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Studies on the Proteins of Fish Skeletal Muscle

6. AMINO ACID COMPOSITION OF COD FIBRILLAR PROTEINS*

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Recent work (Connell, 1958*b*) has shown that the peculiar instability of cod as compared with rabbit myosin cannot be explained with reference to the picture of the molecular weight or shape of the fish protein revealed by sedimentation, diffusion and viscosity. It was therefore decided to see whether any features of the amino acid composition of cod myosin distinguished it from rabbit myosin.

The other main fibrillar protein, actin, has been included in the investigation since it was also of interest to compare the amino acid composition of this protein from two different species. In addition, cod 'natural' actomyosin, that is actomyosin prepared directly from cod muscle, has been analysed both because of its importance as a model substance and because an idea of the relative amounts of myosin and actin in the complex can be obtained by comparing their amino acid compositions. Since the method of Hasselbach & Schneider

(1951) cannot be applied to fish because the solution used to extract myosin also extracts considerable amounts of actin (J. J. Connell, unpublished work), the indirect determination of myosin and actin in natural actomyosin would seem to be the only way of measuring the amounts of myosin and actin in fish muscle.

MATERIALS AND METHODS

Proteins. Myosin, actin and actomyosin were prepared from the skeletal muscle of cod (*Gadus callarias* L.) killed just beforehand as described by Connell (1958*b*). Myosin and actomyosin were precipitated from the final buffer solution by dialysis at 0° against many changes of distilled water for several days, and the precipitates were dried with acetone, ethanol-ether (1:3, v/v) and finally in air at room temperature. The actin was purified twice by removing unpolymerized material in the preparative ultracentrifuge, the final pellet of F-actin being depolymerized in 0.1 M-adenosine triphosphate solution, pH 8 (1 ml./10 mg. of protein), and salt removed by dialysis against distilled

* Part 5: Connell (1958*b*).

water for 4 days at 0°. The protein was then precipitated by adding 0.1N-HCl until the pH of the solution was about 4; the precipitate was washed twice with acetone and then with ethanol-ether (1:3, v/v); it was finally dried in air at room temperature.

The preparations of myosin, actin and actomyosin had 0.97, 0.29 and 1.94% of ash and 16.41, 16.40 and 16.50% of nitrogen (moisture- and ash-free), respectively.

Hydrolysis. Samples (30–50 mg.) of the proteins were weighed into 8 mm. × 75 mm. Pyrex test tubes and 0.5 ml. of freshly distilled constant-boiling HCl was added to each. The mixture was then frozen and the tube evacuated with an oil pump and sealed whilst still evacuated. The tubes were heated for 24 or 48 hr. in an oven at $110 \pm 0.5^\circ$, and the contents filtered to remove humin and made up to volume. The hydrolysates were kept frozen until analysed, when portions were removed and dried over NaOH pellets in a vacuum desiccator before application to the column of ion-exchange resin.

Determination of amino acids. The amino acids were separated by elution chromatography on columns of powdered (finer than 200 mesh) Amberlite IR-120 ion-exchange resin (CG-120, type II as supplied by British Drug Houses Ltd.) according to Moore, Spackman & Stein (1958). Fractions (2 ml.) of eluate were collected and analysed according to Moore & Stein's (1954) method with the exception that stannous chloride (0.08%) was used as the reducing agent.

Tryptophan was determined on samples of the intact protein by the method of Spies & Chambers (1949), and cystine plus cysteine by performic acid oxidation (Schram, Moore & Bigwood, 1954) followed by hydrolysis of the protein and separation of the cysteic acid on a 0.9 cm. × 15 cm. column of IR-120 (Moore *et al.* 1958).

A determination of the amino acids in a 24 hr. hydrolysate of crystalline bovine-serum albumin (Armour Laboratories, Chicago, Ill., U.S.A.) gave a result which was in satisfactory agreement with the published analyses for this protein (Moore & Stein, 1949), the recovery of nitrogen being 100.7%.

RESULTS

The amino acid compositions of cod myosin, actin and 'natural' actomyosin are given in Table 1. The results for threonine, serine and amide nitrogen were obtained by linearly extrapolating to zero time the results for 24 and 48 hr. hydrolyses. The arginine contents of the myosin and actomyosin 48 hr. hydrolysates were about 10% higher than those for the 24 hr. hydrolysates; the former values are given in Table 1. The results for all the other amino acids (except tryptophan and cystine plus cysteine) are the means of determinations on 24 and 48 hr. hydrolysates. Replicates generally agreed within 3%.

Also shown in Table 1 are the amino acid compositions of rabbit myosin and actin, and the percentage of cod myosin in cod actomyosin calculated from the results on each amino acid assuming that myosin and actin are the sole constituents of actomyosin.

DISCUSSION

It is evident from the results shown in Table 1 that the amino acid compositions of cod myosin and cod actin are closely similar to those of the rabbit proteins. Even if the other evidence (Connell, 1954, 1958*a, b*) is disregarded there can be little doubt that the cod-muscle proteins whose analyses are presented here are genuine counterparts of the proteins prepared from rabbit muscle. The figures for the cod proteins tend as a whole to be slightly lower than those for the rabbit proteins, particularly for lysine and glutamic acid. Part of this discrepancy probably can be ascribed to the rather low total recoveries of nitrogen of the cod-protein analyses (about 95%). A number of other small differences between the cod and rabbit results may be noted: aspartic acid is lower in cod actin, proline is higher in both cod proteins and amide is higher in cod myosin. A separate determination of the amide nitrogen of cod myosin by the method of Chibnall, Mangan & Rees (1958) gave a value of 81 g.mol. of amide nitrogen/10⁵ g. of protein, which is closer to the value for rabbit myosin. The value of the tryptophan content of cod actin is lower than the value for rabbit actin given by Kominz, Hough, Symonds & Laki (1954), but this difference is perhaps not significant since both Tsao & Bailey (1953) and Perry (1947) give the value for rabbit as 6 g.mol. of tryptophan/10⁵ g. of protein.

Myosin and actin therefore join the growing number of proteins which when isolated from different sources have amino acid compositions that are very alike; at least for two species, myosin fulfils the prediction which Bailey (1954) made for it in this respect. If differences between the amino acid compositions of cod and rabbit myosins are responsible for the different stabilities of the two proteins then these differences must be small and incapable of being detected by the present methods of amino acid determination.

The values for the proportion of myosin in cod 'natural' actomyosin calculated from each amino acid determination appear at first glance to vary enormously. However, it is clear that, since the error in the determination of each amino acid is approximately the same, the accuracy of the value calculated in this way will depend upon the differences between the quantities of individual amino acids in the myosin and actin. Where these differences are less than 1 g. of amino acid nitrogen/100 g. of protein nitrogen, that is for serine, alanine, valine, methionine, phenylalanine, tryptophan and cystine, the calculated values are so inaccurate as to be useless. If the values calculated from these amino acids are excluded and the remaining values weighted according to the difference between the quantities of individual

Table 1. Amino acid composition of cod fibrillar proteins compared with rabbit myosin and rabbit actin

	g.mol./10 ⁵ g. of protein									
	Myosin					Actin				
	Rabbit					Rabbit				
	(g. of N/100 g. of protein N)	Myosin (1)	Actin (2)	Acto-myosin (3)	Percentage of cod myosin in cod actomyosin $\frac{(3)-(2)}{(1)-(2)} \times 100$	Cod	(Kominz, Hough, Symonds & Laki, 1954)	Bailey (1948)	Cod	(Kominz, Hough, Symonds & Laki, 1954)
Aspartic acid		7.21	6.08	6.94	76	85	85	67	71	82
Threonine		3.04	4.81	4.04	44	36	41	43	56	59
Serine		3.87	4.71	4.61	12	45	41	41	55	56
Glutamic acid		12.31	7.56	10.94	71	145	155	150	89	101
Proline		2.51	4.35	2.60	83	25	22	17	51	44
Glycine		3.77	5.51	4.25	73	44	39	25	65	67
Alanine		6.03	5.80	6.03	100	71	78	73	68	71
Valine		3.75	4.22	3.99	49	44	42	22	49	42
Methionine		1.67	2.42	1.20	—	20	22	23	28	30
Isoleucine		2.95	4.89	3.42	76	35	42	119	57	57
Leucine		6.52	5.50	6.35	83	77	79		64	63
Tyrosine		1.26	2.77	1.43	89	15	18	19	32	32
Phenylalanine		1.98	2.30	1.96	—	23	27	26	27	29
Lysine		12.14	7.37	11.01	76	72	85	81	43	52
Histidine		3.33	5.26	3.95	68	13	15	15	20	19
Arginine		12.99	14.22	13.00	100	38	41	42	41	38
Tryptophan		0.62	1.29	0.75	80	3.7	3.9	3.9	7.6	10
Cystine/2		0.61	0.97	0.81	45	7.2	8.6	12	11.4	11.2
Amide N		8.45	5.20	8.15	90	99	86	86	61	66
Total		94.65	95.28	95.43						

amino acids in myosin and actin, the values calculated from the lysine and leucine figures receiving the greatest and least weights respectively, the average value obtained is about 73 %. The error in this weighted average cannot be calculated simply but is probably about 6–7 %. The ratio of myosin to actin in cod 'natural' actomyosin is therefore between 2 and 4:1; this is also probably close to the ratio between the two proteins in whole cod muscle since cod 'natural' actomyosin makes up practically the whole of the fibrillar protein of cod (Dyer, French & Snow, 1950) and therefore contains all the myosin and actin of the muscle. It appears that the ratio of myosin to actin in cod muscle is similar to the ratio of close to 3:1 found for rabbit muscle by Hasselbach & Schneider (1951). However, the actual quantities of myosin and actin in cod muscle are likely to be greater than those in rabbit muscle since the amount of fibrillar protein is greater in cod than in rabbit (for summary see Bailey, 1954).

SUMMARY

1. The amino acid compositions of cod myosin, cod actin and cod 'natural' actomyosin have been determined.
2. Cod myosin and cod actin closely resemble the corresponding rabbit proteins in amino acid composition.
3. The ratio of myosin to actin in cod 'natural' actomyosin was found to lie between 2 and 4:1 and therefore approximates to the corresponding ratio in rabbit muscle.

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A Quantitative Method for the Chromatographic Separation of 17-Oxo Steroid Sulphates from 17-Oxo Steroid Glucuronides: with Observations on the Behaviour of Conjugated Corticosteroids on the Same System

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Steroid metabolites are excreted in the urine mainly as glucuronide or sulphate conjugates. Although it is possible to distinguish between these two forms of conjugation by specific hydrolytic

procedures and to separate them physically by solvent partition after acetylation (Kellie & Wade, 1956), neither process gives the steroid conjugates in unmodified form. The former process liberates free steroids and the latter acetylated glucuronides which can no longer be hydrolysed by β -glucuronidase or oxidized by bismuthate. A method has been evolved in which, by taking advantage of the adsorption of the conjugates on neutral alumina,

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