MYOSIN FROM CARDIAC MUSCLE

by

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In connection with physico-chemical investigations on myosin, actin and actomyosin from skeletal muscle of rabbit made at this institute by SNELLMAN and coworkers, and recently summarized in a paper by SNELLMAN AND GELOTTE[1], it seemed to be of interest to undertake similar investigations on the same proteins from other muscular tissues. There is reason to believe that physiological differences between smooth, skeletal and cardiac muscle correspond in some way to differences in the contractile proteins. One may also hope that such comparative investigations will be able in the future to throw some light upon the problem of muscular contraction.

Myosin and actin from cardiac muscle have been investigated earlier, but obviously not in detail. Thus SZENT-GYÖRGYI[2] refers to investigations by RÓZSA, who has made viscosimetric measurements on myosin and actomyosin. From these he draws the conclusion that there are no or very small differences between the contractile proteins in heart and skeletal muscle. Similar measurements by KASAVINA AND BALYASNAYA[3] show that artificial actomyosin from cardiac myosin and actin has a lower contracting power than the corresponding protein complex from skeletal muscle. BAILEY[4] has reported that cardiac myosin has a lower adenosine triphosphatase activity than ordinary myosin.

EXPERIMENTAL

For preparing myosin from cardiac muscle the method given by SZENT-GYÖRGYI[2] for the preparation of myosin from skeletal muscle has been used in a slightly modified form. For the present investigations sheep hearts were used, but the same results are obtained with hearts of calf, cow and pig.

The heart of the killed animal is removed as rapidly as possible and cooled to 0° C. The muscle of the right and left ventricle is freed of fat and minced in a cooled meat mincer. The minced muscle is extracted with three volumes 0.5 *M* KCl + 0.1 *M* potassium phosphate, p_H 7, at 0° C during 10 minutes. The mixture is filtered through a cloth and an equal volume of redistilled water at room temperature is added. The solution is stirred for about two hours at room temperature, while most of the actomyosin precipitates. The precipitate is centrifuged down and the clear supernatant is cooled to about + 4° C. Then two volumes of redistilled water of the same temperature are added slowly with gentle stirring. At this salt concentration, which corresponds to 0.08 *M* KCl, all the myosin is precipitated. The myosin is now allowed to settle, decanted after some hours and centrifuged. The myosin is then dissolved in 0.5 *M* KCl, p_H 7. The buffer system is 0.05 *M* primary and 0.05 *M* secondary potassium phosphate. This solution is used as solvent in all experiments, unless mentioned otherwise.

With this method it has not been possible to obtain cardiac myosin in the same crystallized

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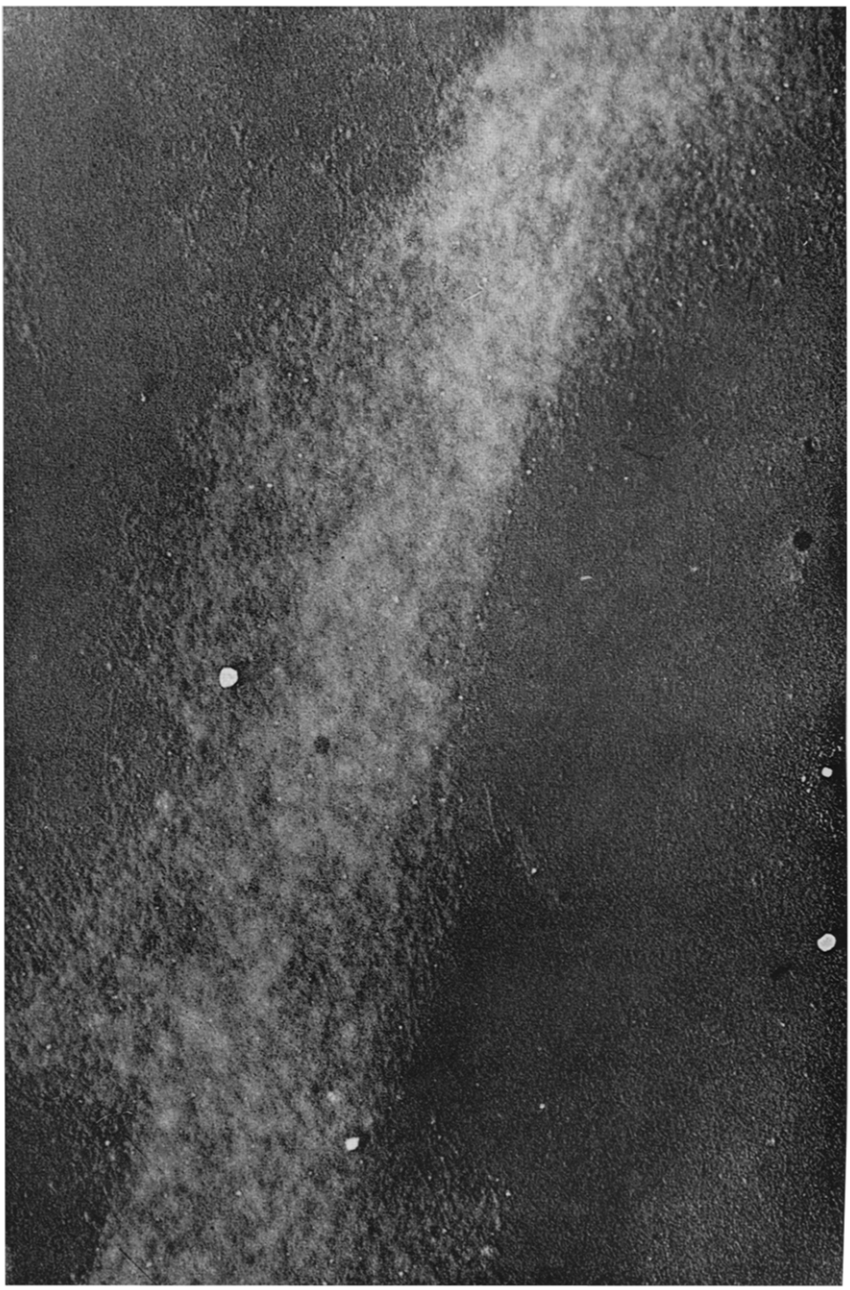

Fig. 1. Electron micrograph of cardiac myosin. Magnification 30,000 ×

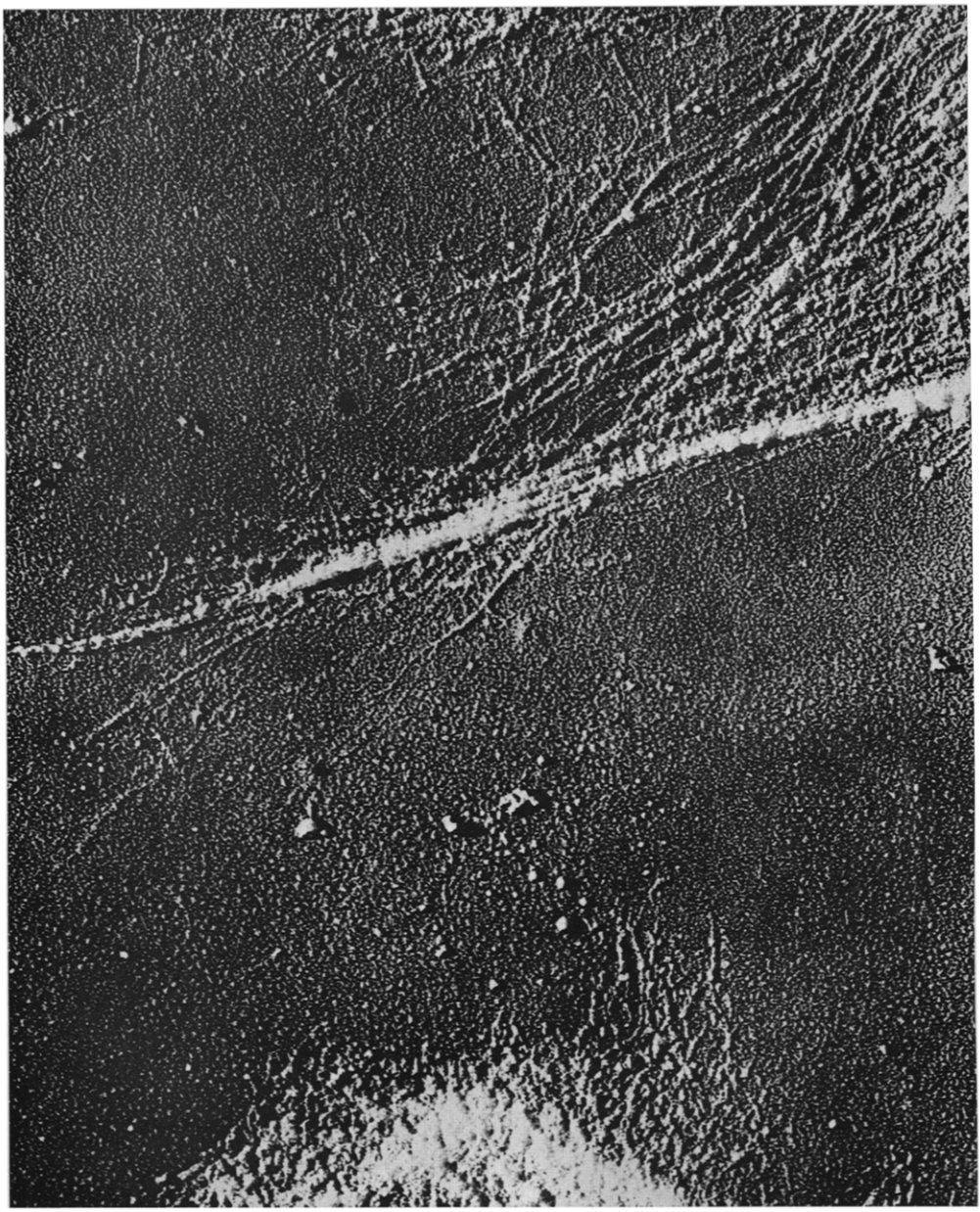

Fig. 2. Electron micrograph of cardiac myosin. Magnification 30,000 ×

form as in the case of myosin from skeletal muscle. Further experiments to prepare a crystallized cardiac myosin were not performed, because it is already fairly pure. In order to free the preparation completely from actin and actomyosin it is sufficient to reprecipitate two or three times with water in the same way as described above. The purity is controlled by viscosimetric measurements, the preparation being considered pure if there is no decrease in viscosity upon addition of ATP. As a test 4.0 ml myosin solution containing 1 mg myosin per ml is taken. The viscosity is measured before and after the addition of 1 mg ATP. Purification by fractionated precipitation with ammonium sulphate is also possible. The pure myosin precipitates at 0° C and pH 7 between 31 and 35% saturation. As will be mentioned later this method, however, cannot be recommended because the protein is broken down to some extent.

The same results and the same yield of myosin are also obtained by the method of WEBER[5]. After the extraction with buffer at 0° C, the minced muscle is filtered off, and the solution is immediately diluted with five volumes of cold water. The precipitate thus formed containing both myosin and actomyosin is dissolved in 0.5 *M* KCl, pH 7. To obtain pure myosin it is sufficient to carry out two or three reprecipitations with water.

The yield of purified myosin obtained by these methods amounts to about 0.3% of the wet muscle used in the preparation. Because of the great sensitivity of myosin for heavy metal ions it is necessary to use redistilled water in all work with this protein. Water filtered through an ion exchanger (Amberlite IR-100) has shown to be quite adequate throughout the whole preparation. The myosin obtained is rather unstable in solution. It can be kept for about one week at 0° C. After a longer storage some of the protein begins to precipitate. Care must be taken not to have it at room temperature for several hours.

Figs 1 and 2 represent electron micrographs of the purified cardiac myosin. By comparing them with the corresponding pictures published by SNELLMAN AND ERDÖS[6] it is seen that cardiac myosin does not crystallize as does myosin from skeletal muscle. It is mostly obtained as a gelatinous precipitate without any structure. Sometimes some sort of aggregation into longer threads is observed (Fig. 2). Whether or not this aggregation occurs around contaminations of F-actin, still present in the solution, is very difficult to say, but it seems probable. This may also be the case for the crystallized myosin. COHEN[7] has shown that elongated molecules are precipitated in a paracrystalline state by small amounts of multivalent anionic colloids *e.g.* heparin, hyaluronic acid and chondroitin sulphuric acid. F-actin has about the same magnitude of charge as these colloids, and it may be similarly responsible for the crystallization of myosin.

The cardiac myosin is quite insoluble in distilled water and in dilute salt solutions. It dissolves readily in 0.5 *M* KCl, but on dilution with water it becomes opalescent and is fully precipitated at an ionic strength of $\mu = 0.1$. As shown by SZENT-GYÖRGYI[2] crystallized myosin is insoluble only at a salt concentration of 0.025 *M* KCl, where it has a solubility minimum. It is soluble both in distilled water and at other salt concentrations.

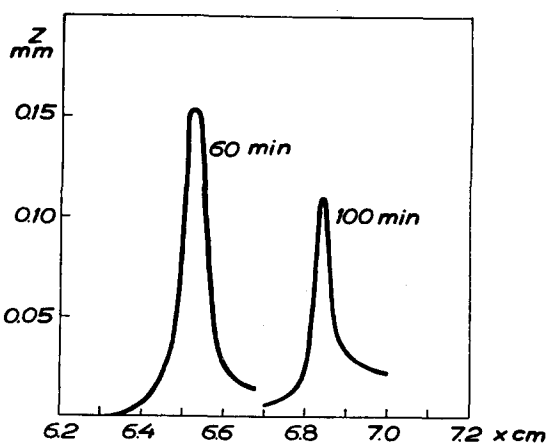

Fig. 3. Sedimentation diagram of cardiac myosin. Conc.: 2.4 mg/ml. Solvent: 0.5 *M* KCl + 0.1 *M* K-phosphate pH 7

Sedimentation diagrams of cardiac myosin show only one component (Fig. 3). This, however, holds only when the myosin solution contains about 1 mg per ml or more. At lower concentrations the myosin seems to be split, and the sedimentation diagrams indicate an inhomogeneous substance. In nearly all experiments the solvent is 0.5 *M* KCl, pH 7, but solutions containing 0.7 *M* KCl are also used. In order to establish whether

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the myosin has undergone any changes during the preparation, sedimentation diagrams have been taken of purified solutions of actomyosin to which ATP (adenosinetriphosphate) has been added immediately before the beginning of the experiment. The actomyosin then dissociates into F-actin, which rapidly sediments to the bottom of the cell, and myosin, which shows the same diagram and sedimentation constant as the purified myosin solution. The sedimentation constant shows a rather large concentration dependance, which indicates that the molecule may be asymmetric, probably some sort of fiber molecule. The molecule may also be hydrated. As seen from Fig. 4 a value for the sedimentation constant at zero concentration cannot be given with accuracy, but it must lie within the limits 6.5–7.5 S. It may have the same value $S_{20} = 7.2$ S as is obtained for myosin from skeletal muscle (1.5).

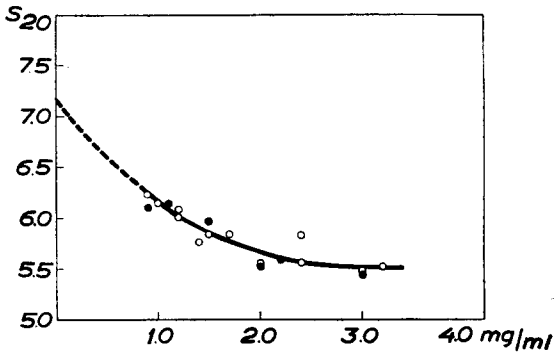

Fig. 4. Variation of the sedimentation constant with concentration of cardiac myosin. Open circles: 0.5 M KCl + 0.1 M K-phosphate p_H 7. Full circles: 0.7 M KCl + 0.1 M K-phosphate p_H 7

The cardiac myosin is also electrophoretically homogeneous. More extensive investigations have not been made. It is impossible to obtain a myosin solution at a p_H corresponding to its isoelectric point 5.4 and also difficult to make measurements at more acid p_H values. Therefore the determinations of the mobility have been made only on the alkaline side of the isoelectric point and at some different ionic strengths. The results are the same as those obtained for crystallized myosin from skeletal muscle[8]. The mobilities thus found, using a protein concentration of 1%, were $u = -1.8 \cdot 10^{-5}$ in 0.5 M KCl + 0.05 M potassium phosphate at p_H 6.0, and $u = -2.7 \cdot 10^{-5}$ cm^2 volt^{-1} sec^{-1} at p_H 7.8.

Cardiac myosin in 0.5 M KCl shows a normal viscosity at lower concentrations up to 1.5 mg/ml, but has an anomalous viscosity at higher concentrations (Fig. 5). The same holds for actomyosin to which some ATP is added. The measurements were made in an OSTWALD viscosimeter at a temperature of 22° C.

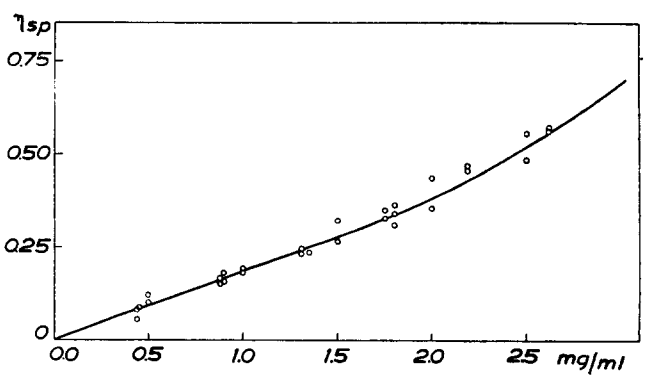

Fig. 5. Viscosity curve of cardiac myosin

BAILEY found a lower adenosine triphosphatase activity for cardiac myosin than for myosin from skeletal muscles. There is no reason to doubt BAILEY's results, but since these investigations have been made with a mixture of myosin and actomyosin, it would be of interest to study the enzymatic properties of the pure myosin. According to earlier results an enhanced activity is found in glycine buffer, and the optimum lies at about

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p_H 9. So all experiments have been performed in 0.5 *M* KCl + 0.5 *M* glycine at p_H 9.

In order to obtain the most correct values another method for the determination of phosphorus has been used. To avoid hydrolysis of the ATP by the acid solutions in FISKE-SUBBAROW's method[9], the measurements have been made according to LOWRY AND LOPEZ[10].

A single incubation mixture, containing 20 mg ATP, 0.08 moles $CaCl_2$, and about 1 mg myosin, dissolved in 20.0 ml 0.5 *M* KCl + 0.5 *M* glycine, p_H 9, is incubated at 25° C. Every fifth minute up to 30 minutes a sample of 2.0 ml is taken from the mixture, inactivated by addition of 2.0 ml saturated ammonium sulphate and cooling to 0° C. After one hour in ice water the precipitated myosin is filtered off and 1.0 ml is taken for estimation of the phosphorus content at a p_H of 4.3. If the substrate is in excess a straight line is obtained when the phosphorus liberated by the enzyme is plotted against the time of incubation. The turnover numbers are calculated from this curve. The unit of activity is taken as the volume in μl of a hypothetical gas equivalent to the amount of phosphorus in μg liberated in one hour by myosin containing 1.0 mg nitrogen.

$$Q_P = \frac{P \cdot \frac{22.4}{31} \cdot \frac{60}{t}}{\text{mg myosin-N}}$$

The activity values are not quite comparable with BAILEY's Q_P values, since he uses an incubation temperature of 37° C. Therefore the phosphatase activity of pure myosin from skeletal muscle of rabbit has been measured under the same conditions. Table I shows the results.

TABLE I

THE ADENOSINE TRIPHOSPHATASE ACTIVITY OF CARDIAC MYOSIN FROM CALF AND SKELETAL MYOSIN FROM RABBIT, AND ITS DECREASE UPON STORAGE

Time after preparation in days	Q_P			
	Cardiac myosin		Skeletal myosin	
	Prep. 1	Prep. 2	Prep. 1	Prep. 2
2	600			
3		540		
4			5400	5500
6	420			
7		380		
9	270			
11			5200	5200
12	260			
13		410		
15	300	280		

Contrary to earlier investigations the adenosine triphosphatase activity is found to be surprisingly constant for different preparations of the same myosin, though a variation of 5–10% is reasonable. When the myosin is stored in solution, even at 0° C, there is some loss of activity, and this is more pronounced for the cardiac myosin. As

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seen from Fig. 6 cardiac myosin looses about 60% of its activity after storage at 0° C during 10 days, and then becomes nearly constant. For myosin from skeletal muscle the decrease in activity during the same time is found to be 6%. When, however, the cardiac myosin is bound to actin in the form of actomyosin the phosphatase activity is about 50% higher at p_H 9, and the decrease in activity on storage is less.

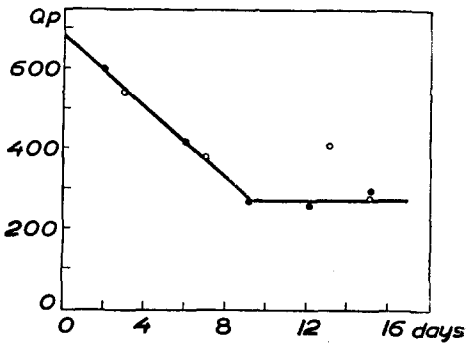

Fig. 6. Adenosine triphosphatase activity of two different preparations of cardiac myosin plotted against the time after preparation. The myosin solutions were stored at 0° C

DISCUSSION

It appears from these experiments that cardiac myosin and myosin from skeletal muscle have about the same properties. Some differences, however, exist. Cardiac myosin is insoluble in pure water and is also less soluble in salt solutions than the myosin from skeletal muscle. Thus the former must have a more hydrophobic character. Both viscosity and ultracentrifugation measurements indicate an elongated molecular structure. The variation in the sedimentation constant with concentration, different from that of ordinary myosin, could be explained as a solvation of the cardiac myosin molecule causing an alternation of the frictional resistance against the sedimentation. This is not probable, however, firstly because the cardiac myosin is more hydrophobic, and secondly because the sedimentation constant does not change upon an increased addition of electrolyte (Fig. 3). There is another possible explanation for this divergence between the two types of myosin. Salting out experiments show that the crystallized myosin from skeletal muscle is not quite pure[1], while the cardiac myosin is free from actin and other impurities. Since the results obtained from the ultracentrifugation experiments are the same as those obtained for fibre molecules, it is more probable that the type of concentration dependance of the sedimentation constant as shown by the cardiac myosin is the correct one. This view is also supported by the fact that ordinary myosin sometimes behaves in the same way. If crystallized myosin is further purified by recrystallization as described by SZENT-GYÖRGYI[2], the ultracentrifugation data give the same results as those for cardiac myosin.

There is, nevertheless, no doubt that cardiac myosin is more labile than myosin from skeletal muscle, and it is possible that some changes take place during the preparation of actin-free myosin. Bearing in mind that myosin is not a unit protein but a complex of several proteins, a change in the molecular structure during preparation is very well possible. In ultracentrifugation experiments with cardiac actomyosin split into myosin and actin by ATP, the myosin part gives the same sedimentation constant as myosin prepared in the manner described above. This, of course, only shows that the molecule does not break down to any greater extent. A rearrangement or a smaller change in the molecule is possible, however.

The loss of adenosine triphosphatase activity during storage of cardiac myosin is a good indication of its instability, but it is difficult to determine whether the low enzymatic activity is a consequence of the preparation. There is reason to believe that cardiac muscle, due to its mode of action, has a lower phosphatase activity *in vivo* than

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skeletal muscle. The great difference found *in vitro* is probably caused by a partial damage of the parts of the molecule responsible for these enzymatic properties. The fact that actomyosin has a higher activity may indicate a protective action of actin on the phosphatase activity during preparation.

A further proof of the instability of cardiac myosin is that it is possible to split off some part of the complex. With the same method as used by GUBA[2] to prepare what SZENT-GYÖRGYI calls a "protin-free" myosin, *i.e.* stirring a myosin precipitate during several hours with 0.025 *M* KCl, a substance containing a nucleotide has been split off from the cardiac myosin and has also been isolated. It has been shown that already during purification of the cardiac myosin by reprecipitation with water and by storage of a myosin solution for some days, relatively great amounts of this substance are split off. It has been found that by fractionated precipitation with ammonium sulphate almost all of this substance is split off. Therefore the reprecipitation with water has been preferred for the preparation of actin-free cardiac myosin. Investigations on this split product as well as analyses are in progress.

By mixing solutions of cardiac myosin and F-actin from skeletal muscle, an actomyosin is obtained which has a lower viscosity than the actomyosin prepared from F-actin and myosin from skeletal muscle. This may depend on the fact that due to its instability, not all the cardiac myosin has the ability to form this complex, but it is evidently another indication of the difference between the two types of myosin. Because of the difficulty to obtain a cardiac actin which polymerizes to F-actin a corresponding experiment to prepare F-actomyosin from myosin and actin from cardiac muscle has failed. This polymerization occurs to a very small extent with actin from heart using the preparation method described by STRAUB[11]. These facts indicate that actin from different muscular tissues also has varying properties, and there is reason to believe that the differences between different muscles are not restricted to myosin only.

One has to suppose that the rather small structural differences, which physicochemical investigations have shown to exist for different kinds of myosin, are of secondary importance for the studied reactions. Since myosin must be regarded as a rather complicated complex of proteins and other substances, questions concerning its nature can be answered only after a thorough investigation of these smaller components. For such a purpose the cardiac myosin may be of importance and perhaps in some respects more suitable than myosin from skeletal muscle.

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SUMMARY

Myosin from cardiac muscle was prepared which seems to be free from contamination by F-actin. The electrophoretic mobility and sedimentation constant are the same as for crystallized myosin from skeletal muscle, but the sedimentation constant has another concentration dependence. The adenosine triphosphatase activity is low and decreases upon storage. The cardiac myosin is unstable in solution and part of the molecule is easily split off. The properties of cardiac myosin are discussed and compared with those of crystallized myosin.

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RÉSUMÉ

Nous avons préparé à partir du muscle cardiaque une myosine qui semble ne pas être contaminée de F-actine. La mobilité électrophorétique et la constante de sédimentation sont les mêmes que pour la myosine cristallisée de muscle strié, mais la constante de sédimentation est une autre fonction de la concentration. L'activité adénosinetriphosphatasique est basse et elle diminue lorsque les préparations sont conservées. La myosine cardiaque est instable en solution et une partie de la molécule est facilement éliminée. Nous avons discuté les propriétés de la myosine cardiaque et les avons comparées à celles de la myosine cristallisée.

ZUSAMMENFASSUNG

Aus Herzmuskel wurde ein Myosin hergestellt, welches frei von F-Aktin-Verunreinigungen zu sein scheint. Die elektrophoretische Mobilität und die Sedimentationskonstante sind dieselben wie für kristallisiertes Myosin aus Skelettmuskel, aber die Sedimentationskonstante zeigt eine andere Abhängigkeit von der Konzentration. Die Adenosin-Triphosphatase-Aktivität ist schwach und nimmt beim längeren Aufbewahren der Präparate ab. Das Herzmyosin ist in Lösung nicht beständig und ein Teil des Moleküls wird leicht abgespalten. Die Eigenschaften des Herzmyosins werden erörtert und mit denen des kristallisierten Myosins verglichen.

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