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ON THE RECONSTITUTION OF RABBIT MYOSIN FROM FAST AND SLOW MUSCLE*

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SUMMARY

Myosin from slow twitch muscle contains a low molecular weight component obtained by treatment with 4 M LiCl, which comprises about 9 % of the total protein, and which differs electrophoretically in polyacrylamide gel from that of the fast muscle. It was found that the addition of light subunit to the remaining heavy matrix resulted in the enhancement of ATPase activity in both myosins. In the crosswise reconstitution experiments, the light subunit from the F-myosin yielded higher ATPase activities than that of S-myosin regardless of whether the heavy subunits from S- or F-myosins are used. These results support the notion that the light subunit, an integral part of the myosin molecule, is exchangeable between different species of myosins.

Myosin–ATPase activity, which plays a rate limiting role with respect to contraction velocity[1,2] varies greatly in different muscles, as shown for example in a comparison between slow (S)- and fast (F)-twitch muscles in the same animal (*cf.* ref. 3). Correlated with this, there are other physicochemical distinctions, *e.g.* in the response of the ATPase to pH, ionic strength, tryptic digestion and sulfhydryl group activation[4–8], and in the fact that S- and F-myosin contain electrophoretically different light subunits[9,10]. These subunits (mol. wt. 20000–30000) had been mainly studied for F-myosin, *e.g.* by STRACHER[11] who separated them from the remaining molecular matrix by gelfiltration in 4 M LiCl and recovered 30 % of the native ATPase upon recombination, and by DREIZEN AND GERSHAM[12] who used a rapid salting-out fractionation and could reconstiture 70 % of the original ATPase activity. In the work reported herein, it was attempted to isolate subunits and heavy matrix from both F- and S-myosin, and to recombine them crosswise.

Myosin was prepared by the standard procedure of this laboratory (*cf.* ref. 3). The only modification was that the minced slow rabbit muscle was extracted with 4 vol. of Guba–Straub solution containing 3–5 mM ATP for 30 min prior to the dilution with cold water. The preparation was essentially free of nucleotides. While the ATPase activity of F-myosin was determined by measuring P_i release as described previously[3], the much lower ATPase of S-myosin was estimated by measuring ADP production

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according to the modified procedure of ESTABROOK AND MAITRA[13] using Boehringer's enzyme assay kits.

In Fig. 1, as in F-myosin[14], the presence of the light subunit in S-myosin treated with 4 M LiCl, 50 mM Tris–maleate (pH 7.5), is evidenced by the slowly sedimenting ultracentrifugal peak which accounts for about 9 % of the total protein. The molecular weight of the light subunit ($25.9(\pm 3.4)\cdot 10^3$) from S-myosin as determined by the high speed sedimentation equilibrium experiments is comparable with that of the F-myosin[12]. The possibility for recombination of these subunits is suggested by the disappearance of this slowly sedimenting peak upon dialysis of an aliquot of the sample against 0.5 M KCl, 50 mM Tris–maleate, pH 7.5. The molecular weight of S-myosin ($415(\pm 35)\cdot 10^3$, determined by the sedimentation equilibrium procedure), appears to be similar to that of F-myosin (H. D. KIM, unpublished data), for which we have frequently confirmed the molecular weight value in this range as first proposed by MOMMAERTS AND ALDRICH[15] and now again repeatedly encountered in the literature (most recently in ref. 16). In agreement with comparable results by others[9,10], the light subunits prepared with 4 mM LiCl treatment of F- and S-myosin appear to be different. In Fig. 2, purified light subunits after LiCl treatment were electrophoretically separated on polyacrylamide gel. While the light subunits from F-myosin contained 3–4 components, only 2 components were found in S-myosin.

In the recombination experiments which were carried out according to the procedure of DREIZEN AND GERSHAM[12], the myosins (within 48 h of preparation) at 15–20 mg/ml in 0.5 M KCl and 2 mM dithiothreitol were mixed with equal volumes of 8 M LiCl, 50 mM Tris–maleate, pH 7.0, and allowed to react for 5 min at 4°. The heavy and light subunits were promptly fractionated by salting out in 75 % saturated potassium citrate–4 M LiCl and centrifuged at 5000 rev./min for 5 min. Under these conditions, the supernatant fraction contained exclusively the water soluble light subunits as

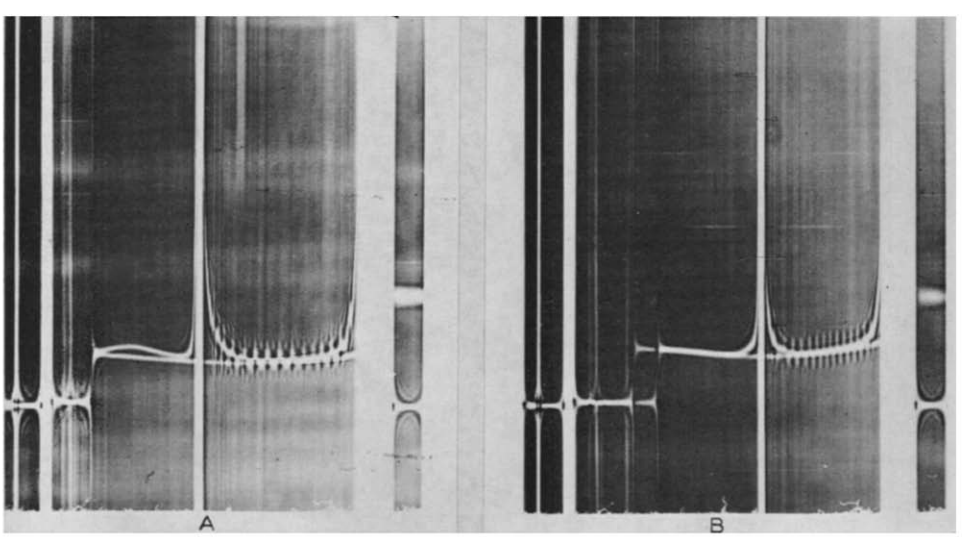

Fig. 1. Sedimentation diagram of LiCl-treated S-myosin, pH 7.5, 4°, phase-plate angle 70°, protein concentration of 11.0 mg/ml. A. Myosin stored in 4 M LiCl, 50 mM Tris–maleate, pH 7.5, for 4 h. at 52640 rev./min for 240 min. B. Sample used in A was dialyzed against 0.5 M KCl, 50 mM Tris–maleate, pH 7.5; at 52640 rev./min for 210 min.

shown by the absence of heavy material in the gel electrophoresis and of protein precipitating at low ionic strength, but the precipitate, the heavy residual matrix, was always contaminated with the light subunit. The recombined sample was dialyzed against 0.5 M KCl, 1 mM dithiothreitol, 50 mM Tris–maleate (pH 7.0) for Ca^{2+}-ATPase determination. Since the duration of treatment in 4 M LiCl and the ratio of light to heavy subunit dictate the degree of recombination, an attempt was made to use the identical conditions for both myosins as much as possible.

Table I shows the results of experiments in which simultaneously and identically prepared heavy matrix and light subunits from S- and F-myosins were recombined. The light subunits had no ATPase but the preparations of the heavy matrix had a sizable residual activity, being contaminated by light units. It was not possible to remove these by prolonged or repeated dissociative treatment with LiCl, because this led to irreversible denaturation, thus we must work with the handicap of a considerable background activity. It is clear, however, that the inclusion of the light subunit resulted in the enhancement of ATPase activities from both S- and F-myosins.

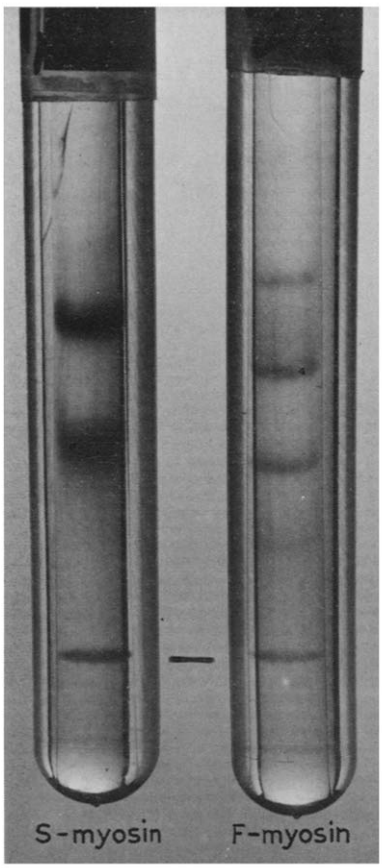

Fig. 2. Acrylamide electrophoresis of myosin light subunit after LiCl treatments, of S- and F-myosin as labeled. Gel electrophoresis was carried out by the modified method of DEYL *et al.*[17] stained with coomassie brilliant blue, using 10 cm, 7.5% gels. Note that the lowest bands (marked with a horizontal line) do not represent separate components, but are the leading edges of the dye marker.

TABLE I

RECONSTITUTED ATPASE ACTIVITY AS PERCENT OF NATIVE MYOSIN

Ca^{2+}-activated ATPase was determined in the medium containing 50 mM Tris–acetate buffer, pH 8.0, 1 mM $CaCl_2$, 1 mM ATP and 0.1 mg protein per ml medium. All reactions were initiated by the addition of ATP at 25°.

Fraction	*ATPase activity* (%)	
	*S-myosin**	*F-myosin***
Control	100	100
Precipitate	41.4 ± 5.0	46.9 ± 3.3
Precipitate + supernatant from S-myosin	60.3 ± 4.7	68.4 ± 4.2
Precipitate + supernatant from F-myosin	78.1 ± 6.3	80.2 ± 3.5
Supernatant	0	0

* Mean values from four experiments; control ATPase of S-myosin: 0.158 ± 0.015 μmoles P_i per mg per min.

** Mean values from five experiments, control ATPase of F-myosin: 0.65 ± 0.05.

Interestingly the light subunit prepared from F-myosin could not only substitute for the light subunit in S-myosin but also gave a higher degree of reconstitution, while conversely the light subunit prepared from S-myosin reconstitutes F-myosin less effectively.

The findings so far do not allow a clear decision whether the high reconstitution obtained with the F-subunits is due to a greater affinity for recombination on their part. The results are all that can be achieved until newer methods of decomposing the myosin are discovered, but they do suggest some degree of participation by the light subunits in determining the ATPase activity of the total molecule, and show, in any case, that these units are exchangeable. One idea underlying our attempts was that they might be a molecular counterpart of the altered myosin–ATPase activities induced by nerve crossing[3], and this is in line with the findings of SAMAHA *et al.*[18] on the reciprocal changes in light subunits (and of pH sensitivity) after cross-innervation.

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