CLXXV. COMPOSITION OF THE MYOSINS AND MYOGEN OF SKELETAL MUSCLE

By KENNETH BAILEY

From the Biochemical Department, Imperial College of Science and Technology, South Kensington, London, S.W. 7

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THE individual proteins of skeletal muscle are sufficiently well characterized to warrant a detailed investigation of their composition. There are in muscle at least three salt-soluble proteins, the globulin myosin, the albumin myogen and a further globulin termed by Weber [1933] globulin X, which separates when pressed muscle juice, free from myosin, is dialysed. Recently, Smith [1937] has postulated the presence of small amounts of another albumin, myoalbumin, which has a lower isoelectric point than that of myogen. Of these proteins, myosin occurs in the greatest proportion; it is characterized by an isoelectric point at *p*H 5·5, and in the absence of salt at neutrality forms a gelatinous thixotropic precipitate. Although its solubilities are typically those of a globulin, it is almost unique in exhibiting double refraction of flow [Muralt & Edsall, 1930], a property possessed by long anisotropic particles such as are found amongst the plant virus proteins. Weber & Stöver [1933] assess the particle weight of myosin as high as $0{\cdot}6$–$1{\cdot}2 \times 10^6$, decreasing in urea solution to 100,000. In terms of the theory of denaturation put forward by Astbury *et al.* [1935], a high-molecular elongated molecule of this nature is configurationally disposed towards denaturation, and the ease with which myosin sols become denatured, on standing, on warming, or by addition of small amounts of alcohol is a characteristic which distinguishes it from many other globulins.

The physical properties of denatured myosin films, correlated with a study of their X-ray diffraction patterns suggest a configuration closely allied to that of keratin [Astbury & Dickinson, 1935]. Thus, according to the degree to which such films are stretched, X-ray patterns resembling the α- and β-keratin states may be obtained. On exposure to steam, the β-form is "set" at the expense of the α-modification as in the case of stretched hair; both types of protein are reversibly elastic, and unstretched myosin films exhibit the phenomenon of supercontraction as does the labile form of keratin in which the cystine and other bridges between adjacent peptide chains have been dissolved. In a highly hydrated elastic mechanism such as myosin, the disulphide cross linkage typical of keratin is hardly conceivable and the incorporation of large amounts of cystine would seem unnecessary. It is known that the cystine content of whole muscle is low, but it seemed of great interest to ascertain whether the myosins prepared from representative species of several metazoic classes contained a uniformly low proportion of cystine, just as the keratins as a group are characterized by a high cystine content. The S content of keratin, however, which is almost wholly attributable to cystine, is by no means uniform, and varies from 1·35 % in porcupine quill to 5·1 % in human hair.

Apart from any analogy with keratin, it is of even greater interest to ascertain whether in the course of evolution a fundamental amino-acid composition has been elaborated for the myosins of all species, or whether differences are

detectable. For this purpose, values have been obtained for total N, amide N, total S, cystine, methionine, tyrosine and tryptophan. The myosins selected were representative of the crustaceans (lobster), fish (whiting), birds (chicken), mammals (rabbit, dog, ox); of the myogens, only that of rabbit has been analysed.

The extent to which analyses of myosin contribute to the analysis of whole muscle may be gauged from existing data on the partition of N between the various proteins of muscle. Earlier figures (e.g. those of Weber [1933] and of Smith [1934]) assess the myosin content of rabbit muscle at a value lower than that recorded by Smith's most recent analysis [1937]. As a percentage of the total intracellular N, it is seen (Table I) that the proportion of myosin in rabbit muscle is comparable with that of fish, but the proportions of stroma in the two cases are different. Discussion of the figures for myogen, globulin X and myoalbumin is not germane to the present investigation, but these should not be accepted without reference to the original papers.

Table I. *Partition of N between the proteins of skeletal muscle*

(g. N/100 g. of total coagulable N)

Muscle	Stroma protein	Intracellular protein	Composition of intracellular fraction (N as percentage of total intracellular N) Myosin	Myogen	Globulin X	Myoalbumin
Rabbit [Smith, 1937]	15	85	67·5	9	22·5	1
Rabbit [Weber, 1933]	17	83	47	26·5	26·5	—
Haddock [Reay & Kuchel, 1936]	3	97	69	18·6*	Residual intracellular 12·4	

* This figure includes globulin X if present in fish muscle.

Existing analyses of pure myosin and myogen are relatively few, and are collected in Table II for comparison with results reported in this paper.

Table II. *Previous analyses of muscle proteins*

(Results expressed as a percentage of protein dry weight)

Protein	Author	N	S	P	Tyrosine	Tryptophan	Cystine
Rabbit myosin	Weber [1933]	—	1·0	0·09	~5	~1·3	—
,,	Todrick & Walker [1937]	15·3	0·94	—	—	—	0·27*
,,	Mirsky [1936]	—	—	—	—	—	0·67
Cow cheek myosin	Folin & Marenzi [1929]	—	—	—	3·92	0·98	—
Rabbit myogen	Weber [1933]	—	0·77	0·028	~6	~1·5	—

* As cysteine.

N and S figures are lower than those obtained by the author, whilst tyrosine and tryptophan figures are higher. According to Todrick & Walker, native myosin at *p*H 7·6 contains 0·27–0·3 % of —SH groups, i.e. approximately 35 % of the total cystine content. Dried preparations of myosin even when freshly prepared give no evidence of —SH groups in the hydrolysate, yet in the denatured preparations of Mirsky & Anson [1934–35], the alleged —SH groups were exposed to temperatures of 108° without apparent destruction. The specificity of many methods for the determination of sulphydryl in denatured and undenatured proteins is on the whole somewhat questionable, and further discussion will be deferred to a future investigation of the —SH groups of myosin.

Experimental

Preparation of proteins

For myosin, the procedure of Edsall [1930] was followed. The buffered KCl extract of the minced muscle was poured into 20 vol. of ice-cold water, and the precipitated protein after centrifuging was redissolved in KCl, filtered through paper pulp and reprecipitated. After centrifuging and washing, it was dried in several grades of alcohol, then in ether and finally *in vacuo* over P_2O_5. All specimens of rabbit and dog myosin were prepared from the hindlimb muscles and longissimus dorsi after perfusion of the animals with ice-cold 1% saline via the thoracic aorta. Only the abdominal and pincer muscles of the lobster and the breast muscle of chicken were utilized. Specimens of fish myosin were supplied by Dr G. A. Reay of the Torry Research Station; some samples of rabbit myosin and all samples of rabbit myogen were supplied by Mr E. C. Bate Smith of the Low Temperature Research Station, Cambridge. The preparation of myogen is described in a recent paper [Smith, 1937].

Sulphur distribution

Methods and conditions of hydrolysis have been described in a previous paper [Bailey, 1937], but several additional comments are necessary.

Methionine. The determination of methionine on specimens of myosin dried by alcohol is not possible, since even on further drying at 108°, traces of alcohol are retained by the protein, and these lead to the production of volatile iodide with HI. If however the protein is allowed to swell in 20 times its bulk of 0·1 *N* NaOH, and after addition of an equivalent amount of HCl, is evaporated to dryness, so removing volatile alcohols, the methionine value is then equal to that obtained after preliminary hydrolysis of the protein with HCl. The possibility of the occurrence of labile methoxyl or ethoxyl groups arising from esterified carboxyl groups was not overlooked. No alcohol could be detected by the sensitive test of Schryver & Wood [1920] when freshly precipitated myosin was heated with dilute alkali, whereas positive tests were obtained with alcohol-dried samples. The amount of alcohol retained by samples of rabbit myosin after drying overnight at 108° is of the order of 0·2%.

Cystine. The colorimetric determination of cystine in myosin, as in all proteins of low cystine content, is rendered less accurate by the colour contributed by the hydrolysate. This error is least in Lugg's modification [1932] of the Folin-Marenzi method, where it is included in the correction for extraneous reducers. The relation between the cystine content of myosin and time of hydrolysis was investigated in the cases of rabbit and chicken myosins (Table III). At all points the Folin-Marenzi-Lugg value exceeds the Sullivan-Lugg, and at 43 hr. both values show a decrease due apparently not only to

Table III. *Relation between the cystine content of myosin and time of hydrolysis*

	Time of hydrolysis hr.	Cystine %	
		Folin-Marenzi-Lugg [Lugg, 1932]	Lugg [1933]
Rabbit myosin	7	0·77	0·65
	15	0·76	0·61
	43	0·53	0·42
Chicken myosin	7	0·90	0·81
	15	0·85	0·74
	43	0·57	0·45

deamination or decarboxylation, but also to reactions involving the disulphide group. 15 hr. were adopted as the standard period of hydrolysis, in conformity with conditions obtaining in the previous paper [Bailey, 1937].

Uniformity of specimens with respect to S distribution

At least two specimens of each myosin type have been analysed, and the results are expressed in an abbreviated form in Table IV. All samples *inter se* show some variation in cystine content, but divergences in the total S, methionine and cystine are most marked in fish myosin. This latter is in fact more difficult to prepare than mammalian myosin, since the KCl sol is often viscous and difficult to filter.

Table IV. *Uniformity of myosins with respect to S distribution*

Results expressed as a percentage of protein dry weight (ash-free)

Protein	Source of protein	Total S	Methionine	Total (*S–S*) Folin-Marenzi-Lugg method	N
Rabbit myosin (AA)	E. C. Bate Smith	1·10	3·4	0·82	16·7
(AB)	,,	1·11	3·4	0·84	16·6
(AC)	J. G. Sharp	—	3·4	0·82	16·7
(AD)	Author	1·10	3·4	0·77	16·7
Dog myosin (BA)	,,	1·06 (Schoeller)	3·3	0·80	16·5
(BB)	,,	1·06	3·35	0·72	16·6
Chicken myosin (CA)	,,	1·10	3·4	0·92	16·7
(CB)	,,	1·12	3·4	0·85	16·6
Fish myosin (DA)	G. A. Reay	1·19 (Schoeller)	3·6	0·98	16·5
(DB)	,,	1·14	3·4	0·85	16·7
(DC)	,,	1·21	3·65	0·94	16·6
Lobster myosin (EA)	Author	1·17	3·4	1·0	16·7
(EB)	,,	1·21	3·45	0·92	16·6
Rabbit myogen (FA)	E. C. Bate Smith	1·29	2·8	1·96	16·6
(FB)	,,	1·26 (Schoeller)	2·9	1·90	16·6

Selecting representative analyses from each group, these results are expanded in Table V.

The total S in mammalian and chicken myosins varies between 1·06 and 1·12 %, but in fish and lobster myosins it rises to 1·21 %. The S of myosin hydrolysates, after removal of humin at *p*H 2·5 is less than that of the protein itself. Direct determination of humin-S and S evolved as H_2S during hydrolysis accounts for 3–4 and 1–2 % of the total S respectively, and these figures are in accord with the observed differences in S content of protein and hydrolysate. The methionine values, with the exception of fish myosin, are uniform. The total disulphide of fish and lobster myosins is greater than that of chicken myosin, and this in turn is higher than those of the mammalian myosins. In all cases the Folin-Marenzi-Lugg method gives results considerably higher than the corresponding Sullivan methods, and even on the assumption that the total disulphide is derived solely from cystine, the average S recovery in terms of $(S-\bar{S})$ and methionine is 85 % of the total S, and 89 % of the S in the hydrolysate; when on the other hand the Sullivan-Lugg value for cystine is utilized, the corresponding figures are each 3 % lower. Little importance can be attached to

Table V. *S. distribution of muscle proteins*

Results expressed as a percentage of protein dry weight

Protein	S on protein	S on hydrolysate after removal of humin at *p*H 2·5	Methionine	Cystine: Folin-Lugg	Cystine: Marenzi-Lugg	Cystine: Sullivan	$\frac{(S-S)+M_s}{P_s}$ %	$\frac{C_s+M_s}{P_s}$ %	$\frac{(S-S)+M_s}{H_s}$ %	$\frac{C_s+M_s}{H_s}$ %
Rabbit myosin (AD)	1·10	1·06	3·4	0·77	0·62	0·53	85·3	81·6	88·5	84·7
Dog myosin (BB)	1·06	1·02	3·35	0·72	0·58	0·50	86·1	82·6	89·5	86·0
Chicken myosin (CB)	1·12	1·06	3·43	0·85	0·74	0·60	86·2	83·7	91·1	88·3
Fish myosin (DC)	1·21	1·15	3·65	0·94	0·83	0·72	85·7	83·3	90·2	87·6
Lobster myosin (EB)	1·21	1·15	3·45	0·92	0·75	0·65	81·7	78·0	86·0	82·1
Rabbit myogen (FA)	1·29	1·22	2·8	1·96	1·86	1·80	87·3	85·3	92·3	90·2
				Average S recovery for myosin			85·0	82·0	89·0	86·0

the Sullivan values in the case of myosin hydrolysates, since the *p*H at which colour development takes place is largely fortuitous, depending on the volume of hydrolysate used in each determination. Any error induced by a lowering of *p*H will be most marked in proteins of low cystine content, as Lee [1935] showed in experiments with casein. The S distribution of rabbit myogen readily distinguishes it from rabbit myosin; the total S and cystine contents are higher, and the methionine lower.

Determination of tyrosine and tryptophan

Lugg's [1937] colorimetric method was adopted. The determination depends upon the simultaneous formation of a soluble tyrosine-mercury and an insoluble tryptophan-mercury complex, which are separated by centrifuging. The tryptophan compound is dissolved in $12N$ H_2SO_4 containing $HgCl_2$ and $HgSO_4$, and both tryptophan and tyrosine are determined in their respective solutions by comparing the colour which develops in presence of nitrite with that of a standard treated in an identical manner. The method is a considerable improvement on the Folin & Ciocalteu [1927] method. Not only have the conditions for the production of the mercury compounds been studied in great detail, but the faint cloudiness which sometimes develops in the tyrosine assays is avoided, and the procedure for determination of tryptophan is entirely changed and simplified. The hydrolysis of the protein was effected by digesting 0·32 g. of protein with 3 ml. of $5N$ NaOH at 100° in a sealed tube for 20 hr. After cooling and diluting, the hydrolysate was adjusted to *p*H 2·5 with $5N$ H_2SO_4, centrifuged and the silica precipitate produced from the glass by alkali erosion was washed 4 times with water and twice with $0{\cdot}1N$ H_2SO_4. The hydrolysate and washings were then diluted to 25 ml. and stored in the dark. 2 ml. of this solution were compared, in the case of myosin hydrolysates, with a standard containing 1 mg. of tyrosine and 0·25 mg. of tryptophan. In the case of edestin (included in the series to allow of comparison with older methods) and of rabbit myosin, there was a slight destruction of tryptophan and tyrosine with longer periods of hydrolysis (Table VI).

Table VI. *Relation between tyrosine and tryptophan contents and period of hydrolysis*

Protein	Period of hydrolysis hr.	Tyrosine %	Tryptophan %
Edestin	20	4·24	1·43
	36	4·13	1·46
	67	4·10	1·36
Rabbit myosin	20	3·38	0·84
	38	3·33	0·83

It is important to note that Folin & Marenzi [1929], using a specimen of Osborne's edestin, obtained figures in excellent agreement with those quoted for the 20-hr. hydrolysis, viz. tyrosine 4·28 %, tryptophan 1·46 %. The values obtained for a series of myosins and rabbit myogen are tabulated in Table VII.

Table VII. *Tyrosine and tryptophan contents of muscle proteins*

Protein	Tyrosine %	Tryptophan %
Rabbit myosin (AC)	3·38	0·84
(AB)	3·38	0·82
(AD)	3·40	0·82
Dog myosin (BB)	3·28	0·76
Ox cheek myosin	3·22	0·84
Chicken myosin (CB)	3·27	0·80
Fish myosin (DB)	3·90	0·82
(DC)	4·30	0·95
(DE)	4·40	1·10
Lobster myosin (EB)	3·58	0·85
Rabbit myogen (FD)	4·21	1·51

Attention may be drawn to the following points: (1) The values obtained for rabbit myosin prepared by different workers show excellent agreement. (2) These values agree reasonably well with those of dog, ox cheek and chicken myosins. (3) The tyrosine contents of fish and lobster myosins are somewhat higher, but different samples of fish myosin do not agree *inter se*. (4) Rabbit myogen is richer in tyrosine and tryptophan than the corresponding myosin, the values being comparable with those of edestin. (5) In comparison with the tryptophan contents of many food proteins, e.g. casein, egg albumin and lactalbumin, the myosins are relatively poor in tryptophan.

Amide-nitrogen

The conditions of hydrolysis for the liberation of amide-N were studied by Vickery [1922] in the case of wheat gliadin, and by Shore *et al.* [1936] in the case of ovalbumin. Amide-N is set free very readily by boiling with dilute acids, but higher values are obtained if peptide hydrolysis of the protein is completed by use of stronger acids; this increase is usually attributed to deamination and to a slight breakdown of arginine. In the computation of the base-binding capacity of a protein the amide value obtained after mild hydrolysis of the protein is usually utilized. 0·3 g. of protein was refluxed with 10 ml. of 2*N* HCl for 3 hr., and the hydrolysate, after cooling, was neutralized to thymolphthalein, and aliquots were subjected to distillation in a micro-Kjeldahl apparatus. Complete hydrolysis for 15 hr. with 5*N* HCl gave an apparent increase of 6–7 % in the amide-N of edestin and rabbit myosin. Results are recorded in Table VIII.

The mammalian and avian myosins again show excellent agreement, whilst fish and lobster myosins, and rabbit myogen give lower values. The total N of

Table VIII. *Amide-N of muscle proteins*

Protein	Total N %	Amide-N as percentage of total N
Edestin	18·4	9·4
,, (complete hydrolysis)	—	10·0
Rabbit myosin (AD)	16·7	7·20
,, (complete hydrolysis)	—	7·66
Dog myosin (BB)	16·6	7·17
Ox cheek myosin	16·6	7·23
Chicken myosin (CB)	16·6	7·00
Fish myosin (DC)	16·6	6·66
Lobster myosin (EB)	16·6	6·75
Rabbit myogen (FD)	16·6	5·53
,, (FE)	16·6	5·30

all myosins and myogen, reported here and in previous tables are, within the limits of experimental error, identical.

Collected results and discussion

The analyses reported in previous sections are collected in Table IX.

Table IX. *Representative analyses of muscle proteins*

(Except where otherwise stated, results are expressed as percentages of protein dry weight)

		Myosins					
	Rabbit myogen	Rabbit	Dog	Ox cheek	Chicken	Fish	Lobster
N	16·6	16·7	16·6	16·6	16·6	16·6	16·6
S	1·29	1·10	1·06	—	1·12	1·21	1·21
Amide N as percentages of total N	5·53	7·20	7·17	7·23	7·00	6·66	6·75
Cystine:							
(Folin-Marenzi-Lugg) (*a*)	1·96	0·77	0·72	—	0·85	0·94	0·92
(Sullivan-Lugg)	1·86	0·62	0·58	—	0·74	0·83	0·75
Methionine (*b*)	2·8	3·4	3·35	—	3·43	3·65	3·45
Tyrosine (*c*)	4·21	3·38	3·28	3·22	3·27	4·30	3·58
Tryptophan (*d*)	1·51	0·82	0·76	0·84	0·80	0·95	0·85
Sum of (*a*), (*b*), (*c*) and (*d*)	10·48	8·37	8·11	—	8·35	9·84	8·80

The sum of total $(S-S)$ groups (as cystine), methionine, tyrosine and tryptophan is highest in rabbit myogen; the corresponding figures for rabbit, dog and chicken myosins are uniform, the maximum variation from the mean of these 3 values being 2%; both lobster and fish myosins give a higher total, but deviation is most marked in the latter. Reviewing the analyses of different specimens of the same myosin, and of specimens of different myosins, it may be concluded that the mammalian and avian myosins, in so far as they have been analysed, show only minor analytical differences, and that these proteins are elaborated according to a precise amino-acid plan. The myosins showing most differences *inter se*, i.e. fish and lobster myosins, also show the greatest analytical deviations from the other myosins examined. But in all myosins, the general order of magnitude of each amino-acid constituent is the same. It should be noted, moreover, that cystine and tryptophan on the one hand, and methionine and tyrosine on the other, are present in the same proportions. As in the keratins there are variations in the cystine content, but the order of variation is not as large. In general, methionine has been elaborated in preference to cystine; of the 37 proteins examined by Baernstein [1932], only 3, exclusive of the muscle preparations, have methionine contents exceeding that of myosin. In

the keratins the conditions are reversed, and only small amounts of methionine occur. Comment has already been made on the fact that in the keratins, cystine plays an integral part in maintaining a specific molecular configuration, and although the denatured form of myosin is structurally related to keratin, and in particular to the supercontracting form of keratin, it is evident that the properties of native myosin and of muscle itself militate against the assignation of a similar role in the case of muscle proteins. The observed differences in the S distribution of these two classes of proteins, the keratins and myosins, are therefore comprehensible.

SUMMARY

1. The total N, amide N, total S, cystine, methionine, tyrosine and tryptophan of a series of myosins from skeletal muscle have been determined. Except for minor analytical differences, mammalian and avian myosins possess a uniform composition, but larger deviations are observed in the case of lobster and fish myosins. In all myosins analysed, the general order of magnitude of each amino-acid constituent is similar, and points to the existence of a fundamental amino-acid plan for the elaboration of these proteins.

2. Rabbit myogen differs markedly in composition from rabbit myosin.

3. The total disulphide content, expressed as cystine, of all myosins examined varies between 0·72 and 1·0 % and the content of methionine between 3·35 and 3·65 %. The average recovery of S in terms of total disulphide and methionine as a percentage of the S in the protein hydrolysate is 89 %. It appears that methionine is elaborated in preference to cystine, and the significance of this fact is discussed in relation to the properties of denatured myosin films which are structurally related to the labile supercontracting form of keratin.

4. The tryptophan content of the myosins in comparison with those of other food proteins (e.g. casein and egg albumin) is relatively low.

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