $I=0.28$ (Portzehl *et al.* 1950; Mommaerts & Parrish, 1951). The intrinsic viscosities of artificial actomyosins made by adding the preparation and cod F-actin together were about 50% of those of 'natural' actomyosins from cod. The intrinsic viscosity of the preparation itself was 2.0 (Fig. 2) at an average shear rate of about 3500 sec^{-1} , and this value did not vary much between shear rates of 2000–7000 sec^{-1} .

Fig. 1. (a) Electrophoretic diagram of cod actomyosin at $I=0.4$, pH 7.4. Ascending limb. Field strength 1.14 v/cm.; duration of electrophoresis, 57 720 sec. Migration from L to R. (b) Electrophoretic diagram of cod myosin at $I=0.3$ and pH 7.4. Ascending (above) and descending (below) limbs. Field strength 2.21 v/cm.; duration of electrophoresis, 95 400 sec. Migration (asc.) from L to R, (desc.) from R to L.

Fig. 2. Intrinsic viscosity of cod actomyosin ($\square$) and cod myosin ($\odot$) at 0° ; pH 7.4, $I=0.5$. η_{sp} = specific viscosity. c = protein concentration in g./100 ml.

Fig. 3. Isoelectric point of cod myosin ($\odot$) and cod actin ($\triangle$). Below pH 4: KCl-Na acetate-HCl buffers; pH 4-6: KCl-Na acetate-acetic acid buffers; above pH 6: KCl-phosphate buffers. Ionic strength in all cases 0.4. u = electrophoretic mobility.

Fig. 4. Diffusion of cod myosin at 0.5° ; $I=0.4$, pH 7.4. c = protein concentration in g./100 ml. $D_{20,w}$ = corrected diffusion coefficient.

Even concentrated solutions of the protein were only faintly opalescent, and precipitation into water with vigorous stirring to a final ionic strength of 0.05 gave a sheen of myosin 'crystals'.

Electrophoretically, the protein was almost homogeneous at pH 7.4 and $I=0.3$ or 0.4, showing a slight asymmetry of the ascending boundary (Fig. 1b). The mobility under these conditions was $-2.90 \times 10^{-5} \text{ cm}^2 \text{v}^{-1} \text{sec}^{-1}$, and the isoelectric point 5.3 at $I=0.4$ (Fig. 3). It was not possible to examine the protein below pH 5 because of its insolubility, but it appeared to be substantially

homogeneous between pH 5 and 7.4. Average acidic and basic groups amounted to 18.2 and 15.5 equivalents per 10^4 g. protein at pH 11.5 and 2.2, respectively.

One sample of the protein when examined in the ultracentrifuge sedimented as a single boundary with sedimentation constants of 9.65 and 17.1×10^{-13} at 0.9 and 0.45% concentration, respectively. At the lower concentration the boundary spread fairly rapidly, indicating some degree of polydispersity. Diffusion experiments were conducted over a wider range of concentration (0.1–0.7%) and only a slight dependence of diffusion coefficient on concentration was observed (Fig. 4). The experiments lasted about 5 days at $+0.5^\circ$; after about 3 days slight skewing of the boundaries could be detected. The extrapolated, corrected diffusion constant was $0.4 \times 10^{-7} \text{ cm.}^2 \text{ sec.}^{-1}$ when calculated by the area-height method.

Hydration was estimated to be 0.5 g. water/g. protein.

Preparation of cod actin

An aqueous extract from acetone-dried cod muscle fibre as prepared by Feuer, Molnar, Pettko & Straub (1948) shows the general properties of a solution of *G*-actin. On addition of salts, the limpid

solution can be polymerized to a viscous solution of *F*-actin showing pronounced double refraction of flow. Polymerization at 0° appears to be very slow, although the content of adenine compounds in the extract is at least as high as in comparable rabbit extracts as judged by the absorption spectrum of the deproteinized extract. The slow polymerization at 0° was made use of in examining electrophoretically some early preparations made from fibre which had been ground before extraction. Dialysis at 0° of the aqueous extract against

$0.3 \text{ M-KCl} + 0.0312 \text{ M-Na}_2\text{HPO}_4 + 0.007 \text{ M-KH}_2\text{PO}_4$, pH 7.4, $I=0.4$ enabled the diagram depicted in Fig. 5a to be obtained. A main boundary of mobility $3.8 \times 10^{-5} \text{ cm.}^2 \text{ v}^{-1} \text{ sec.}^{-1}$ —presumably *G*-actin—and at least two others are apparent, one a slower 'shoulder' of the main boundary, and another more rapid discrete boundary of mobility— $5.5 \times 10^{-5} \text{ cm.}^2 \text{ v}^{-1} \text{ sec.}^{-1}$ —possibly tropomyosin (cf. Dubuisson, 1950a; Hamoir, 1951a). It was found that the specific viscosity of the preparation was improved (Fig. 6) by omitting the Na_2CO_3 treatment of the Feuer *et al.* (1948) method as suggested by Tsao & Bailey (1953), and the impurities were concurrently reduced to a low level (Fig. 5b). In this case the actin was inactivated before electrophoresis by dialysis against water for several days; electrophoresis of *G*-actin could also be achieved by prior depolymerization with

Fig. 5. (a) Electrophoretic diagram of a cod actin extract according to Feuer *et al.* (1948) $I=0.4$, pH 7.4. Ascending limb. Field strength 0.94 v/cm.; duration of electrophoresis, 78 720 sec. Migration from R to L. Protein concentration, 1.26 mg./ml. (b) Electrophoretic diagram of a modified Feuer *et al.* (1948) extract. $I=0.4$, pH 7.4. Ascending limb. Field strength 0.88 v/cm.; duration of electrophoresis 71 280 sec. Migration from L to R. Protein concentration, 0.50 mg./ml. (c) Same as 5b, another extract. Field strength 0.78 v/cm.; duration of electrophoresis 64 880 sec. Migration from L to R. Protein concentration, 0.6 mg./ml.

Fig. 6. Specific viscosity of cod *F*-actin preparations in 0.1 M-KCl at 0° . Δ , Modified Feuer *et al.* (1948) extract (cod); $\square$, Feuer *et al.* (1948) extract (cod); $\odot$, 30% acetone extract (cod) (Tsao & Bailey, 1953); ---, Feuer *et al.* (1948) extract (rabbit). c = protein concentration in g./100 ml. η_{sp} = specific viscosity.

0.6M-KI (Dubuisson, 1950c). Efforts were made to improve the preparations as far as their specific viscosity was concerned but without success. The butanol method of Tsao & Bailey (1953) does not appear to be applicable to cod muscle; 30% acetone at 0° or at 20° failed to extract more than small quantities of protein from butanol-treated, acetone-dried fibre, and the resulting polymerized form had a low specific viscosity (Fig. 6) and actomyosin-forming power. In any case, the use of butanol seems not to be necessary, since cod actin solutions are invariably water-clear and show no trace of colour or specific absorption between 300–500 m μ . even when concentrated.

An attempt was made to purify *F*-actin by sedimenting it free of lighter impurities in a high-speed centrifuge (Mommaerts, 1951). When *F*-actin was spun at 25 000 *g* for 2 hr. in an angle centrifuge at 0°, a concentration gradient was obtained rather than a compact pellet. The transparent protein at the bottom of the centrifuge tube was extremely viscous and intensely birefringent, but considerable amounts of *F*-actin still remained even at the top of the tube as evidenced by viscosity and birefringence. It is not certain whether this is due to variations in weight of the *F*-actin microfibrils, if such exist in solution, or to failure completely to eliminate vibrations or convection during the centrifuging. It has also been pointed out that the explanation may lie in the smaller ability of cod actin when compared with rabbit actin to give the type of solution 'structure' which Tsao & Bailey (1953; Tsao, 1953) suggest is formed by rabbit actin dimers. The behaviour of a 'weak structure' on centrifuging might be expected to be different from that of a 'strong structure'. Whatever the reason the specific viscosity of the protein from the bottom quarter of the tube was not noticeably different from that of the original solution.

In view of the seeming difficulties attendant on removing the last traces of impurity, the main properties of cod actin have been determined on aqueous extracts of muscle fibre obtained by the method of Feuer *et al.* (1948) omitting the final treatment with Na₂CO₃. The yield obtained by extracting 1 g. fibre with 20 ml. water for 30 min. at room temperature was about 3–5 mg./ml. The protein was concentrated by acetate precipitation at pH 4.7 and re-solution in aqueous NaHCO₃.

Properties of cod actin

The electrophoretic properties of the *G*-form have been outlined above. Some preparations appeared to be very homogeneous at pH 7.4 (Fig. 5c), but there was some indication of separation into two boundaries in prolonged runs. Electrophoresis of the *F*-form was complicated by thixotropy (Tsao & Bailey, 1953) but at low concentration the bulk of

the protein moved with a single, if deformed, boundary the mobility of which was reasonably reproducible. The average mobilities of *G*- and *F*-actin were -3.9×10^{-5} and about -6.5×10^{-5} cm.² v⁻¹ sec.⁻¹, respectively, at pH 7.4, *I* = 0.4. The protein gave very opalescent solutions between pH 3 and 6, and therefore the isoelectric point, pH 4.7–4.8 (Fig. 3), could only be determined by measuring the movement of the blackening on the photographic plate. Average acidic and basic groups were 26.3 and 15.3 equivalents per 10⁴ g. protein at pH 11.5 and 2.2, respectively. The intrinsic viscosity of the *G*-form in 0.6M-KI + 0.006M-Na₂S₂O₃, pH 5.6, was 0.075 both at 0° and 25° (Fig. 7).

Ultracentrifugal analysis of a sample in

0.3M-KCl + 0.0312M-Na₂HPO₄ + 0.007M-KH₂PO₄, pH 7.4, *I* = 0.4, which was thought to be de-polymerized but which evidently contained some *F*-actin (not detectable by the double refraction of flow test used), showed a very heavy, polydisperse component and a much lighter component. The latter moved so slowly away from the meniscus that

Fig. 7. Intrinsic viscosity of cod *G*-actin in 0.6M-KI. ○, 25°; △, 0°. *c* = protein concentration in g./100 ml.; η_{sp} = specific viscosity.

Fig. 8. Osmotic pressure of cod *G*-actin in 0.6M-KI at 0°. π = osmotic pressure in mm. Hg; *c* = protein concentration in g./100 ml.

its sedimentation coefficient could not be determined but was estimated to be about 1.0×10^{-13} . Diffusion experiments in $0.6\text{ M-KI} + 0.006\text{ M-Na}_2\text{S}_2\text{O}_3$ on *G*-actin lasting 3–4 days gave a value at both $+0.5^\circ$ and 25° of $2.3 \times 10^{-7}\text{ cm}^2\text{ sec}^{-1}$ for the corrected diffusion constant calculated by the height-area method. This value varied randomly by $\pm 10\%$ between concentrations of 0.3–0.6% protein. The diffusion constant calculated by the method of moments was about 15% higher than this, and shape-analysis of the diffusion curves showed that the preparations were somewhat polydisperse. Hydration and partial specific volume were estimated to be 0.46 g. water/g. protein and 0.735, respectively.

Osmotic pressure measurements (Fig. 8) on a single sample in $0.6\text{ M-KI} + 0.006\text{ M-Na}_2\text{S}_2\text{O}_3$ pH 5.6 at 0° gave a reduced osmotic pressure (Adair & Robinson, 1930) of 1.0–1.10, representing a particle weight of about 160000.¹

The tryptophan and tyrosine contents were found to be 1.0 and 5.0%, respectively (protein nitrogen 16.0%).

DISCUSSION

It is clear that arguing from the case of rabbit myosin, the ultracentrifugal results on cod myosin, and to a lesser extent the diffusion results, are in conflict with the other properties of the protein. The sedimentation constant of rabbit myosin is well established as about 7×10^{-13} (Snellman & Erdős, 1948b; Portzehl *et al.* 1950; Johnson & Landolt, 1951; Mommaerts & Parrish, 1951), and approximately the same value may be inferred from Hamoir's (1951c) results on carp actomyosin in the presence of ATP, but the values for the sedimentation coefficient of cod myosin correspond almost exactly to those found for rabbit and cod actomyosin at the same concentrations. Again, the diffusion constant of cod myosin is about one-half that found for rabbit myosin, and in between the values quoted for rabbit actomyosin by Bergold (1946) (0.52) and by Ziff & Moore (1944) (0.30). Yet, in all the other properties examined, the cod preparation is strikingly similar to rabbit myosin. Thus, its solutions have the same appearance as solutions of rabbit myosin, its solubility is similar, and it behaves towards ATP and *F*-actin in a similar way. The failure of cod myosin to show a fall in viscosity on addition of ATP can hardly be due to inactivation during preparation or to the inefficacy of the ATP, because cod actomyosin preparations made under similar conditions show a large effect. Electrophoretically the protein also behaves like myosin, although here the distinction is not so clear-cut, since the electrophoretic behaviour, isoelectric point and numbers of acidic and basic

groups of rabbit myosin and actomyosin are not very different. The intrinsic viscosity of the protein clearly differentiates it from actomyosin (Fig. 2), and the value is similar to that obtained for rabbit myosin (Guba & Straub, 1943; Portzehl *et al.* 1950) and for rat and mouse myosins (Miller, Golder, Eitelman & Miller, 1952).

It is unfortunate that the paucity of ultracentrifugal data precludes a conclusive judgement on the identification of the cod protein with rabbit myosin. If they are the same type of protein and the ultracentrifuge results for cod are truly characteristic, then their molecular dimensions must differ widely, since the extrapolated sedimentation coefficient of the cod protein will be far higher than 7×10^{-13} . There is a possibility that the sample examined by ultracentrifuging was denatured: Portzehl *et al.* (1950) have shown that L-myosin on keeping denatures to give a fairly well-defined component sedimenting at about 15×10^{-13} , and certainly cod myosin can hardly be kept longer than 10 days *post mortem* without signs of denaturation. This possibility is very unlikely in the case of the results other than the ultracentrifugal ones, some of the preparations being examined 2–3 days *post mortem*.

An axial ratio for cod myosin of about 50 may be computed from the viscosity data assuming a prolate ellipsoidal model and hydration of 0.5 g. water/g. protein. This figure is of the same order as that derived from viscosity data on rabbit myosin, but the molecular weight of the cod protein must then be at least double that of the rabbit protein.

One of the original aims of this work, namely to demonstrate the existence in cod muscle of a protein corresponding to myosin in respect of properties and molecular size and shape, has, thus, not been fully achieved. The problem, indeed, seems to be more complex than might have been imagined, and can probably only be resolved by a more detailed ultracentrifugal examination. The failure to extract actin-free myosin with the same ease as in the rabbit, rat or mouse, cannot be due to an entire depletion of ATP from the muscle before extraction since ATP can be prepared from cod muscle which is in essentially the same post-mortem condition, and also cod actin contains amounts of ATP (or at least adenine compounds capable of promoting polymerization of the actin) equivalent to those found in rabbit actin. It may be that the ATP is not effective in the same way as it is in intact rabbit muscle in aiding extraction of myosin (Roth, 1947); certainly pyrophosphate is not effective in this connexion, and it is clear that Hasselbach & Schneider's (1951) method of exhaustively extracting actin-free myosin from a coarse mince, must fail when applied to fish, since even fairly short extractions under their conditions yield solutions of appreciable ATP-sensitivity. In some preliminary experiments

Table 1. *Properties of cod myosin and cod actin compared with those of rabbit myosin and rabbit actin*

	Cod		Rabbit	
	Myosin	Actin	Myosin	Actin
$S_{20,w} \times 10^{13}$	9.65 ($c^* = 0.9$) 17.1 ($c = 0.45$)	1.0 (?) ($c = 0.7$)	7.1a ($c = 0$)	3.2a ($c = ?$)
$D_{20,w} \times 10^7 \text{ cm}^2 \text{ sec}^{-1}$	0.4 ($c = 0$)	2.3 ($c = 0.3-0.6$)	0.87a ($c = 0$)	
Mol. wt. (from osmotic pressure in 0.6M-KI)	—	160 000	—	140 000b
Minimum mol. wt.†	—	40 000	—	35 000c
Intrinsic viscosity	2.0	0.075‡	2.2a	0.20b
U§	-2.9	(F-) -6.5 (?) (G-) -3.9	-2.9d	(F-) -6.3e (G-) -4.6e
Isoelectric point	5.3	4.7-4.8	5.4f	4.7-4.8 (?)

* c = protein concentration in g./100 ml.

† Calculated from tyrosine and tryptophan contents.

‡ In 0.6M-KI.

§ Electrophoretic mobility $\times 10^5 \text{ cm}^2 \text{ V}^{-1} \text{ sec}^{-1}$ (buffer for cod proteins: KCl-phosphate pH 7.4, $I = 0.4$; buffer for rabbit proteins: 0.25M-NaCl + 0.052M phosphate, pH 7.4, $I = 0.4$).

|| For buffers see experimental section.

a Summarized in Weber & Portzehl (1952).

b Tsao (1953).

c Tsao & Bailey (1953).

d Dubuisson (1950b).

e Dubuisson (1950c).

f Snellman & Erdős (1948a), buffer: 0.1-0.5M-KCl + 0.05M K veronal-acetate.

(Connell, unpublished) it has been noted that pyrophosphate both in the presence or absence of added magnesium and at 0° or 25° reduces the viscosity of cod actomyosin solutions only partially when compared with the reduction achieved by ATP.

It has been concluded (Weber & Portzehl, 1952) that the extractability of *F*-actin from muscle depends to a large extent on the state of the insoluble muscle structures which, unless disrupted in some way, impede the removal of the long *F*-actin microfibrils. This may well be an important factor in the case of extraction of *F*-actin in cod, because the amount of stroma is very low in fish, and its 'permeability' may be greater. In addition, the form in which actin is present in fresh fish muscle may be different, that is, its microfibrils may be shorter or thinner than those in rabbit.

Cod actin closely resembles rabbit actin; it can exist in both the *G*- and *F*-forms, and its physical and chemical properties are similar to those of rabbit actin (Table 1). The high mobility of actin on the alkaline side of its isoelectric point is reflected in the large number of acidic groups demonstrable at pH 11.5 (26.3 equivalents/10⁴ g. protein); actin, together with tropomyosin, must be among the most highly charged proteins. The osmotic pressure data show that the molecule of cod *G*-actin must exist as the dimer in 0.6M-KI (Tsao, 1953), and the similarity at 0° and at 25° of intrinsic viscosity and diffusion coefficient shows that the axial ratio cannot change much between these two temperatures. The estimated sedimentation coefficient of

1.0×10^{-13} for the one sample of *G*-actin does not appear to be reliable, since when taken together with the value of $2.3 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ for the diffusion constant it yields a value of about 40 000 for the molecular weight—much lower than that derived from osmotic pressure. Better agreement is obtained if the value of the sedimentation coefficient found for rabbit *G*-actin is used, namely about 3.2×10^{-13} (Snellman, Erdős & Tenow, 1949; Portzehl *et al.* 1950; Mommaerts, 1952), giving a molecular weight of about 130 000. Also, the axial ratio assuming a prolate ellipsoidal model and correcting for hydration as suggested by Oncley (1941), would be 25 using the values $D_{20,w}^0 = 2.3 \times 10^{-7}$ and $S_{20,w} = 3.2 \times 10^{-13}$, which agrees rather well with Tsao's (1953) value of 24 for an end-to-end dimer. However, this result is not supported by the very low intrinsic viscosity of 0.075 which when incorporated into Simha's (1940) equation, corresponds to an axial ratio of 5.5 for a hydrated prolate ellipsoid. The disparity between the values calculated from sedimentation-diffusion and viscosity data is very much greater than is usually encountered, but may be due to an unsuitable choice of hydrodynamic model. The data do not justify application of the theory of 'equivalent ellipsoids' (Scheraga & Mandelkern, 1953), but it is interesting to note that the use of the 'string of beads' model of Kuhn recently discussed by Shulman (1953) for fibrinogen results in the fairly close axial ratios of 5 (using $\eta = 0.075$) and 7 (using $S_{20,w} = 3.2 \times 10^{-13}$ and $D_{20,w}^0 = 2.3 \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$).

SUMMARY

1. Proteins resembling rabbit myosin and rabbit actin have been prepared from cod muscle.

2. Although most of the properties of cod and rabbit myosin are the same there is considerable apparent disparity between their ultracentrifugal properties.

3. Some possible causes of this disparity have been examined.

4. It is more difficult to prevent the concurrent extraction of actin when preparing fish myosin than with rabbit: possible reasons for this have been discussed.

5. The molecular size and shape of cod *G*-actin have been investigated.

6. In 0.6M potassium iodide solution, cod *G*-actin appears to exist as the dimer, though its exact molecular dimensions are uncertain.

The author is indebted to Dr R. A. Kekwick for the ultracentrifugal analyses. The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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