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ASSOCIATION AND DISSOCIATION OF THE THICK AND THIN FILAMENTS WITHIN MYOFIBRILS IN CONDITIONS OF "CONTRACTION" AND "RELAXATION"*

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SUMMARY

1. The current assumption that the low ATPase activity of relaxed myofibrils is represented by the ATPase activity of myosin which has been set free during the dissociation of actomyosin was investigated. For this purpose, the ATPase activity of relaxed skeletal myofibrils of the rabbit and of the crab *Maia squinado* has been compared with the activity of contracted fibrils and of purified rabbit myosin in conditions of varying ionic strength, pH and concentrations of MgATP (*i.e.* $MgATP^{2-}$ + $MgHATP^{-}$) and Mg^{2+}.

2. Contraction and relaxation of the fibrils was induced by changing the concentration of Ca^{2+} from about $5 \cdot 10^{-5}$ to below $1 \cdot 10^{-8}$ M.

3. In all conditions studied, the ATPase activity of relaxed fibrils was about 6–8 times less than that of the contracted fibrils, but it remained a typical actomyosin ATPase.

4. Quantitatively and qualitatively, this ATPase differs from the ATPase of myosin. For instance, its dependence on pH is the reverse of that of the myosin ATPase.

5. Calculation showed that the fibrils are dissociated by 90% in conditions of relaxation. Since the ATPase activity of myosin was merely some 2% of the actomyosin activity, the major part of the ATPase of fibrils, even at a dissociation of 90%, is bound to show the properties of the ATPase of actomyosin.

6. However, a dissociation of 90% cannot be distinguished from a dissociation of 100% by means of physical methods (viscosity, superprecipitation, resistance to stretch, *etc.*). This explains why physical methods indicate a "full" dissociation of actomyosin although, enzymatically, the ATPase is still of the actomyosin type.

7. The possible reasons are discussed for the discrepancy between the 100-fold increase in the ATP turnover and the 1000-fold increase in energy turnover of the living muscle during the transition from relaxed to active state. The most problable explanation seems to be an ATPase activity of myosin which is too high by a factor of ten as compared to the energy turnover of living muscle at the resting state. This high activity cannot be caused by a contamination of the myosin by Ca^{2+}-insensitive actomyosin.

Abbreviations: EGTA, ethyleneglycoldiaminotetraacetic acid.

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INTRODUCTION

The theory that the contractile proteins of muscle are dissociated when the muscle is in "relaxed state" and that they are associated in the states of contraction and rigor[1] has been held for a long time as being a satisfactory explanation to account for all observations made on muscle. The resistance to stretch of muscle and of glycerol-extracted fibres is very high during contraction and rigor, whereas it drops to almost zero in the relaxed state[2]. Again, recent X-ray diffraction studies[3] and electron micrographs[4,5] show that the cross bridges of the myosin filaments are free during relaxation and are bound to the actin filaments during contraction and rigor. These findings also agree with the enzymatic observations; glycerol-extracted fibrils split ATP about 10 times as fast in conditions of contraction as in conditions of relaxations[1,6,7]. Furthermore, contracted fibrils hydrolyze ATP as rapidly as Mg^{2+}-activated actomyosin ATPase does, while the splitting velocity of the relaxed fibrils is as low as the Mg^{2+}-inhibited ATPase of myosin[1]. However, this chemical argument for the "association" of actomyosin during contraction and its "dissociation" during relaxation is not wholly convincing, since neither a proper investigation to see if the low ATPase acitivity of relaxed fibres and fibrils actually coincides "quantitatively" with the also low activity of purified myosin nor any study to see if the ATPase of relaxed fibrils corresponds "qualitatively" to that of myosin has been made.

Such a test has become essential since PERRY AND LEADBEATER[8] and PERRY AND COTTERILL[9] have shown that acto-heavy meromyosin may have a viscosity, indicating full dissociation but possessing a Mg^{2+}-stimulated ATPase activity larger than pure heavy meromyosin. Consequently, this paper describes the systematic comparison between the ATPase activities of purified myosin and of extracted fibrils in conditions of relaxation and contraction at varied ionic strength, pH and concentrations of the free Mg^{2+} and of the MgATP complex (*i.e.* $MgATP^{2-} + MgHATP^{-}$). Contraction and relaxation of the fibrils were produced by adjusting the level of the free Ca^{2+} to $5 \cdot 10^{-5}$ M and $< 1 \cdot 10^{-8}$ M, repectively[29].

RESULTS

Dependence on ionic strength

Fig. 1 shows dependence of the ATPase activities on ionic strength (pH 7.0) of contracted and relaxed rabbit fibrils (Curves 1a and 1), of contracted and relaxed crab fibrils (Curves 2a and 2) and of rabbit myosin* (Curves 3a and 3)**. Fig. 2 shows the corresponding curves at pH 6.5.

These results show the following similarities: (1) The fibrillar ATPase activity in conditions of relaxation always amounts to about the same fraction (*i.e.* 1/7 with

* Till now it was not technically possible to obtain crab myosin.

** To compare the ATPase activities of relaxed fibrils and of myosin, it is only necessary to have the curve of myosin without Ca^{2+} (Curve 3). The activity curve of myosin in the presence of 0.05 mM Ca^{2+} (Curve 3a) is also shown because unexpectedly it does not coincide with Curve 3. This may be caused by a slight Ca^{2+}-activation of the myosin ATPase in spite of the excess of Mg^{2+} present or it may reveal traces of actomyosin contaminating the myosin.

rabbit fibrils at pH 7.0) of the activity in conditions of contraction*. (2) The ATPase activity of all the fibrils decreases to 1/6 or 1/10 when the ionic strength increases from 0.05 to 0.2. Both of these findings are consistent with earlier observations and are also valid for rabbit and crab fibrils at pH 7.0 and pH 6.5.

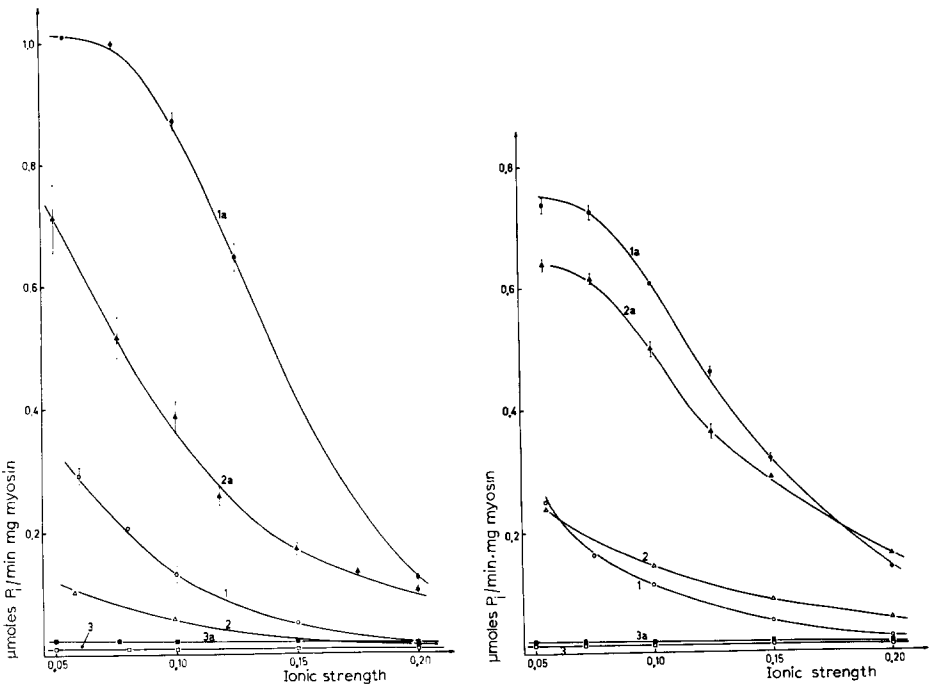

Fig. 1 and 2. Dependence of ATP splitting by fibrils and myosin on ionic strength at pH 7 (Fig. 1) and pH 6.5 (Fig. 2).** Curves 1 and 1a: rabbit fibrils: O—O, $[Ca^{2+}] < 1 \cdot 10^{-8}$ M, ●—●, $[Ca^{2+}] \simeq 5 \cdot 10^{-5}$ M. Curves 2 and 2a: crab fibrils: △—△, $[Ca^{2+}] \simeq 5 \cdot 10^{-8}$ M; ▲—▲, $[Ca^{2+}] \simeq 7 \cdot 10^{-5}$ M. Curves 3 and 3a: rabbit myosin; □—□, $[Ca^{2+}] < 1 \cdot 10^{-8}$ M; ■—■, $[Ca^{2+}] \simeq 5 \cdot 10^{-5}$ M. Vertical bars represent S.E. (where they are not indicated, their size coincides with the size of the curve sign). Added [ATP] = added $[MgCl_2]$ = 1 mM. Temp., 20°. See MATERIALS AND METHODS for further details.

In contrast to the fibrils, the activity of the myosin ATPase does not depend to any great extent on the ionic strength in this range (Curves 3 and 3a in Figs. 1 and 2). The activity of the relaxed fibrils, is always much higher than that of myosin, especially at low ionic strengths (*cf.* Curves 1 and 2 with 3 in Figs. 1 and 2). Thus the ATPase of relaxed fibrils shows the typical features of the actomyosin-type ATPase and not those of the myosin-type with respect to its velocity and dependence on ionic strength.

* This is fully valid only at ionic strengths between 0.2 and 0.075 since at still lower values the very high ATPase rate of the contracted state approaches the absolute maximum unlike the very low rate of the relaxed state. An exception to this is the ATPase of crab fibrils at pH 7.0 (*cf.* Curve 2a in Fig. 1).

** For the sake of comparison of the activities of myosin and fibrils, the rate of the fibrillar ATPase has been related to myosin in all figures. This is feasible by simple doubling the fibrillar activity found because the content of myosin within the fibrils is about 50% (ref. 10).

Dependence on pH

Rather similar behavior is displayed by the different ATPase activities of the contractile substrate of the rabbit muscle at varied pH's. The rate of the myosin ATPase decreases by about one half when the pH increases from 6.0 to 8.0 (Fig. 3, Curves 3 and 3a), whereas the rate of the fibrillar ATPase increases 2-fold in both contracted and relaxed states (Fig. 3, Curves 1 and 1a)*.

With respect to their dependence on pH, the fibrillar ATPases go in the opposite direction to the myosin ATPase. The splitting velocity of relaxed fibrils (pH 6.0) is about 5 times as high as that of the myosin ATPase and at pH 8.0 almost 30 times as high (*cf.* Curve 1 with 3). Nevertheless, the rate of the relaxed fibrils amounts to a mere one eighth of that of contracted fibrils over the whole range tested (*cf.* Curve 1 with 1a).

A very similar dependency on pH applies to the crab fibrils which in the relaxed state (Curve 2) exhibit an ATPase entirely different from the myosin ATPase (Curve

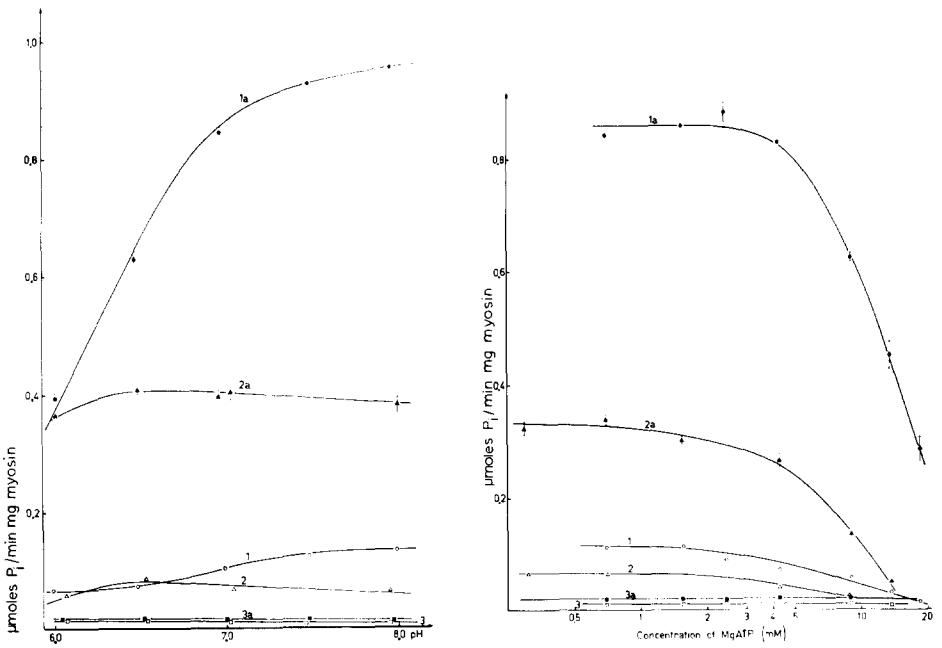

Fig. 3. Dependence of ATP splitting by fibrils and myosin on pH at an ionic strenght of 0.1. Curves 1, 2 and 3 with $(Ca^{2+}] < 5 \cdot 10^{-8}$ M. Curves 1a, 2a and 3a with $[Ca^{2+}] \simeq 5 \cdot 10^{-5}$ M. Curves 1 and 1a rabbit fibrils, 2 and 2a crab fibrils, 3 and 3a rabbit myosin. Vertical bars represent S.E. Added [ATP] = $[MgCl_2]$ = 1 mM. Temp., 20°. See MATERIALS AND METHODS for further details.

Fig. 4. Dependence of ATP splitting by fibrils and myosin on the concentration of MgATP at pH 7.0 and ionic strenght of 0.1. Curves 1, 2 and 3 with $[Ca^{2+}] < 5 \cdot 10^{-8}$ M; Curves 1a, 2a and 3a with $[Ca^{2+}] = 2 \cdot 10^{-5}$–$5 \cdot 10^{-5}$ M. Curves 1 and 1a rabbit fibrils, 2 and 2a crab fibrils, 3 and 3a rabbit myosin. Vertical bars represent S.E. Added [ATP] = $[MgCl_2]$ increasing from 1 to 15 mM (Curves 1 and 1a), to 20 mM (Curves 3 and 3a) or from 0.5 to 10 and 15 mM (Curves 2 and 2a), respectively. Temp., 20°. See MATERIALS AND METHODS.

* This dependence on pH of the fibrillar ATPases agrees well with the pH dependence of the ATPase of actomyosin gel found by BÁRÁNY AND BÁRÁNY[11]. However, the existing information about the myosin ATPase was obtained at much higher ionic strength with or without Ca^{2+}-activation (*cf.* ref. 12).

3). However, the opposite direction when compared to the myosin ATPase is restricted to the pH range between 6.0 and 6.5 because only in this range does the fibrillar activity increase slightly. Though the fibrillar ATPase exceeds the myosin ATPase over the pH range from 7 to 8 (*cf.* Curve 2 with 3), it is but one sixth of the activity of contracted fibrils throughout the whole range of the curve (*cf.* Curve 2 with 2a).

Dependence on the concentration of MgATP complex

In the measured range between 0.7 and about 14 mM MgATP, the velocity of hydrolysis by myosin remains constant (Fig. 4; Curves 3 and 3a). In the case of fibrils (rabbit and Maia), however, constant activity exists only between 0.3 and 3 mM MgATP and the splitting velocity falls off steeply as the concentration of the MgATP complex* is further increased, in conditions of contraction and of relaxation (Fig. 4; Curves 1, 1a, 2, 2a). The absolute values of the fibrillar activities in conditions of

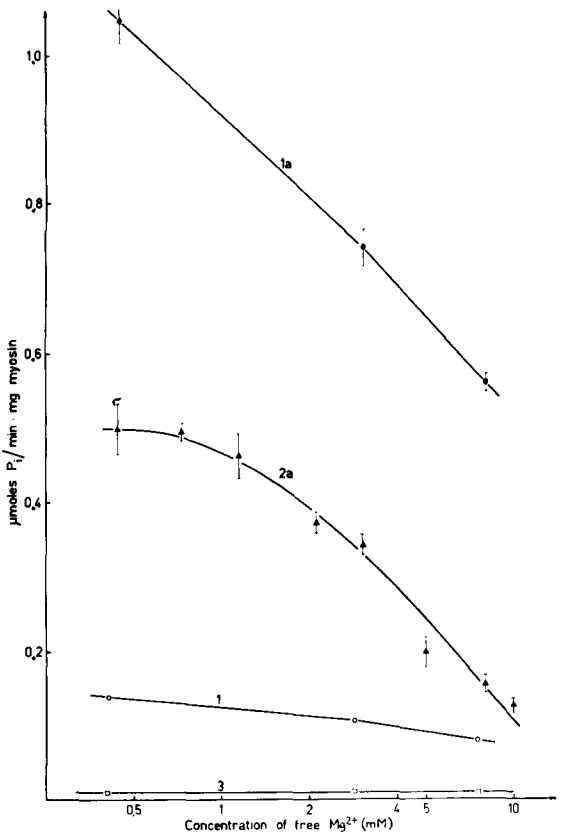

Fig. 5. Dependence of ATP splitting by fibrils and myosin on the concentration of Mg^{2+} at pH 7.0 and ionic strength of 0.1. Curves 1 and 1a: rabbit fibrils; ○—○, $[Ca^{2+}] < 1 \cdot 10^{-8}$ M; ●—●, $[Ca^{2+}] = 5 \cdot 10^{-5}$–$9 \cdot 10^{-5}$ M. Curve 2a: crab fibrils with $[Ca^{2+}] \simeq 10^{-5}$ M. Curve 3: rabbit myosin with $[Ca^{2+}] < 1 \cdot 10^{-8}$ M. Added [ATP] = 2 mM, added $[MgCl_2]$ increasing from 2 to 10 mM (Curves 1, 1a and 3) or 12 mM (Curve 2a), respectively. Temp., 20°. See MATERIALS AND METHODS.

* These experiments clearly show that an inhibition of the fibrillar ATPase activity by over optimal concentrations in the physiological range also occurs when neither ATP nor Mg^{2+} are present in excess (*cf.* refs. 13 and 14).

contraction to those in relaxation are higher by a factor of 6 in Maia to 10 in rabbit (at least in the well-measureable range from 0.3 to 10 mM MgATP). At optimal concentrations of MgATP, on the other hand, the ATPase activities in conditions of relaxation are still 3–5 times higher than those of myosin alone (*cf.* Curves 1 and 2 with 3 of Fig. 4). In the presence of over-optimal concentrations of MgATP, this difference progressively disappears as the activity of the contracted fibrils declines.

Dependence on the concentration of Mg^{2+}

A change in the Mg^{2+} concentration is only possible in that range in which the concentration of added Mg^{2+} is higher than that of the added ATP. In this case, the concentration of MgATP remains practically the same. During the experiments in Fig. 5, 1.6 mM MgATP corresponding to a Mg^{2+} concentration of 0.4 mM, whose value can be increased up to 8 mM* by the addition of $MgCl_2$, is always present. This gives in both relaxed and contracted fibrils a decrease in the ATPase activity of about 55 % of the original value (Fig. 5; Curves 1a and 1). The same change in concentration of Mg^{2+} does not alter the ATPase activity of Ca^{2+}-free myosin (Fig. 5; Curve 3). The absolute values of the fibrillar hydrolysis in the contracted state are always about 7 times higher than those in relaxed state (*cf.* Curve 1a with 1). The velocity of splitting in conditions of relaxation, even at the highest Mg^{2+} concentration, is still 8 times that of pure myosin (*cf.* Curve 1 with 3). Here again, as judged from the graded dependence on the Mg^{2+} concentration, the fibrillar ATPase in conditions of relaxation is found to be characteristically of the actomyosin type.

The velocity of ATP hydrolysis by fibrils of *Maia squinado* in conditions of contraction decreases more markedly with increasing Mg^{2+} concentration, *i.e.* to 30 %, as does the activity of rabbit fibrils (Fig. 5; Curve 2a). Although the corresponding curve in conditions of relaxation is lacking the curve for contraction is given here because such measurements on crab fibrils have not been published previously.

DISCUSSION

In all experiments, the ATPase activity of relaxed fibrils behaves quantitatively as a 6–8 fold "diluted" activity of contracted fibrils. On the other hand, the relaxed state activity is quantitatively and qualitatively totally different from the activity of pure myosin. Occasionally even, the direction of its dependency on the chosen parameter is opposite in relaxed fibrils and myosin, respectively (*cf.* Fig. 3). Hence, the observation of PERRY AND LEADBEATER[8] and PERRY AND COTTERILL[9] that the heavy meromyosin ATPase may become an acto-heavy meromyosin ATPase without a physically observable formation of a complex can be placed on a very broad basis and can be expanded to the physiological interaction between myosin and actin.

Consequently, the question arises as to how far actin and myosin in relaxed fibrils actually are associated? Since the ATPase activity of myosin represents a mere 1.5–2 % of the activity of actomyosin (*cf.* Figs. 1–5), it is concluded that an almost complete dissociation of the actomyosin complex reduces the activity of

* The increase of the Mg^{2+} concentration from 0.4 to 8 mM raises the MgATP concentration from 1.6 to 1.97 mM and decreases the ATP concentration correspondingly from 0.4 down to 0.03 mM (*cf.* MATERIALS AND METHODS).

the actomyosin ATPase without inducing a comparable myosin activity. To estimate the "degree" of dissociation it is thus sufficient to take into account only the reduction of the splitting velocity between the fully associated fiber and that of the relaxed fiber. Taking account of the very small activity of myosin ATPase during partial dissociation, the computation of the dissociated part (degree of dissociation, X) takes the form

$$X = \frac{\text{AM—Sp}}{\text{AM—M}}$$

where Sp represents the measured ATPase activity of relaxed fibrils, AM that of fully associated fibrils and M is the activity of pure Ca^{2+}-free myosin.

The degree of dissociation (X) is calculated for the standard conditions in which relaxation is usually measured by physical means (*e.g.* loss of tension, absence of superprecipitation, *etc.*), namely at an ionic strength of 0.1, at pH 7 or 6.5 and at a concentration of MgATP of 1–2 mM. For this purpose, the splitting activity of relaxed fibrils (Sp) at the above ionic strength, pH and MgATP concentration is chosen from Curves 1 of Figs. 1–5. The splitting activity (AM) of completely associated fibrils is obtained from the rates of contracted fibrils or more specifically from their maximum rates. Full association may not exist in all conditions but most probably does so at the hydrolytic maximum. The results in Table I show that for the standard conditions of relaxation, the calculated degree of dissociation of actomyosin amounts to about 89 % in all experiments and that a mere 11 % of the actomyosin is still associated.

TABLE I

DEGREE OF DISSOCIATION OF ACTOMYOSIN IN RELAXED FIBRILS OF THE RABBIT IN STANDARD CONDITIONS

Computed from Fig. No.	*pH*	*Degree of dissociation* (%)
1	7.0	88
2	6.5	86
3	6.5	94
3	7.0	91
4	7.0	88
5	7.0	88
Mean		approx. 89

It is clear that a dissociation of 90 % cannot be distinguished from a dissociation of 100 % by the measurement of superprecipitation, electron microscopy or X-ray diffraction. The only physical method sensitive enough consists of measuring the disappearance of tension of extracted single fibers during the transition from conditions of contraction to conditions of relaxation. Even with this method, however, full and incomplete relaxation (H.PORTZEHL, unpublished experiments) was sometimes found. Also, it is quite possible that, in spite of the persistent 10 % contraction,

the tension fully disappears because a small elongation of the remaining 90% of the fiber is induced by this local contraction.

Thus, the discrepancy, first found by PERRY AND LEADBEATER[8] and PERRY AND COTTERILL[9] and confirmed here, between the results of chemical and physical investigations of the degree of dissocation is possible to explain. Consequently, the somewhat strained assumption[8,9] that in certain conditions actin and heavy meromyosin or, in this study, myosin may be associated chemically but not physically is unnecessary because this assumption does not consider the fact that the physical phenomena are governed exclusively by the "dissociated" part of the actomyosin system while the chemical properties belong to its "associated" fraction.

Therefore, is has to be assumed that extracted fibrils contain in conditions of relaxation still some small remnant of actomyosin. In the living resting muscle, however, even this small remnant is most probably dissociated. EBASHI[15] has presented evidence that relaxation induced by removal of Ca^{2+} is a function of "natural tropomyosin". It is not improbable, as judged by the investigations of SCHAUB[16], and SCHAUB *et al.*[17], that this protein will be damaged or partially extracted during the preparation of the fibrils. This is illustrated by the observation that the dissociated, *i.e.* relaxed, part of the actomyosin in fibrils becomes markedly smaller after long-term storage in 88.5% glycerol at $-15°$ (see Table II).

If this assumption is valid, then the activity of the myosin ATPase in conditions of relaxation is 100 times lower than that of the actomyosin ATPase in conditions of contraction. In living muscle, however, there is a difference of at least 1000 between the metabolism of the relaxed and contracted states. This means that there is a 10-times difference in metabolism between the fibrils and the living muscle.

In order to explain this discrepancy between the living muscle and the isolated contractile system, the following three possibilities are suggested. (1) The ATPase activity of contracted fibrils is 10 times "lower" than the turnover of the living contracted muscle; (2) the ATPase activity of myosin in conditions of relaxation is 10 times "higher" than the turnover of the resting muscle; and (3) the ATPase activities of contracted fibrils as well as of myosin deviate significantly from the turnover of living muscle during contraction and rest.

A closer examination of these possibilities reveals that the ATPase activity of contracted fibrils is not 10 times lower but only 3 times lower when it is computed from the maintenance heat* of the isometrically contracted living muscle at standard length[18]. When it is also considered that the measurement of the ATPase has been carried out at a fibrillar length of about 25% of the standard length[19], it is very probable that the ATP turnover of the contracted fibrils almost equals the energy turnover of the living contracted muscle; for like the development of tension[20,21] the maintenance heat strongly decreases with decreasing length[21]. Thus, it is possible that the maintenance heat at a length of about 30% comprises only one third of its original value.

If these assumptions are valid, then the ATP turnover of contracted fibrils does not differ from that of the contracted living muscle. However, the ATPase

* Since the rate of ATP hydrolysis is derived from the production of inorganic phosphate measured during several minutes it is permissible to compare the ATP turnover with the maintenance heat only because shortening heat or activation heat have no further influence upon the the hydrolytic rate.

activity of myosin in conditions of relaxation remains virtually 10 times higher than it is in the living muscle, as shown from measurements of the resting heat[22].

It is tempting to explain this high ATPase activity of myosin by assuming a contamination of 1–2 % of Ca^{2+}-insensitive actomyosin. This can be excluded on the grounds that 90 % of the ATPase activity found would be caused by the Ca^{2+}-insensitive actomyosin and only about 10 % by myosin which in turn would induce the same dependency on the parameters used as is exhibited by the fibrillar ATPase. This is, however, not the case (*cf.* Curve 3 with 1 and 1a in Figs. 1–5). The following assumption seems rather more probable, namely that the living muscle contains an inhibitor of ATPase which is active in the resting state and which in the course of isolation and purification of myosin will be denatured and/or lost in much the same way as "natural tropomyosin".

The summary of the evidence presented here leads to the probable conclusion that the ATP turnover of isolated contractile systems increases by a factor of about 100 during the transition from the resting state to the active state and not by a factor of 1000* as in the case of the energy turnover in the living muscle because the ATPase activity of isolated myosin is 10 times higher than the resting turnover of living muscle.

MATERIALS AND METHODS

ATP was bought as the disodium salt at P–L Biochemicals (Milwaukee, Wisc.). Ethyleneglycoldiaminotetraacetic acid (EGTA) was a gift of Geigy (Basel). All other chemicals were of analytical grade obtained from Merck (Darmstadt).

Preparation of the fibrillar suspensions

The fibrils from the leg muscles of the crab *Maia squinado* Rondelet were prepared by the method of PORTZEHL *et al.*[23]. The fibrils from rabbit muscle were obtained from thin bundles of psoas muscle fibers which had been stored for up to 9 months in 88.5 % glycerol. When required they were scissored into 150 ml of cold 0.1 M KCl *plus* 0.5 mM $KHCO_3$ (pH approx. 7). This mixture was minced for 3 min at maximum speed in a precooled blender (Braun), diluted with 150 ml of 0.5 mM $KHCO_3$ and centrifuged with 1900 × *g* (mean) for 12 min at 0°. After treating the sediment twice in the same manner, it was taken up in 3 vol. of 88.5 % glycerol and stored at −16° for periods of over a year without loss of its ATPase activity (*cf.* Table II). For use, the glycerol was washed out by suspending the rabbit or crab fibrils in either 20 vol. of 0.1 M KCl (pH approx. 7) or 40 vol. of 0.02 M KCl (pH approx. 7), depending on the required salt content of the incubation mixture. After centrifugation, the sediment was homogenized for 20 sec in 0.05 M or 0.02 M KCl (pH approx. 7), and the final protein content ranged between 0.1 and 0.2 %.

Preparation of myosin

The procedure of PORTZEHL *et al.*[24] was used in a slightly modified form. The scissored rabbit muscle was homogenized for 90 sec in 1.5 vol. of cold 0.6 M KCl

* The energy turnover of living muscle of course may increase far more than 1000 times during the transition, depending on the conditions of contraction, *e.g.* about 3000 times[22] in isometric condition at standard length or about 8000 times during a twitch (compited from data of A. V. HILL[22]).

TABLE II

DIMINUTION OF THE DEGREE OF DISSOCIATION DURING LONG-TERM STORAGE OF THE FIBRILS

Prep. No. of fibrils	*Storage time in 85% glycerol at −15°*	*Degree of dissociation* (%)*
Rabbit		
1	1–8 days	90
	2 months	89
2	13 months	88
3	14 months	88
4	42 months	76
Crab		
3	12–34 days	96
4	20–60 days	92
6	10 months	92
8	15 months	92
7	24 months	78

* Contrary as in Table I, the splitting rates (AM) used here were measured at $I = 0.05$.

in a blender (Braun) and then an other 1.5 vol. of 0.6 M KCl were added. After a 15-min extraction, the supernatant myosin was 4 times reprecipitated. The resulting solutions of myosin contains practically no actomyosin (sensitivity toward ATP, 1–4%) or only traces of actomyosin (sensitivity toward ATP in one case 14%, *cf.* ref. 24) and shows no significant alteration in ATPase activity.

Since the preparations of both fibrils and myosin possess rather different absolute ATPase activities, care was taken to perform all experiments concerning one parameter with the same preparation.

Determination of ATP splitting

The crab fibrils were incubated at 20° in a medium containing ATP* and $MgCl_2$*, 0.02 M imidazole (Figs. 1 and 4) or 0.015 M Tris–maleinate *plus* 0.1 mM $CaCl_2$ or 1–2 mM EGTA (potassium salt) and KCl for the adjustment of the ionic strength. For rabbit fibrils and myosin, the medium was the same except for 0.01 M (Fig. 4) or 0.015 M Tris–maleinate.

The hydrolysis of ATP was measured as P_i by the method of FISKE AND SUBBAROW[25] as modified by ROCKSTEIN AND HERRON[26]. The splitting rate was determined by sampling five aliquots over a period of several min. The splitting rates proved to be linear for up to 90% of hydrolyzed ATP; usually, however, not more than 20% of the ATP was hydrolyzed in any one experiment.

The calculation of the concentrations of the different ionic species of ATP, Mg^{2+} and Ca^{2+} was carried out using the formula for EGTA as previously described[27]. The values of the stability constants were those given by NANNINGA[28], corrected for pH (α_H; see ref. 27) but not for ionic strength. Therefore, the calculated concentrations are strictly valid at an ionic strength of 0.1. The ionic strength of the solutions will not, however, be exact should the stability constants of ATP change

* The concentrations are given in the legends to the figures.

markedly with ionic strength. However, this restriction will not influence the results, since the error will be of the same magnitude for both contraction and relaxation. The influence of EGTA on the concentrations of Mg^{2+} and of MgATP was shown by calculation to be not more than 1 %.

Protein was determined by the semimicro-Kjeldahl method using a factor of 6.25 for the fibrils and 6.05 for myosin.

For the measurement of pH, a precision compensator Metrohm E 322 was used.

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