SUBFRAGMENT 1 OF COW CAROTID MYOSIN

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1. Introduction

This enzymatically active subunit of smooth muscle myosin has not so far been isolated. Previous work on cow carotid myosin [1] and on chicken gizzard myosin [2] has revealed that these myosins are, as cardiac myosin [3, cf. 4] much more resistant to tryptic digestion than rabbit skeletal myosin. Furthermore, if the L-meromyosin (LMM) obtained behaves on the whole similarly to its skeletal counterpart, the H-meromyosin (HMM) aggregates in the course of the digestion into a faster sedimenting component of higher intrinsic viscosity [1]. This alteration can be avoided by the addition of 2 mM β-mercaptoethanol or by taking advantage of the stabilizing influence of actin [5]. As cow carotid HMM is rapidly overdigested and the corresponding subfragment 1 (HMM S-1) is fairly stable in the presence of actin, homogeneous solutions of this subunit can be prepared after tryptic digestion of cow carotid actomyosin.

2. Materials and methods

Each preparation of actomyosin was isolated from 300–600 g of minced cow carotids previously freed of adventitia as described earlier [6]. A 0.8% solution dissolved in 0.12 M tris, 0.1 M HCl, 0.25 M NaCl (pH 7.4) was digested with trypsin (Worthington) at a ratio of 450:1 by weight in a water bath at 25°; a threefold quantity of soybean trypsin inhibitor (Worthington) was added routinely after 60 min. For the following experimental procedures, the readers are referred to previous publications: preparation of cow carotid F-actin [7]; estimation of the protein content [6]; sedimentation velocity runs [8]; vertical starch gel electrophoresis at pH 8.6 [9]; Ca-ATPase activity at pH 7 and in the presence of 0.05 or 0.5 M KCl [5]; liberation of the light chains in the presence of monoiodoacetate by succinylation [8, 10] or by treatment at pH 11 [11]. The sedimentation equilibrium centrifugations and the corresponding diffusions were carried out at 5°. To calculate the ultracentrifugal results, use was made of the partial specific volume of rabbit skeletal HMM S-1 of 0.742 at 20° [12]. Amino acid analysis was made as before [6] but after hydrolysis for 24, 48 and 72 hr at 107°. The physiological fluid column was used for the detection of 3-methylhistidine [13].

3. Results

3.1. *Isolation*

The proteolytic digestion of cow carotid actomysin is followed by ultracentrifugation in the presence of 0.2% ATP and 10 mM Mg^{2+}, by viscometry and by Ca^{2+}-ATPase activity determinations. All subsequent purification steps are carried out in the presence of 2 mM β-mercaptoethanol. In order to isolate the actosubfragment 1, the 60 min digest is centrifuged twice for 1 hr at 106,000 *g* (Spinco Model L-50), the complex being redissolved with a homogenizer. F-Actin is removed by a first centrifugation in the presence of 0.6% ATP and 10 mM Mg^{2+}, for 150 min at 165,000 *g* and by a second one without ATP for 60 min at 106,000 *g*. The crude subfragment 1 (fig. 1, upper diagram) is freed from the slow sedimenting impurity of about 2.8 S corresponding apparently to

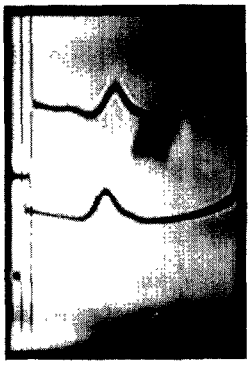

Fig. 1. Ultracentrifugal pattern of 0.3% (w/v) subfragment 1 before (upper diagram) and after (lower diagram) phosphate fractionation. Conditions of ultracentrifugation: 64 min at 59,780 rpm in a phosphate-NaCl buffer of pH 7.1 and ionic strength 0.35, double sector cells, angle 55°.

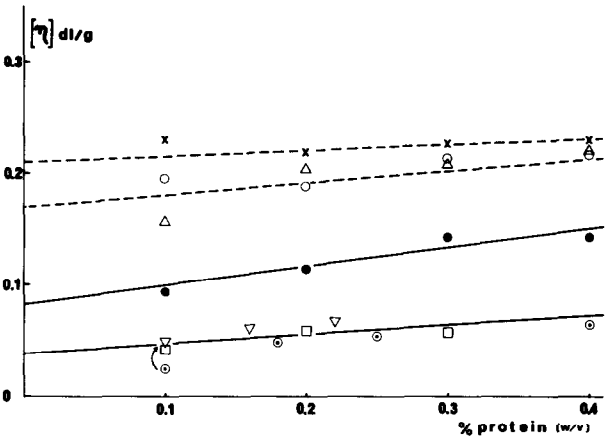

Fig. 2. Intrinsic viscosity of the subfragment 1 before (△, ○, x, ●) and after filtration (▽, ⊙, □) on Sephadex G-200, in a phosphate-NaCl buffer of pH 7.1 and ionic strength 0.35, 2 mM β-mercaptoethanol.

subfragment 2 [14] by phosphate fractionation in view of its higher stability in the presence of phosphate than in that of ammonium sulphate; the solution is made 1.75 M by addition of a cold 3.6 M K_2HPO_4, 0.4 M NaH_2PO_4 solution and the precipitate separating out on the top of the centrifuge tube by centrifugating 20 min at 23,500 *g* is isolated by filtration on a sintered glass filter (fig. 1, lower diagram). A final purification step similar to that used for subfragment 1 of rabbit skeletal myosin [15, 16] is achieved by filtration on Sephadex G-200. From 28 to 47% of unretarded material made of aggregated subfragment 1 separates from the main peak which is concentrated by vacuum dialysis with Selecta Ultrathimbles no 100 (Schleicher and Schüll, Dassel, Germany).

3.2. *Homogeneity and properties*

The purified subfragment 1 sediments as a single peak devoid of the slower and faster components of fig. 1. Sedimentation equilibrium centrifugations at 6,164 rpm and 5° give plots of log *C* versus r^2 which are linear over the greater part of the 1.9 mm solution column. Starch gel electrophoreses give a unique wide band whose trailing is likely due to a slight adsorption on the starch gel.

The comparison of our results with data of the literature (table 1) shows an excellent agreement of the various characteristics determined by ultracentrifugation at low or high speed. But the intrinsic viscosity of cow carotid HMM S-1 extrapolates to only 0.04 ± 0.002 dl/g (mean of three preparations) at zero concentration (fig. 2). Higher and less reproducible figures extrapolating to 0.17–0.21 dl/g (three preparations) or 0.08 dl/g (one preparation) are obtained before gel filtration.

Apart from this peculiarity, cow carotid HMM S-1 behaves as the other subfragments 1. It combines with cow carotid F-actin giving a complex of high viscosity sedimenting rapidly, which is split by ATP.
Its succinylation or treatment at pH 11 in the presence of monoiodoacetate liberates a low molecular weight fraction which can be detected by ultracentrifugation at high speed, as in the case of the corresponding HMM [8]. Its Ca-ATPase activity at neutral pH is about twice that of cow carotid myosin as well in the presence of 0.05 M as in the presence of 0.5 M KCl and shows the slight increase with the ionic strength typical of the smooth muscle myosins [2, 5, 17].

3.3. *Composition*

Some specific variations shown in table 2 appear to distinguish the cow subfragments 1 from their rabbit skeletal counterpart: their contents in arginine and aspartic acid are higher and that in tyrosine lower. Cow carotid HMM S-1 appears on the other hand to contain less alanine but its main difference lies as far as we can judge from the available analytical data in the lack of 3-methylhistidine.

Table 1
Physical-chemical parameters of HMM S-1 of various origins.

	Cow carotid	Cow heart [3]	Rabbit skeletal muscle
Extrapolated sedimentation coefficient $\times 10^{13}$	5.7	~5.6	5.8 [14] 5.75 [15] 5.95 [16, 20] 5.78 [21]
Concentration dependence of S*	$k = 0.015$	–	$k = 0.021$ [14] $k = 0.019$ [16] $k = 0.037$ [21]
Molecular weight $\times 10^{-3}$	110 ± 5	110 ± 10	115 ± 5 [14] 117 – 121 [15] 118 [22]
$D^{5}_{20,w}$ mg/ml $\times 10^{7}$**	3.89	–	3.60 [12] 3.73 [16]
Extrapolated intrinsic viscosity (dl/g)	0.040 ± 0.002	–	0.08–0.11 [12] 0.064 [14] 0.09 [15, 21]

* Corresponding to the angular coefficient k of the formula $1/S = 1/S_0 + kc$ where c is given in g per 100 ml.
** Measured by ultracentrifugation at low speed except in Mueller [12].

Table 2
Amino acid compositions of HMM S-1 of various origins (expressed as residues/10^5 g).

Amino acid	Cow carotid*	Cow heart (Tada et al., 1969) [3]	Rabbit skeletal muscle (Mueller, 1965) [12]	(Young et al., 1965) [15]	(Jones and Perry, 1966) [16]	(Lowey et al., 1969) [14]
Lys	80 ± 8	90	64	94	71	83
His	15 ± 2	17	13	16	15	18
Arg	45 ± 3	42	25	29	32	34
Asp	107 ± 1	99	88	89	91	85
Thr	49 ± 0	40	47	46	50	49
Ser	36 ± 1	39	41	33	43	41
Glu	130 ± 3	175	130	131	126	117
Pro	32 ± 2	25	26	21	30	37
Gly	52 ± 1	44	53	51	55	61
Ala	63 ± 3	72	72	70	67	70
Cys	11 ± 0	9.3	10	–	10	11
Val	42 ± 0	34	38	44	51	55
Met	30 ± 0	26	27	28	29	28
Ile	41 ± 1	38	37	48	52	53
Leu	74 ± 2	100	71	89	73	75
Tyr	21 ± 2	17	26	25	31	34
Phe	48 ± 3	33	43	41	46	52
Try	–	4.6	–	–	–	–
3-Methylhis**	0	–	–	–	–	–
Total	876	904.9	811	855	872	903

* Average composition from 2 separate preparations.
** Rabbit skeletal HMM S-1 contains 1 residue per mole or 129,000 g [13].

4. Discussion

Although trypsin has been used initially for the isolation of HMM S-1 from HMM, papain has been preferred more recently because it also splits the bonds attaching the enzymic globules to the structural part of HMM [3, 14] and allows the isolation of HMM S-1, without impairment of the ATPase activity, from cow cardiac myosin which is resistant to proteolytic digestion [3]. As cow carotid myosin belongs to this type of myosins [1], the protection of the active site by actin also appears to be a valuable method in the prevention of this alteration. The subunit isolated in this way retains apparently unaltered the ability of this part of the parent myosin to bind actin and to split ATP. Its molecular parameters also appear to be very similar to those of the other subfragments 1 except from the point of view of its shape. The present results are at variance with earlier viscosimetric determinations suggesting that HMM S-1 is a prolate ellipsoid of revolution [15] or a somewhat elongated globular particle [12]. As globular proteins, such as hemoglobin and chymotrypsin, have an intrinsic viscosity of about 0.035 dl/g [18], our results suggest that cow carotid HMM S-1 has a globular or nearly globular shape similar to that shown by electon micrographs [14, 19]. Further work will show if this discrepancy is related to a difference in shape or to an overestimation of the viscosity due to the presence of aggregated material.

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References

[1] F.Huriaux, Angiologica 2 (1965) 153.
[2] M.Bárány, K.Bárány, E.Gaetjens and G.Bailin, Arch. Biochem. Biophys. 113 (1966) 205.
[3] M.Tada, G.Bailin, K.Bárány and M.Bárány, Biochemistry 8 (1969) 4842.
[4] S.V.Perry, Progr. Biophys. Mol. Biol. 17 (1967) 327.
[5] A.Gaspar-Godfroid, Biochim. Biophys. Acta 167 (1968) 622.
[6] F.Huriaux, J.-F.Pechère and G.Hamoir, Angiologica 2 (1965) 15.
[7] A.Gaspar-Godfroid, G.Hamoir and L.Laszt, Angiologica 4 (1967) 323.
[8] F.Huriaux, G.Hamoir and H.Oppenheimer, Arch. Biochem. Biophys. 120 (1967) 274.
[9] R.K.Scopes, Biochem. J. 107 (1968) 139.
[10] H.Oppenheimer, K.Bárány, G.Hamoir and J.Fenton, Arch. Biochem. Biophys. 120 (1967) 108.
[11] R.H.Locker and C.J.Hagyard, Arch. Biochem. Biophys. 120 (1967) 454.
[12] H.Mueller, J. Biol. Chem. 240 (1965) 3816.
[13] J.Johnson, C.I.Harris and S.V.Perry, Biochem. J. 105 (1967) 361.
[14] S.Lowey, H.S.Slayter, A.G.Weeds and H.Baker, J. Mol. Biol. 42 (1969) 1.
[15] D.M.Young, S.Himmelfarb and W.F.Harrington, J. Biol. Chem. 240 (1965) 2428.
[16] J.M.Jones and S.V.Perry, Biochem. J. 100 (1966) 120.
[17] D.M.Needham and J.M.Cawkwell, Biochem. J. 63 (1956) 337.
[18] C.Tanford, Physical Chemistry of Macromolecules (J.Wiley, New York, 1961) p. 394.
[19] H.S.Slayter and S.Lowey, Proc. Natl. Acad. Sci. U.S. 58 (1967) 1611.
[20] H.Mueller and S.V.Perry, Biochem. J. 85 (1962) 431.
[21] E.Eisenberg, C.R.Zobel and C.Moos, Biochemistry 7 (1968) 3186.
[22] H.Oppenheimer, K.Bárány and J.Fenton, Arch. Biochem. Biophys. 132 (1969) 355.