ULTRACENTRIFUGAL STUDIES OF F-ACTOMYOSIN

by

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NEEDHAM *et al.*[1] found that the viscosity of myosin solutions in 0.5 M KCl decreases if small amounts of adenosinetriphosphate (ATP) are added to the solutions. BANGA AND SZENT-GYÖRGYI[2] found that if the muscle is extracted for a longer time (24 hours) a highly viscous myosin preparation is obtained. If ATP is added to such a preparation the viscosity decreases considerably. STRAUB[3] showed that the high viscosity was due to the fact that another substance, "actin", went into solution with the myosin to form a highly viscous complex, actomyosin.

SZENT-GYÖRGYI *et al.*[4] have shown further that if dilute solutions of myosin and polymerized actin (F-actin) are mixed the mixture immediately shows a large increase in viscosity, indicating that the myosin and the F-actin have reacted in some way. They called the complex thus formed F-actomyosin. It is dissociated under certain conditions by the action of ATP, with an accompanying decrease of the viscosity.

Actomyosin has some of the properties of crystallized myosin and actin but also many new properties. We do not wish to go into all these differences, but will only mention the following: Actomyosin can be contracted, precipitated and dehydrated by the action of ATP in certain definite salt concentrations. These properties are the basis for considering actomyosin as the contracting substance in muscle. SZENT-GYÖRGYI has assumed, from the beginning, that no definite stoichiometrical relation exists between actin and myosin in F-actomyosin, although there is an optimal ratio of one part actin to 2.5 parts myosin.

The object of our experiments has been the investigation of the behaviour of actomyosin (also called actomyosin only) in the ultracentrifuge, and to try to find if actin and myosin react in a stoichiometrical ratio.

ULTRACENTRIFUGAL ANALYSIS OF F-ACTOMYOSIN

F-actomyosin was prepared by mixing myosin and F-actin a few minutes before the experiment. F-actin was prepared by dialysing the unpolymerized actin (G-actin) over night at 4° C against the buffer solution that would be used, with the addition of 0.001 M $MgCl_2$. Determinations of the viscosity were carried out at the same time as the ultracentrifugations. During the measurements the temperature was 20° C.

The first experiments were carried out with the oil turbine ultracentrifuge[5] at 30000 rpm (centrifugal field = $65 \cdot 10^3$ g). Most often two components were observed, the sedimentation constants of which were not considerably far apart. The sedimentation

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References p. 526.

constants were very dependent upon the concentration. The extrapolated sedimentation constants (conc. $\rightarrow$ 0) were of the order 200 S. The concentration calculated from the sedimentation diagram was much too low as compared to that which could be expected.

The following experiments were carried out in the equilibrium centrifuge (a low speed ultracentrifuge with direct motor drive[5]) at 18000 rpm (centrifugal field $= 19 \cdot 10^3$ g) and 10000 rpm (centrifugal field $= 6 \cdot 10^2$ g).

It was hereby shown that the greater part of the actomyosin sedimented as a gel. Because this actomyosin gel sedimented so quickly it had not been detected in the runs at 30000 rpm, where it sedimented while the centrifuge was accelerating.

The gel formation seemed to be much greater with the synthetically prepared actomyosin than with that prepared directly from muscle by prolonged extraction.

Some experiments have also been carried out with actomyosin in the presence of ATP. In these experiments, 0.01 M $MgCl_2$ and 1 g potassium salt of ATP were added to the actomyosin solutions. The solutions were buffered with veronal buffer to p_H 6.8. The solutions contained 3 mg of myosin in 100 ml. The actomyosin usually splits ATP but under the experimental conditions used by us no such splitting of ATP takes place (temperature 20° C). Time of the experiment 2 hours. 0.01 M $MgCl_2$ added to prevent the splitting).

The sedimentation diagrams show in the presence of ATP the components with the very high sedimentation constants disappear while the characteristic diagrams for myosin[7] and F-actin appear. (About the behaviour of F-actin in the ultracentrifuge will later be reported). The dissociation of F-actomyosin into these components clearly takes place under the conditions given.

INVESTIGATION OF THE RATIO F-ACTIN TO MYOSIN IN F-ACTOMYOSIN

The fact that we found in earlier experiments[6] that the solutions prepared by extraction of the muscle for 24 hours contained myosin and actomyosin indicates that a mixture of myosin and actin does not, as previously assumed, react to form an actomyosin of completely indefinite proportions. In order to investigate the question more closely ultracentrifuge and viscosity investigations were made with synthetic actomyosin prepared from the crystallized myosin and F-actin.

At first we tried to separate the substances by differential centrifugation. Myosin has a much lower sedimentation constant than actomyosin and F-actin, so no difficulties are encountered in its separation from the other substances. In these experiments the BEAMS preparative centrifuge was used. After the centrifugation the sediment was separated from the supernatant. The sediment was redissolved in the buffer and viscosimetric measurements according to STRAUB[3] were made of both the solutions.

From experiments with different amounts of actin added to a certain amount of myosin we found that 1) if myosin and actin are mixed in an optimal proportion (1 part actin to 3 parts myosin) the whole protein content sediments to the bottom as actomyosin and no protein is found in the supernatant (no cloudiness occurs with trichloracetic acid); 2) if less actin is present than corresponds with the optimal ratio free myosin remains in the solution while the actomyosin sediments to the bottom; 3) if more actin is present than corresponds with the optimal ratio, free F-action is found in the supernatant. The last experiment was carried out with a certain amount of difficulty, because the difference between the sedimentation constants of F-actin and F-actomyosin is not

References p. 526.

very large. We would add that, in the case that myosin and actomyosin are both present, there are always traces of actomyosin in the supernatant. It is therefore not possible to separate myosin completely from actomyosin by this method.

We have also tried to determine the amount of myosin which is found in solutions containing actomyosin by adding varying amounts of F-actin to a specified amount of myosin, and by determining the myosin concentration from the sedimentation diagrams. The result of these investigations are seen from Table I.

TABLE I

DETERMINATION OF THE MYOSIN CONTENT FROM ULTRACENTRIFUGAL EXPERIMENTS WHEN DIFFERENT AMOUNTS OF ACTIN ARE ADDED TO A FIXED AMOUNT OF MYOSIN (3 mg/ml)

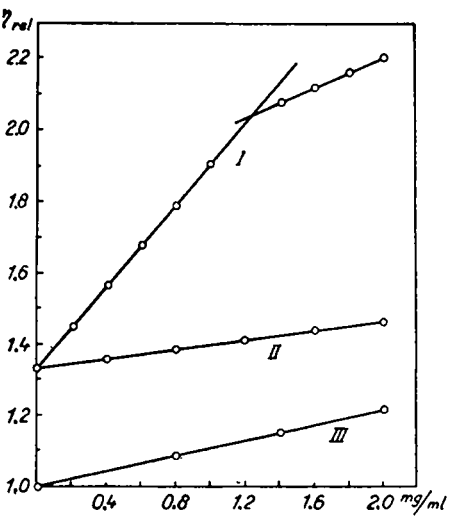
Added amount of actin in mg/ml	s_{20}	Amount of myosin calculated from the sedimentation diagram in mg/ml
0	5.7	3.0
0.2	6.0	2.4
0.4	6.1	1.8
0.6	6.6	1.2
0.8	+	Myosin component observable, but not measurable with certainty
1.0	—	Actin component visible in these two cases
1.2	—	

The values of the sedimentation constants agree with the values which we obtained in the study of the relation between concentration and sedimentation constant of the crystallized myosin[7]. The component can therefore be identified with myosin. The concentration decreases in well-defined equal steps as the actin content increases. It is clear that the actin binds a definite amount of myosin, and, when there is a deficiency of actin, part of the myosin is left in solution. At a certain actin concentration (optimal) no myosin is found in the solution and all the protein sediments as actomyosin. If more actin is added a new component appears which we could recognize as F-actin (other unpublished investigations).

The optimal proportion was in this case: 1 part actin to 3 parts myosin.

The problem was also studied viscosimetrically in order to see if it was possible, in this way, to determine the stoichiometrical proportions between actin and myosin when actomyosin is formed.

The same mixtures were used as in the ultracentrifuge experiments, except that the solutions were diluted to twice their volume. The relative viscosity measurements were carried out according to the method given by STRAUB[3]. The measurements were made at 20° C since the ultracentrifuge determinations were carried out



Viscosity curves. Ordinate: relative viscosity. Abscissae: F-actin added in mg/ml. I. Viscosity curve of a myosin solution with different amounts of actin added; II. Viscosity of the solution when ATP is added; III. Concentration dependence of the viscosity of F-actin

References p. 526.

at that temperature. When the relative viscosity of a solution had been determined, 1 mg of ATP was added and the measurement repeated. The measurements were completed by measuring the relative viscosity of a series of actin solutions of different concentrations. The results of the measurements are given in Fig. 1. Since the solutions show thixotropy the measurements were repeated until constant values were obtained.

The relative viscosity of the mixtures is increased by an increased amount of actin. The curve is linear and has a break at a certain amount of actin indicating that a change has taken place in the proportions at this point. After the break the relative viscosity increases very nearly at the same rate as in the case of actin. If the discontinuity constitutes the endpoint of the formation of actomyosin, it corresponds to a compound containing 1 part actin to 2.5 parts myosin.

It appears from these measurements that myosin and actin combine very nearly stoichoimetrically. Both methods used are subject to certain errors whereby an absolute agreement is not to be expected.

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SUMMARY

F-actomyosin has been investigated in the ultracentrifuge. It is a polydisperse substance. Two different main components are visible. One fraction has a very pronounced gel-like character and sediments very quickly. The other fraction shows a sedimentation picture characteristic of large long-chain molecules.

Experiments carried out indicate that actomyosin is a stoichiometric compound of actin and myosin. The values obtained from ultracentrifuge and viscosity data indicate that actomyosin contains 1 part actin to 2.5–3 parts myosin.

RÉSUMÉ

La F-Actomyosine a été étudiée à l'ultra-centrifugeuse; c'est une substance polydisperse. Deux principaux composés différents peuvent être observés. Une fraction possède un caractère très prononcé de gel et se sédimente très rapidement; l'autre fraction montre une sédimentation caractéristique des molécules à longues chaines. Les expériences faites montrent que l'actomyosine est un composé stoechiométrique de l'actine et de la myosine. Les valeurs obtenues à partir des résultats de l'ultra-centrifugation et des mesures de viscosité indiquent que l'actomyosine contient une part d'actine pour 2.5 à 3 parts de myosine.

ZUSAMMENFASSUNG

F-Aktomyosin wurde in der Ultrazentrifuge untersucht; es ist eine polydisperse Substanz. Man kann zwei verschiedene Hauptfraktionen unterscheiden, von denen die eine ausgesprochenen Gelcharakter hat und sehr rasch sedimentiert, während die andere das für lange Kettenmoleküle charakteristische Sedimentationsbild zeigt.

Die Versuchsergebnisse weisen daruaf hin, dass Aktomyosin eine stöchiometrische Verbindung von Aktin und Myosin ist. Die Ergebnisse der Ultrazentrifugation und der Viskositätsmessungen lassen darauf schliessen, dass Aktomyosin für je 1 Teil Aktin 2.5–3 Teile Myosin enthält.

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