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The Subunit Structure of Gizzard Myosin

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The subunit structure of gizzard myosin

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[Plates 13 and 14]

Gizzard myosin consists of two major subunits, 'heavy chains', with chain masses of about 200000 and two minor components 'light chains' with chain masses of 20000 and 17000. Quantitative densitometry indicates that the two types of light chains occur in non-stoichiometric amounts in a myosin molecule, both in purified myosin preparations and in the whole muscle. Chemical studies on the isolated light chains reveals little chemical similarity between the two types, and preliminary sequence work on the thiol peptides of these light chains further suggests that they may be chemically distinct from those of skeletal and cardiac myosins. Although the exact function of the light chains remains speculative, there is evidence to suggest that the presence of one of the light chains is not required for the hydrolytic activity of this myosin.

1. Introduction

The main structural features and physiochemical parameters of smooth muscle myosins appear to be very similar to those of rabbit skeletal myosin (Hanson & Lowy 1964; Shoenberg 1965; Cohen, Lowey & Kucera 1961; Barany, Barany, Gaetjens & Bailin 1967; Hamoir 1968; Wachsberger & Kaldor 1971) which indicates that this myosin also has a rod-like portion with globular 'heads'. These rods and globular S-1 subfragments isolated by enzymic cleavage of gizzard myosin have dimensions which are very similar to those of rabbit myosin (Kendrick-Jones, Szent-Györgyi & Cohen 1971; Huriaux 1970; Bailin & Barany 1971). The S-1 subfragment contains the ATPase activity and the actin binding properties and this enzymic activity, as in the parent myosin molecule, is considerably lower than that of skeletal myosin and its subfragment (Bailin & Barany 1971). The similarities in the basic structure of myosin and its subfragments from gizzard and rabbit muscles suggest that the differences in enzymic activity may be due to rather subtle differences in the subunit structure of these myosins. To test the validity of this hypothesis, the subunit structure of chicken gizzard myosin was examined.

2. Composition and stoichiometry of the myosin subunits

Chicken gizzard myosin preparations prepared by the usual procedures (Barany *et al.* 1967; Bailin & Barany 1971) are frequently contaminated by variable amounts of actin, tropomyosin and other muscle proteins. These contaminants and the ribonucleoprotein component associated with this myosin can be removed by ion-exchange chromatography in the presence of pyrophosphate (Richards, Chung, Menzel & Olcott 1967) (figure 1). Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (SDS) by the procedure of Weber & Osborne (1969) of this purified myosin shows a major component, the 'heavy chains', with a chain mass of about 200000 similar in size to the heavy chains of rabbit myosin, and two minor components 'light chains' with chain masses of about 20000 and 17000 respectively (figure 2, plate 13). Actin is present in these myosin preparations in amounts usually less than 5%. Quantitative densitometry of 5 and 10% gels of the myosin was carried out by the procedure outlined by Lowey & Risbey (1971) using actin as the marker protein to correct for staining differences in the two types of gels and for errors in sample volumes applied to the gels. The

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results indicate that the light chains represent about 16 % of the myosin (table 1). A similar value has been obtained by chromatographic separation of the light and heavy chains on Sephadex G_{100} in 0.1 % SDS. A comparison of the electrophoretic mobilities of the individual light chains with those of proteins of known molecular mass gives values of 20 000 and 17 000 ± 5 % for the chain masses of the G_I and G_{II} light chains; thus there could be 2 mol of G_I light chain and 2 mol of the G_{II} light chain in a myosin molecule, assuming the molecular mass of the myosin is 470 000. The values obtained by quantitative gel densitometry however indicate that there is significantly more of the G_I than G_{II} light chains (table 1). These values should therefore be regarded as tentative until the stoichiometry of these light chains can be definitely established by other independent procedures, such as the 'radio isotope dilution' technique (Weeds & Lowey 1971).

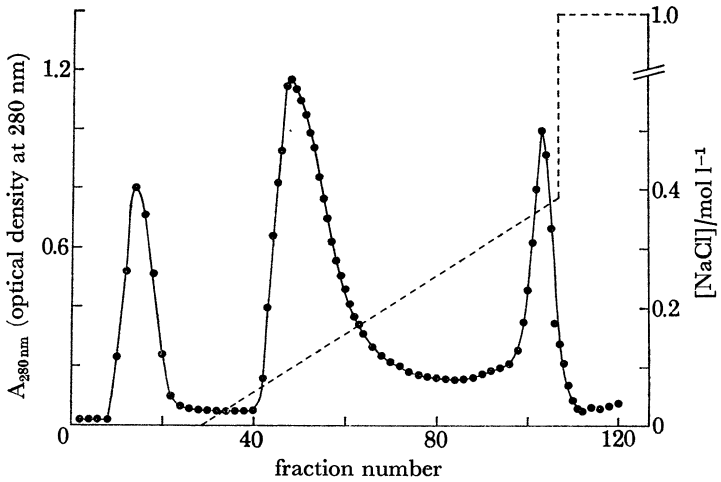

FIGURE 1. Purification of gizzard myosin by DEAE cellulose chromatography. Impure gizzard myosin (protein concentration about 1 %) equilibrated in 0.04 mol/l sodium pyrophosphate pH 7.5, containing 1 mmol/l $MgCl_2$, applied to a 40 cm × 4 cm DEAE cellulose (Whatman DE 52) column and eluted with a linear gradient of 0 to 0.5 mol/l NaCl in 0.04 mol/l sodium pyrophosphate pH 7.5 (1000 ml). The yield of purified myosin was 50–60 %.

TABLE 1. STOICHIOMETRY OF THE LIGHT CHAINS

		percentage light chains/myosin	moles light chain/mole myosin assuming 470 000 mol. mass
SDS Sephadex G_{100} chromatography			
total light chains		15.8	—
densitometry of SDS acylamide gels			
myosin	light chain $_{(20\,000)}$ G_I	10.6	2.34
	light chain $_{(17\,000)}$ G_{II}	5.8	1.66
whole muscle	light chain G_I	11.5	2.64
	light chain G_{II}	6.1	1.75

An estimate was made of the light chain content of whole gizzard muscle by gel densitometry to determine whether during preparation and subsequent column purification any of the light chain material has been preferentially lost. The myosin light chains are not however the only low molecular mass components in the whole muscle, proteins which have chain weights similar to those of the vertebrate troponin system also migrate in this region (figure 2, plate 13). This makes it extremely difficult to assess the baseline for densitometry, so that the values quoted in table 1 for the light chain components of the whole muscle are only approximate.

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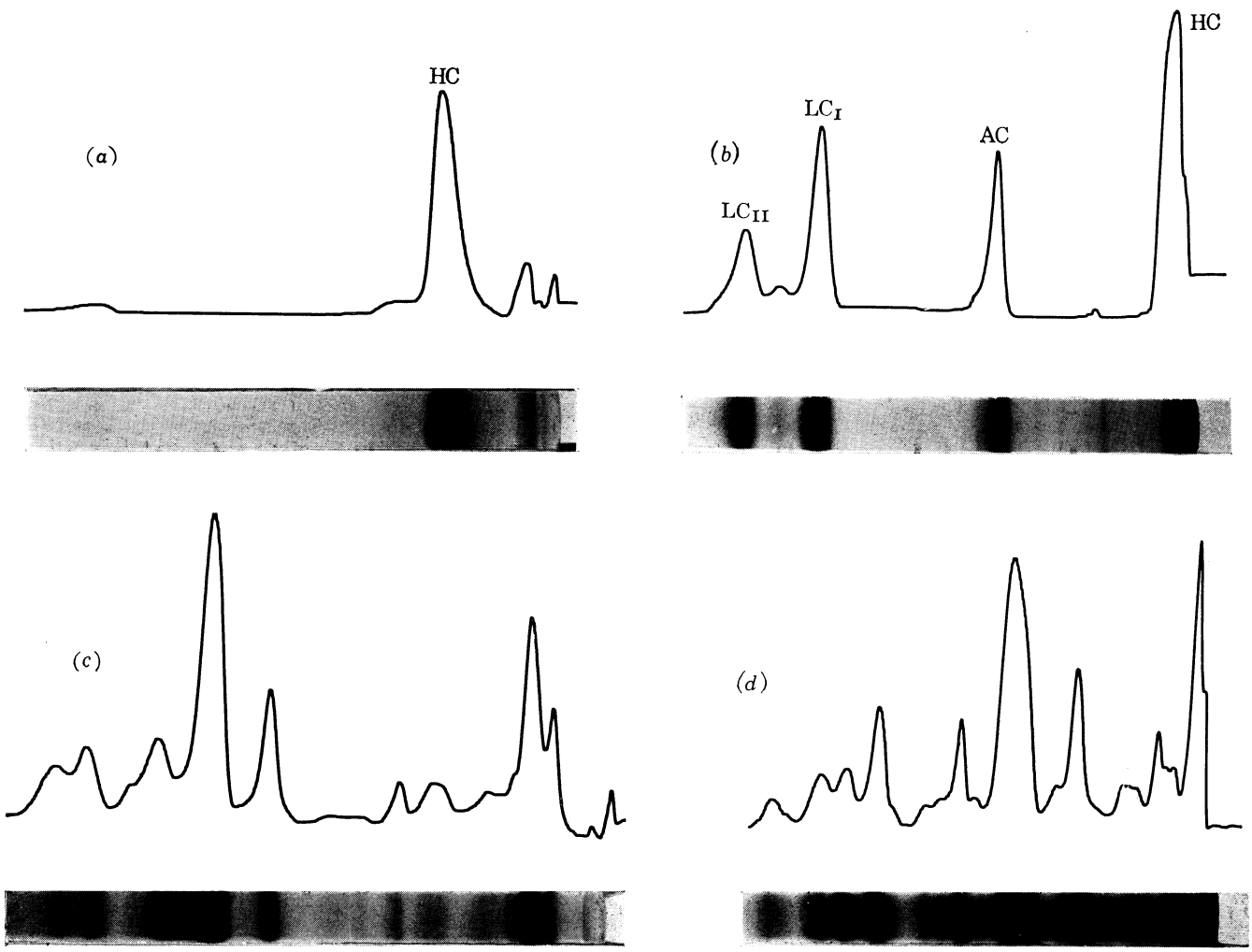

FIGURE 2. Densitometry of purified myosin and whole muscle preparations: (*a*) myosin (5% gel); (*b*) myosin (10% gel); (*c*) whole muscle (5% gel); (*d*) whole muscle (10% gel). HC, heavy chain of myosin; AC, actin; LC, light chains of myosin. The 5% gells were stained with Fast green FCF and the 10% gels with Coomassie brilliant blue. The stain intensities were measured using a Joyce-Loble chromoscan densitometer.

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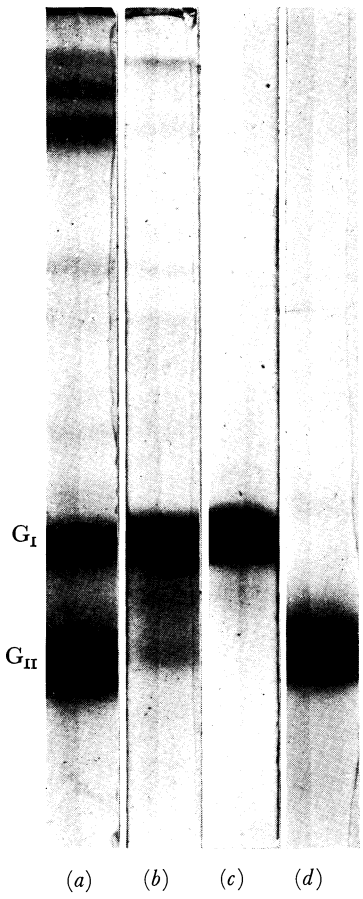

FIGURE 3

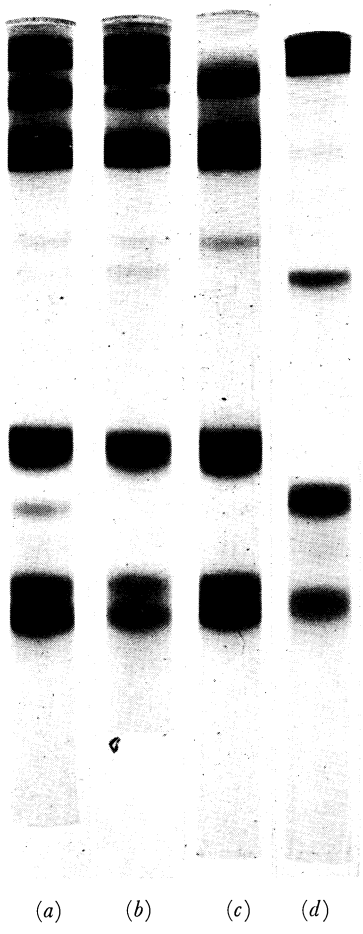

FIGURE 5

FIGURE 3. SDS acrylamide gels of gizzard light chain preparations (10% gels). (*a*) Supernatant from gizzard myosin incubated in 4 mol/l urea for 1 h at room temperature, and the 'undissociated myosin' precipitated by 12-fold dilution with cold water. Showing predominantly the G_{II} light chain. (*b*) Supernatant from the urea treated myosin after treatment at alkaline pH and precipitation of the heavy chains by dilution with 12 vol. of cold water at pH 6.5. Showing predominantly the G_I light chain. (*c*) G_{II} light chain preparation isolated by Sephadex G100 chromatography in SDS. (*d*) G_I light chain preparation isolated by the same procedure as (*c*). The gels have been considerably overloaded with protein to demonstrate the amount of contamination present in these preparations.

FIGURE 5. SDS-acrylamide gel electrophoresis of the S-1 subfragment of gizzard myosin (10% gels). The S-1 fractions were prepared by soluble papain digestion of gizzard myosin at a papain to myosin ratio of 1:200 (by mass) at 23 °C (Lowey *et al.* 1969). (*a*) 4 min digest, material soluble at low ionic strength. (*b*) 8 min digest, material soluble at low ionic strength. (*c*) The S-1 subfragment from these digests purified by DEAE cellulose chromatography according to the procedure of Lowey *et al.* (1969). (*d*) Purified gizzard myosin. The gels were stained with Coomassie brilliant blue. Note the presence of 'HMM' heavy chains at about 140000 to 150000 and the faint trace of the G_I light chain in the initial digest supernatants, which are absent in the purified S-1 preparation. The S-1 subfragment shows a major heavy chain component with a molecular mass of about 80000 and a small amount of a heavier component, a low molecular mass component of about 25000 which is probably due to degradation in the heavy chains, a component of molecular mass about 18000 and the G_{II} light chain of 17000.

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Nevertheless, the ratio of the light chains to heavy chain is about the same in the whole muscle as in the purified myosin preparations indicating that the light chains are not selectively lost during myosin purification.

Although the exact stoichiometry of the light chains in gizzard myosin remains unresolved the major difference in the subunit structures of gizzard and rabbit myosins appears to be their light chain components.

3. Chemical studies on the isolated light chains

Isolation and purification of the light chains

The lower molecular mass light chain (G_{II}) can be preferentially removed from the myosin by incubation in 4 mol/l urea, with an irreversible loss of enzymic activity, under the conditions outlined by Weeds & Pope (1971). The undissociated G_I light chain was released from the urea treated myosin by alkaline pH (Gaetjens *et al.* 1968) or by treatment with guanidine hydrochloride (Gershman, Stracher & Dreizen 1969). The individual light chain preparations were contaminated by variable amounts of the other light chain and by traces of the heavy chains (figure 3, plate 14). These preparations were chromatographed on DEAE cellulose using phosphate buffers at pH 6.0 (Weeds & Lowey 1971) and on G_{150} and G_{100} Sephadex in the presence of SDS, in an attempt to remove the contaminating material and separate the two types of light chains. Owing to the small differences in net charge and mass between the two light chains observed on ordinary and SDS acrylamide gels, the absence of a clear-cut separation of the light chains on either chromatographic system was not unexpected. The light chains run as a single peak in both the Sephadex and DEAE cellulose systems. Analysis of the peak fractions from the G_{100} Sephadex chromatography by gel electrophoresis, however, revealed a partial fractionation of the two types of light chains (figure 3). The fractions containing solely one type of light chain were pooled, treated with Dowex in the presence of 6 mol/l urea to remove the SDS (Weber & Kuter 1971) and carboxymethylated with radioactive iodoacetic acid (Weeds & Lowey 1971) to facilitate identification of the thiol groups.

Amino acid compositions

The amino acid compositions of the two light chains are shown in table 2. The number of half cystine residues in each light chain was checked by amino acid analysis of non-carboxymethylated light chains after performic acid oxidation and by liquid scintillation counting of the radioactive carboxymethylated light chains; all three methods indicate that the G_{II} light chain contains three half-cystine residues and the G_I light chain about one half-cystine residue. On the basis of these analyses the minimum molecular masses of the G_I and G_{II} light chain are 20000 and 17000 respectively, values which are in good agreement with those measured from the SDS acrylamide gels. A comparison of the amino acid compositions of the two light chains indicates no obvious similarity in their chemical compositions.

Tryptic peptide maps of the light chains

Two-dimensional peptide maps were prepared by the procedure described by Weeds & Hartley (1968) from tryptic digests of the carboxymethylated light chains (figure 4). These peptide maps were specifically stained to detect peptides containing tyrosine, histidine and arginine residues and autoradiographed to detect the radioactive carboxymethylcystine (CMCys) containing peptides.

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A comparison of these peptide maps suggests no obvious chemical similarity between the two proteins. The number and distribution of the peptides from each protein especially those peptides which were selectively stained, further suggests that the degree of homology of sequences in the two light chains must be rather limited.

TABLE 2. AMINO ACID COMPOSITIONS OF GIZZARD MYOSIN LIGHT CHAINS

amino acid	G_I light chain	G_{II} light chain
lysine	13.8	9.1
histidine	4.0	2.0
arginine	8.8	5.0
carboxymethyl cysteine	1.3	3.0
aspartic acid	24.0	18.0
threonine	10.7	8.3
serine	9.7	6.3
glutamic acid	22.7	30.2
proline	7.6	3.9
glycine	12.3	13.1
alanine	12.7	7.4
valine	6.0	10.3
methionine	7.9	7.2
isoleucine	7.8	4.1
leucine	10.0	12.0
tyrosine	3.3	3.0
phenylalanine	11.0	7.8
molecular mass	20000	17000

These analyses are the averaged values for both 24 and 72 h hydrolysates, except that the values of threonine and serine were obtained by extrapolation to zero time hydrolysis; those of valine and isoleucine were taken from the 72 h hydrolysates and those of carboxymethylcysteine from the 24 h hydrolysates.

The number of tyrosine (3) and histidine (2) containing peptides in the peptide map of the G_{II} light chain is consistent with its amino acid composition, however the number of arginine (6) and radioactive thiol (4) containing peptides and the total number of peptides is slightly greater than expected. These discrepancies may be due to multiple tryptic cleavages.

The analytical results predict that the peptide maps of the G_I light chain should contain one radioactive thiol, four histidine, nine arginine and three tyrosine containing peptides in a total of twenty-three peptides; the maps are in reasonable agreement with these predictions, although only two tyrosine and two rather weakly radioactive thiol peptides were observed. The positions of these weakly radioactive peptides, which were not detected by ninhydrin, correspond to those of two of the radioactive peptides in the G_{II} light chain map, which suggests that they may be due to cross-contamination by the G_{II} light chain. The lack of overlap between peptides of the two light chains stained with ninhydrin suggests, however, that the level of cross-contamination must be relatively low (about 100 nmol of protein were used for each peptide map, thus ninhydrin should detect impurities in the range of 5 %). The absence of a strong radioactive peptide raises the possibility that the G_I light chain, despite the analytical results, may not contain a thiol residue. To answer the question of the number of thiol peptides in each light chain, these peptides were isolated and purified by paper electrophoresis from chymotryptic and tryptic digests of both light chains according to the procedure outlined by Weeds & Pope (1971). Subsequent analysis indicated that three thiol peptides are present in roughly equal amounts in the G_{II} light chain, while none of the thiol peptides isolated from the G_I light chain are present in significant amounts. Preliminary sequence analysis of these thiol peptides indicates that they appear to be chemically distinct from those of cardiac and skeletal

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muscle myosin light chains. Although these chemical studies suggest that the light chains of gizzard myosin may be unique, further more detailed work on their chemical structure is required to establish definitely their 'identity'.

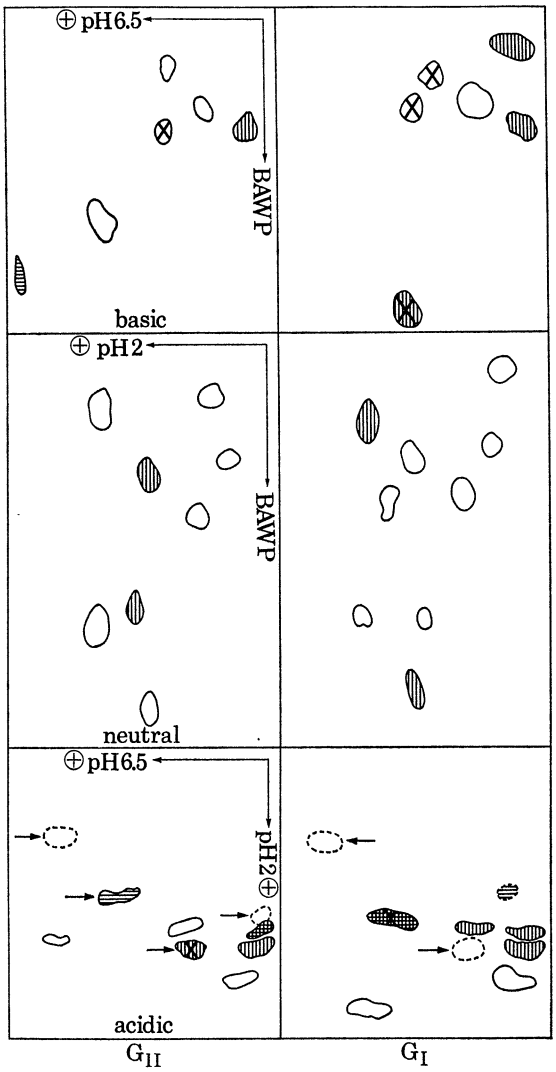


FIGURE 4. Two-dimensional tryptic peptide maps of the light chains. The peptide maps were produced by initially separating the peptides in the first dimension by electrophoresis at pH 6.5. The acidic peptides were rerun in the second dimension by electrophoresis at pH 2.1. and the basic peptides by descending chromatography in butan-1-ol, acetic acid, water, and pyridine (BAWP). The neutral peptides from the initial pH 6.5 electrophoresis were separated by electrophoresis at pH 2.1 and then by descending chromatography in BAWP. □, Peptides stained with the ninhydrin-cadmium reagent; ▥, peptides staining for arginine; ▤, peptides staining for tyrosine; ⊠, peptides staining for histidine. The arrows indicate peptides containing radioactive carboxymethylcysteine residues, which were detected by autoradiography. The peptides with the dotted outlines indicate those not detected by ninhydrin.

The 'role' of the light chains

It has been proposed (Frederiksen & Holtzer 1968; Gershman *et al.* 1969) that the light chains of rabbit skeletal myosin are required for the ATPase and ADP binding of this myosin, since their removal leads to a loss of these functions. Similarly, removal of the light chains of gizzard myosin, under a variety of conditions, leads to an irreversible loss of ATPase activity. It has

also been shown that if the light chains are dissociated from rabbit myosin in mild conditions with 4 mol/l lithium chloride, they can be reassociated with the heavy chains to recover enzymic activity (Stracher 1969; Dreizen & Gershman 1970). These dissociation and recombination experiments were repeated with gizzard myosin under the same mild conditions. The observations that the dissociated heavy chains tended to aggregate and denature rapidly and that analysis on acrylamide gels indicated a variable amount (15 to 25 %) of the light chain material remaining on the heavy chains, made it impossible to demonstrate unequivocally reassociation of light chains with the heavy chain fraction and a resulting recovery of ATPase activity.

TABLE 3. THE EFFECT OF A VARIETY OF REAGENTS ON THE Ca^{2+} ATPASE AND LIGHT CHAINS OF GIZZARD MYOSIN

reagent	treatment	Ca^{2+}ATPase μmol ATP mg^{-1} min^{-1} at 25 °C	release of light chains
DTNB	DTNB-treated myosin	0.217 (0.266)	no
EDTA	EDTA-treated myosin	0.203 (0.210)	no
urea	myosin in 2 mol/l urea (5 min)	0.370	no
	diluted 100-fold	0.198	no
	myosin in 4 mol/l urea (5 min)	0.011	—
	diluted 100-fold	0.021	G_{II} light chain

The figures in parentheses are the values for the control untreated myosins.

The Ca^{2+} ATPase activities were measured in 30 mmol/l KCl, 10 mmol/l Ca^{2+}, 0.5 mmol/l ATP at pH 7.6 in a pH stat using 0.02 mol/l NaOH as titrant. The release of light chains was monitored by 10 % SDS acrylamide gel electrophoresis.

The chemical and stoichoimetric studies indicate differences in the chemical structure and in the relative amounts of the two light chains, which suggest that they may also be functionally different. To investigate this possibility a number of specific reagents were tried which are known to selectively remove light chains from other myosins without destroying their ATPase activities. The thiol reagent 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) which removes a particular light chain from rabbit skeletal myosin without altering its enzymic activity (Weeds 1969; Gazith, Himmelfarb & Harrington 1970) removes very little of the light chain material and has no effect on the ATPase activity of gizzard myosin. EDTA which in mmol/l concentrations removes a regulatory light chain from molluscan myosins with a resulting loss of calcium regulatory activity, but without altering their ATPase activities (Kendrick-Jones, Szent-Királyi & Szent-Györgyi 1972), similarly has no effect either on the light chains or the ATPase of gizzard myosin (table 3). The most compelling evidence for functional differences in the light chains is provided by the S-1 subfragment which on acrylamide gels shows a strong G_{II} light chain band but only a faint trace of the G_I light chain (figure 5, plate 14). The other low molecular band on the gels is thought to be due to degradation in the heavy chains and may be the 'difference peptide' between the two species of heavy chains seen in these preparations. Since the S-1 subfragment has full ATPase activity and actin binding (Ca^{2+} ATPase specific activity is threefold higher than that of the original myosin), it is reasonable to suppose that this G_I light chain is not required for these functions.

The G_{II} light chain appears to be involved in the hydrolytic activity of the myosin since its removal by urea (at concentrations from 3.5–4 mol/l) leads to an irreversible loss of ATPase activity (table 3). At lower concentrations (2 to 2.5 mol/l) urea activates the Ca^{2+} ATPase of

gizzard myosin (Barany *et al.* 1967) without any significant release of light chain material as indicated by the analytical ultracentrifuge. Since this activation is reversible when the urea is removed, one can speculate that urea may alter the conformation of the myosin so that the light chains are unable to regulate correctly the ATPase activity.

These findings are consistent with the hypothesis that the light chains may be involved in determining the contractile response of the muscle.

References (Kendrick-Jones)

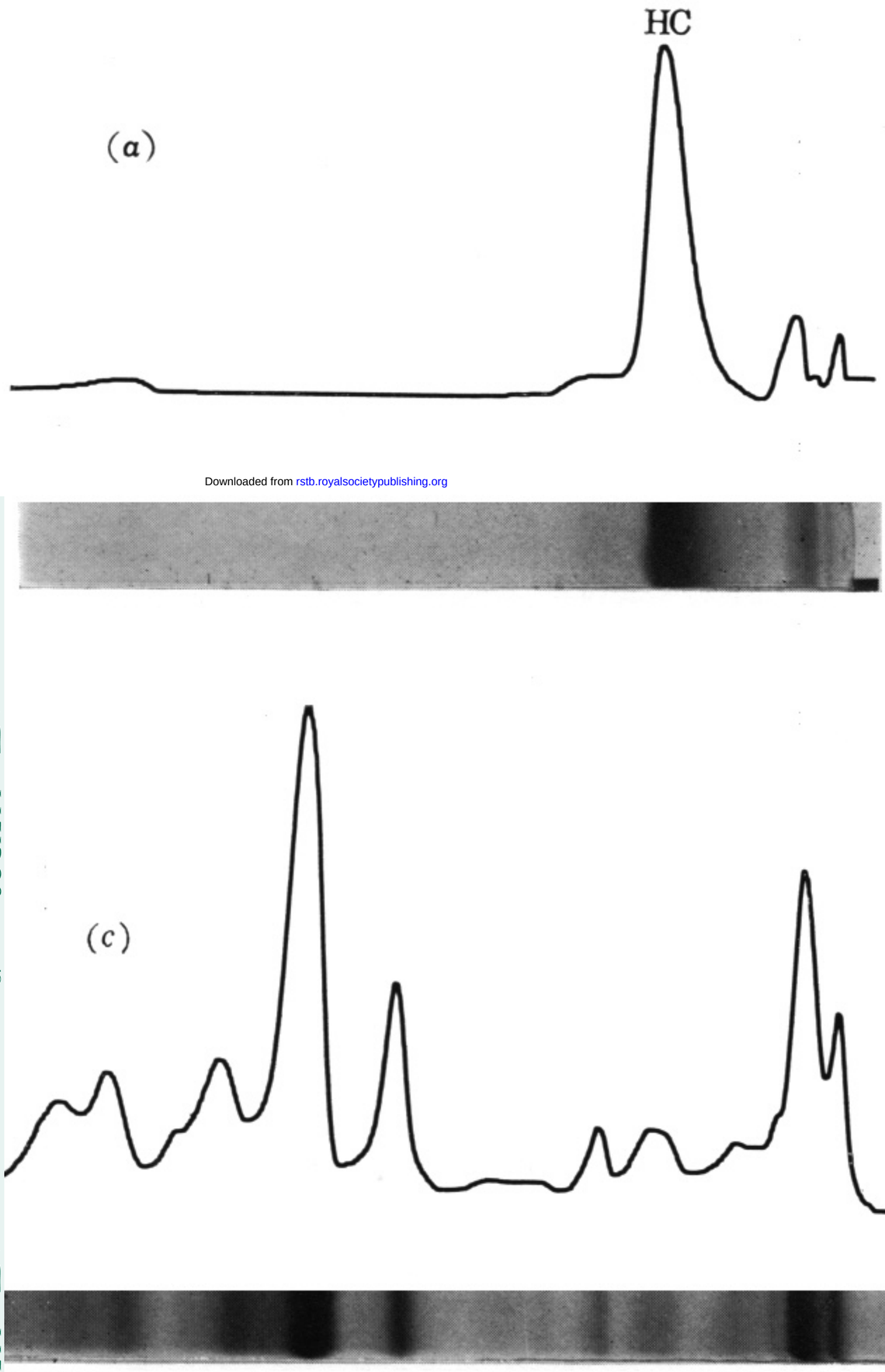
Bailin, G. & Barany, M. 1971 *Biochim. biophys. Acta* **236**, 292–302.
Barany, M., Barany, K., Gaetjens, E. & Bailin, G. 1967 *Archs Biochem. Biophys.* **133**, 205–221.
Barany, M. & Close, R. I. 1971 *J. Physiol., Lond.* **213**, 455–474.
Cohen, C., Lowey, S. & Kucera, J. 1961 *J. biol. Chem.* **236**, PC 23.
Dreizen, P. & Gershman, L. C. 1970 *Biochemistry* **9**, 1688–1693.
Frederiksen, D. W. & Holtzer, A. 1968 *Biochemistry* **7**, 3935–3950.
Gaetjens, E., Barany, K., Bailin, G., Oppenheimer, H. & Barany, M. 1968 *Archs Biochem. Biophys.* **123**, 82–96.
Gazith, J., Himmelfarb, S. & Harrington, W. F. 1970 *J. biol. Chem.* **245**, 15–22.
Gershman, L. C., Stracher, A. & Dreizen, P. 1969 *J. biol. Chem.* **244**, 2726–2736.
Hamoir, G. 1968 *Angiologica* **6**, 190–226.
Hanson, J. & Lowy, J. 1964 *Proc. R. Soc. Lond.* B **160**, 523–524.
Huriaux, F. 1970 *F.E.B.S. Lett.* **10**, 194–197.
Kendrick-Jones, J., Szent-Gyorgyi, A. G. & Cohen, C. 1971 *J. molec. Biol.* **59**, 527–529.
Kendrick-Jones, J., Szentkirályi, E. M. & Szent-Györgyi, A. G. 1972 *27th Cold Spring Harbor Symposium on the mechanism of muscle contraction.* (In the Press.)
Lowey, S. & Risby, D. 1971 *Nature, Lond.* **234**, 81–84.
Lowey, S., Slayter, H. S., Weeds, A. G. & Baker, M. 1969 *J. molec. Biol.* **42**, 1–29.
Richards, E. G., Chung, C-S., Menzel, D. B. & Olcott, M. S. 1967 *Biochemistry* **6**, 528–540.
Shoenberg, C. F. 1965 *Nature, Lond.* **206**, 526–527.
Stracher, A. 1969 *Biochem. biophys. Res. Commun.* **35**, 519–525.
Wachsberger, P. & Kaldor, G. 1971 *Archs Biochem. Biophys.* **143**, 127–137.
Weber, K. & Kuter, D. J. 1971 *J. biol. Chem.* **246**, 4504.
Weber, K. & Osborne, M. 1969 *J. biol. Chem.* **244**, 4406–4412.
Weeds, A. G. 1969 *Nature, Lond.* **223**, 1362–1364.
Weeds, A. G. & Hartley, B. S. 1968 *Biochem. J.* **107**, 531–548.
Weeds, A. G. & Lowey, S. 1971 *J. molec. Biol.* **61**, 701–725.
Weeds, A. G. & Pope, B. 1971 *Nature Lond.* **234**, 85–88.

Discussion

J. C. Ruegg (*Institut für Zellphysiologie, Ruhr-Universität, Bochum, Germany*): As pointed out by Hamoir, the high solubility of smooth muscle contractile protein and the elusive character of smooth muscle myosin filaments may have a common origin. It is obviously important, then, to investigate carefully on the one hand – as Shoenberg did – the conditions which stabilize myosin filaments and to examine, on the other hand, also the solubility characteristics of myosin and actomyosin.

Arterial actomyosin is soluble at low ionic strength in presence of ATP and Mg, the actomyosin ATPase is fully inhibited and myosin exists in the form of dimers or trimers, even at 10^{-5} mol/l Ca^{2+}. But as the concentration of Mg increases gradually to 10 mmol/l the protein is insolubilized and the actomyosin ATPase is activated unless trace calcium is reduced to less than 10^{-8} mol/l by means of EGTA. Shoenberg demonstrated formation or stability of myosin filaments in presence of Mg ions (10 mmol/l $MgCl_2$) and trace Ca^{2+}. In the absence of troponin the protein is insoluble and fully activated with and without trace calcium present.

In vitro the filamentous state of insoluble smooth-muscle myosin depends on trace Ca^{2+}, and calcium ions appear to be necessary for a high Mg- and actin-activated myosin ATPase activity.



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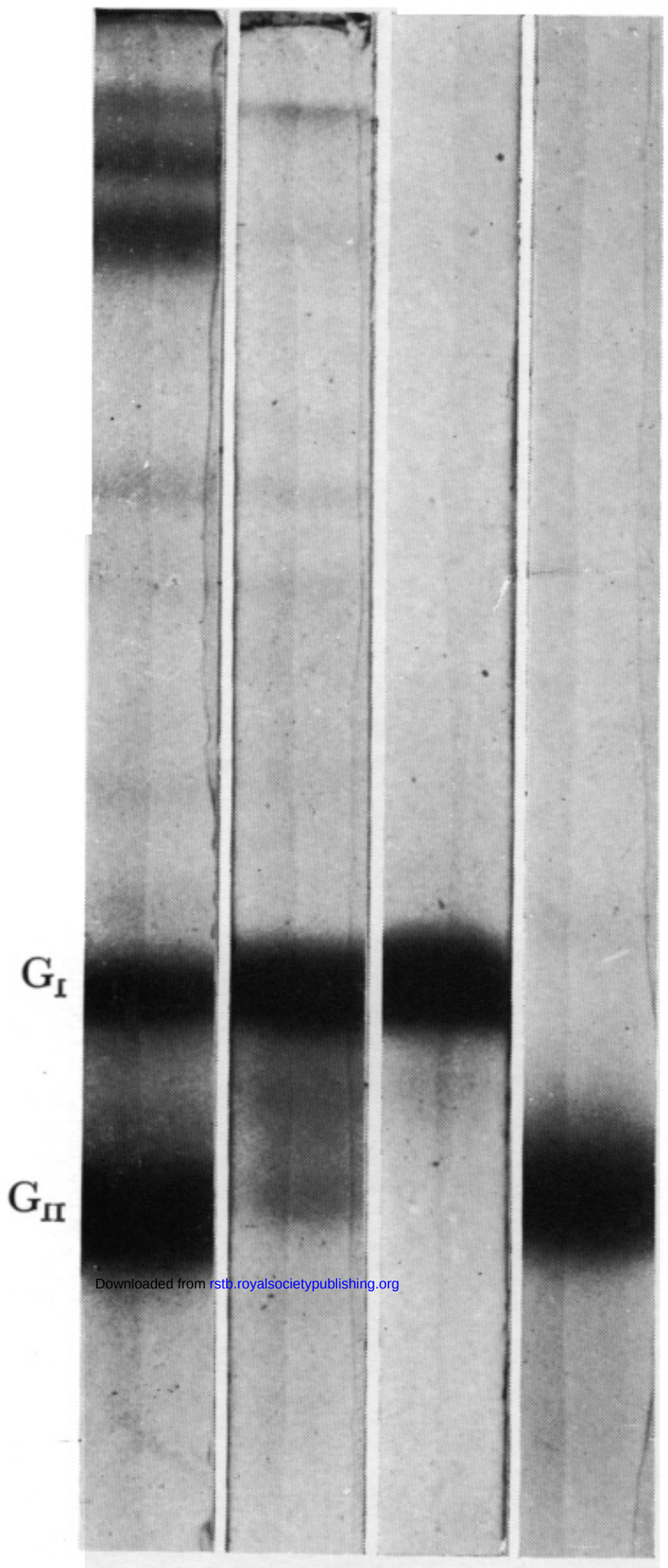

FIGURE 3

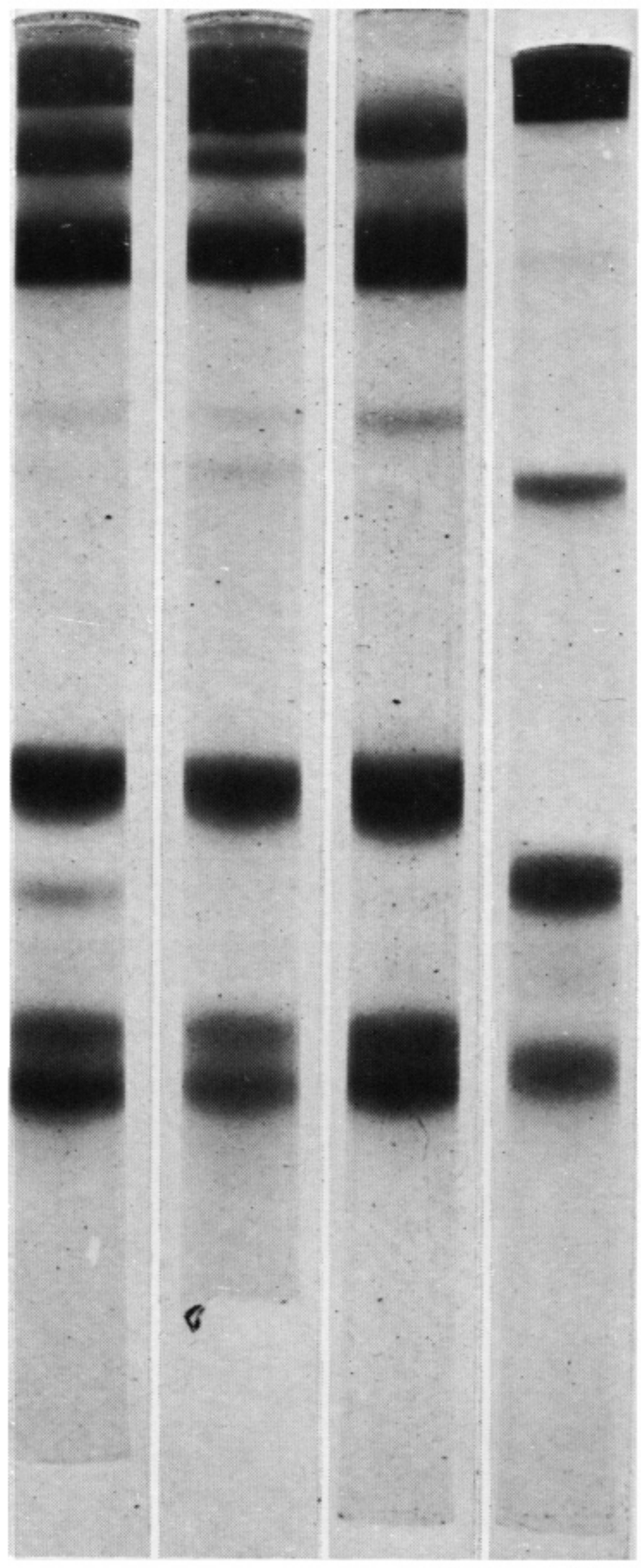

FIGURE 5

FIGURE 3. SDS acrylamide gels of gizzard light chain preparations (10% gels). (*a*) Supernatant from gizzard myosin incubated in 4 mol/l urea for 1 h at room temperature, and the 'undissociated myosin' precipitated by 12-fold dilution with cold water. Showing predominantly the G_{II} light chain. (*b*) Supernatant from the urea treated myosin after treatment at alkaline pH and precipitation of the heavy chains by dilution with 12 vol. of cold water at pH 6.5. Showing predominantly the G_I light chain. (*c*) G_{II} light chain preparation isolated by Sephadex G100 chromatography in SDS. (*d*) G_I light chain preparation isolated by the same procedure as (*c*). The gels have been considerably overloaded with protein to demonstrate the amount of contamination present in these preparations.

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