ULTRACENTRIFUGAL STUDIES OF THE MYOSIN SOLUTIONS OF GREENSTEIN AND EDSALL

by

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Several ultracentrifugal* studies of myosin solution, prepared by approximately the same method as that of GREENSTEIN AND EDSALL[1] have already been made. The results seem, however, to be widely different.

Thus SVEDBERG[2] (1930) concluded, from investigations performed with the ultracentrifuge, that myosin was among the proteins which were found to be heterodisperse and unstable. SCHRAMM AND WEBER[3] reported the presence of two components in their preparations with sedimentation constants s = 6.2** and s = (20–30). On the other hand ZIFF AND MOORE[4] could only detect one component in their preparation with s = 9.5 in a 0.57% solution and s = 12.0 in a 0.33% solution.

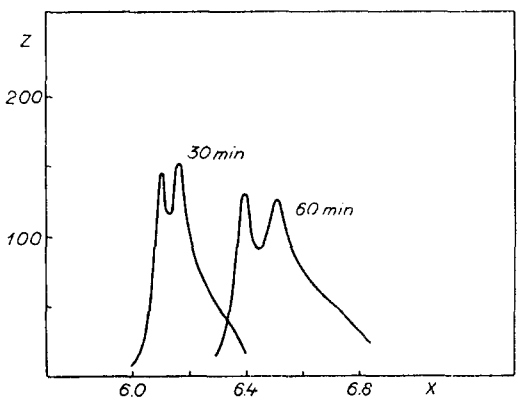

Fig. 1. Sedimentation diagram for the myosin of EDSALL AND GREENSTEIN (2 h) conc. 0.3%. 0.5 M KCl. pH 7.6

Some years ago we therefore began an investigation of myosin solution which were made by approximately the same method as that of GREENSTEIN AND EDSALL, in order to see which protein components we would be able to detect in different cases. At that time it was the best method known for the preparation of myosin solutions. Some time later the work of SZENT-GYÖRGYI[5] became available, including his method for preparing crystallised myosin.

We wish to mention here that depending on the extraction time BANGA AND SZENT-GYÖRGYI[6] have found that two different kinds of myosin can be prepared. Short extraction time yields the solution of Myosin A, prolonged extraction the very viscous Myosin B. When these kinds of myosin are dissolved in 0.5 M KCl the viscosity of Myosin A shows a small decrease on adding adenosin triphosphate (ATP) to the solution. Myosin B, however, responds to the addition of ATP with a great decline of the viscosity.

We know now since the investigations of SZENT-GYÖRGYI[5] and his collaborators, that there are two different kinds of proteins, actin and crystallised myosin. Actin can

* We shall not discuss the theory and technique of ultracentrifugation. For these matters consult SVEDBERG AND PEDERSEN, *The Ultracentrifuge*, Oxford University Press (1940).

** The sedimentation constants are given in Svedberg units (10^{-13}).

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appear in two different forms: unpolymerised (so-called G-actin) and polymerised to long fibrils (so-called F-actin). Myosin and actin together can build up a complex, actomyosin. G-actomyosin (with G-actin) has low viscosity and F-actomyosin (with F-actin) has high viscosity. The latter seems to be the best known of the two actomyosins. They can be dissociated by ATP under certain conditions.

EXPERIMENTAL

In our investigations we used the check-muscle of the cow. 10–15 min after the death of the cow the muscle was frozen in dry ice. It was then minced in frozen state.

The muscle was extracted with 6 parts of 1 M KCl buffered with the same buffer solution wich was used throughout the preparation. We used extraction times of 20 min, 2 h and 24 h. The extract was allowed to stand at – 10° C for three hours. There upon it was melted and vigorously stirred and filtered through a filtermesh. The solution was diluted with water of 0° C until the volume had increased 20-fold. The myosin precipitated and the excess fluid was poured off and the rest centrifuged. The precipitate was dissolved in a little 1 M KCl solution and was dialysed against 0.5 M KCl. The preparation was made at 0° C. In some cases the myosin was precipitated once, in others three times. There was no difference between the two samples in the ultracentrifuge. The concentration of the myosin was determined by KJELDAHL analysis.

At first we made an investigation of myosin extracted during two hours. The solutions were buffered with SÖRENSEN's phosphate buffer (0.07 n).

With these solutions we obtained sedimentation diagrams which generally showed two main components. The shapes of the curves showed that they might contain several components. Sometimes we obtained diagrams with only one component, but these diagrams showed irregularities and the sedimentation constants were often between those expected for the two main components. We therefore have reason to believe that these solutions contained both of our main components but in such a relation that it was not possible to separate them.

The sedimentation constants of our two main components were $s_0 = 7.2$ and $s_0 = 12$, extrapolated to the concentration zero. The concentration dependence of the constants was not so large (see Fig. 2). The value $s_0 = 7.2$ corresponds to that which we found later for SZENT-GYÖRGYI's crystallised myosin. These two components always appear between p_H 5.7–9.3 or in the range in which we have measured.

The component with sedimentation constant 7.2 was better defined than that with $s_0 = 12$. As well as these two components we often had some material with more rapid sedimentation, which was probably actomyosin.

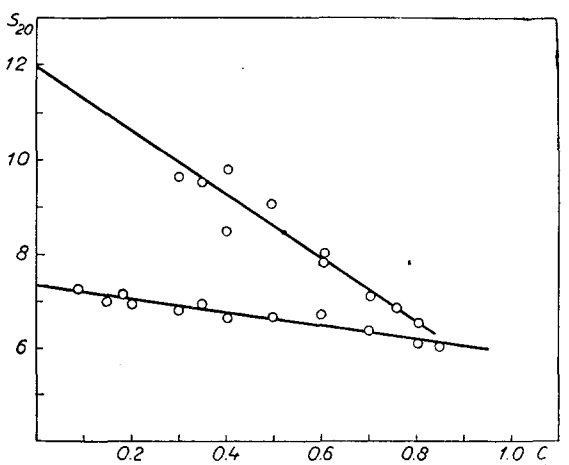

Fig. 2. Variation of s_{20} for the two components of the myosin of EDSALL AND GREENSTEIN wth the concentration of the solution

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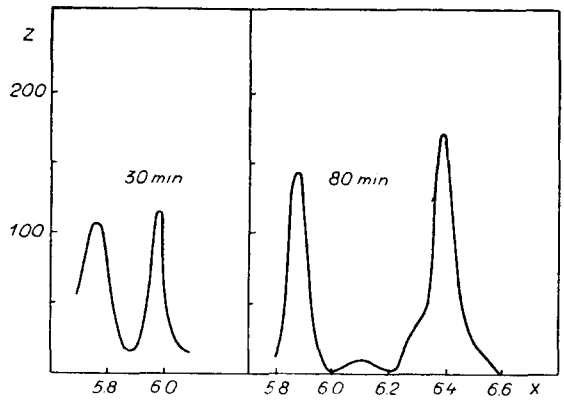

Fig. 3. Sedimentation diagram for a myosin of EDSALL AND GREENSTEIN (24 h). Thrice precipitated with 0.5 M KCl. pH 7.6

At p_H 5.7 the myosin is not very soluble and the part which goes into solution has a lower sedimentation constant.

We also made some experiments with an extraction time of 20 min, and there we obtained much of a very polydisperse material, but the myosin component was still predominant.

An investigation was also made with myosin extracted during 24 hours. This myosin corresponds to SZENT-GYÖRGYI's Myosin B. We have made the preparations using phosphate and glycine buffer.

Phospate buffers (0.07 n) were used between p_H 6.2 and 8.2. In all solutions the component $s_0 = 7.2$ was visible. The component $s_0 = 12$ was not or only very slightly detectable.

In all solutions one more rapid component with $s_0 = 50$ appeared, greatly depending upon the concentration. The component had the same sedimentation constant at different speeds of the ultracentrifuge, so it did not seem to have any jelly-like properties. We have later studied actomyosin in the ultracentrifuge, which was made from purified actin and crystallised myosin, and it seems to contain more of a jelly-like component than of this component. The p_H range for the stability of the actomyosin seemed to be 6–7.8. Above that there was still actomyosin but then it was partly decomposed and less clearly defined.

As well as these components we often observed components with lower sedimentation constants (s = 2–5). These components had the same sedimentation constants as we found later for the inactive material in actin. We have not studied this component more closely.

Glycine buffers (0.08 n). In preparations with these buffer solutions (p_H 8.8–9.4) we often had several components. Those which we call the myosin components could be split into three different components with the sedimentation constants 7.2, 12 and 15.

The actomyosin component also was not always the same as in phosphate buffer. Here we partly had the usual component with s = 50, partly a component which sedimented very rapidly and with

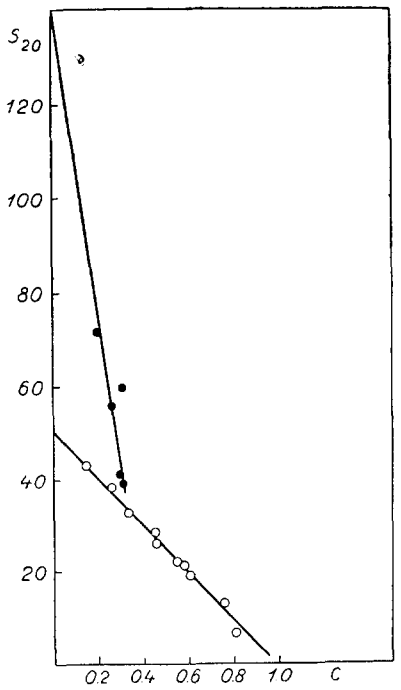

Fig. 4. Variation of s_{20} for the heavier components of the myosin of EDSALL AND GREENSTEIN (24 h) with the concentration of the solution

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a much stronger dependence upon the concentration. In glycine buffer the actomyosin was more resistant to p_H changes. It was only decomposed at p_H 9 and then disappeared. When adenosin triphosphate was added to solutions of actomyosin, this component disappeared.

Our experiments did not exhibit any better reproducibility. Especially the different components with the intermediate sedimentation constants show a certain scattering in the way they appear. It may partly depend upon the state of the muscle from which the preparation was made and partly on small differences in the technique of preparation. In glycine buffers we also get more complicated phenomena than in the other buffers which we have tried.

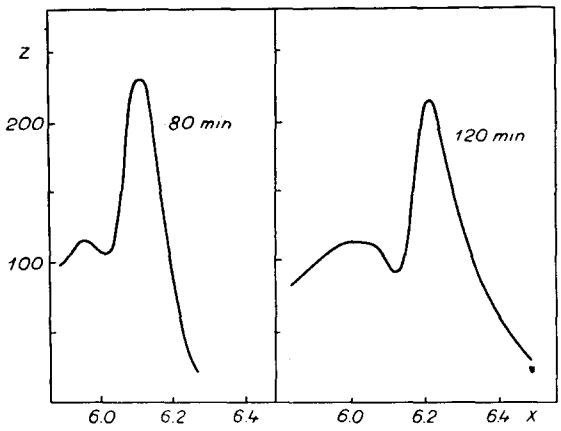

Fig. 5. Sedimentation diagram for the myosin of EDSALL AND GREENSTEIN treated with ATP 0.5 M KCl

DISCUSSION

We know now from other investigations which we wish to report later that the component which has the smallest sedimentation constant belongs to the inactive material in actin and that the set of components with s = 7.2 belongs with certainly to crystallised myosin or to G-actomyosin. The component with the larger sedimentation constant belongs to F-actomyosin. It is interesting to notice that we never obtained any sedimentation constants situated between the lower and the higher one. It has been supposed that F-actomyosin is not a definite complex of actin and myosin but a continous aggregation between F-actin and myosin up to an optimal ratio, but in such a case one would not have expected such a well-defined component. Furthermore, if actomyosin is extracted in such a way that a continuous particle distribution is obtained, such a well-defined component cannot, from a statistical point of view, be expected. It seems that actomyosin dissolves into pieces which are scattered around a certain maximum. We may expect F-actomyosin to be built up in a definite manner out of actin and myosin. We shall later give further evidence for this assumption.

We cannot draw any conclusions concerning the monodispersity of our different components. Other experiments must decide this question. SCHRAMM AND WEBER claim that their solutions were monodisperse, but it is not possible to draw that conclusion from their measurements.

That our sedimentation constants differ from those of previous investigators depends partly upon the fact that we have studied the concentration dependence of the sedimentation constants and partly upon the fact that the resolving power of the optical system (LAMM's scale method) which we have used was better.

In the case of ZIFF AND MOORE, who only found one component (probably actomyosin) in the ultracentrifuge, their electrophoresis patterns show two components. It seems possible that the smaller component which they detected after prolonged electrophoresis was the myosin component which had escaped their observation in

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the ultracentrifuge. In our investigations the myosin component always appeared together with the actomyosin component.

The great difference between our investigations and others is that what one may think corresponds to myosin (and perhaps even to G-actomyosin) is composed of more than one component. Further investigations are needed to settle the question of the nature of these components.

We wish to express our best thanks to professor THE SVEDBERG for enabling us to carry out this work. The investigation has been supported financially by the Rockefeller Foundation.

SUMMARY

An ultracentrifugal analysis of the myosin solution of GREENSTEIN AND EDSALL shows that several components can appear. Some of these can possibly be attributed to actin, myosin and F-actomyosin. The nature of some components with intermediate sedimentation constants is not yet settled. Glycine buffers seem to alter the solutions in a definite manner so that several components appear. F-actomyosin was always deposited with a rather high sedimentation constant so it seems that native F-actomyosin must always be composed in a definite manner.

RÉSUMÉ

L'analyse par ultracentrifugation de la solution de myosine de GREENSTEIN ET EDSALL montre que plusieurs constituants peuvent se former. Les uns correspondent probablement à l'actine, la myosine et la F-actomyosine. La nature des autres, dont la constante de sédimentation est intermédiaire, n'est pas encore définie. Les tampons à base de glycocolle modifient les solutions d'une manière telle que plusieurs constituants apparaissent. La F-actomyosine se sédimente avec une constante relativement élevée, ce qui montre que la F-actomyosine naturelle doit toujours être constituée d'une façon homogène.

ZUSAMMENFASSUNG

Eine Ultrazentrifugalanalyse der Myosinlösung von GREENSTEIN UND EDSALL zeigt, dass verschiedene Komponenten auftreten können. Einige dieser Komponenten können möglicherweise dem Aktin, Myosin und F-Aktomyosin zugeschrieben werden. Die Art mancher Komponenten mit intermediären Sedimentationskonstanten ist noch nicht sichergestellt. Glykokollpuffer scheinen die Lösung auf eine bestimmte Weise zu verändern, so dass mehrere Komponenten erscheinen. F-Aktomyosin setzte sich immer mit einer ziemlich hohen Sedimentationskonstante ab. Natives F-Aktomyosin ist also, wie es scheint, immer auf eine bestimmte Art zusammengesetzt.

REFERENCES

[1] J. GREENSTEIN AND J. T. EDSALL, *J. Biol. Chem.*, 133 (1940) 397.
[2] T. SVEDBERG, *Kolloid-Z.*, 51 (1930) 10.
[3] G. SCHRAMMM AND H. H. WEBER, *Kolloid-Z.*, 100 (1942) 242.
[4] M. ZIFF AND D. H. MOORE, *J. Biol. Chem.*, 153 (1944) 653.
[5] A. SZENT-GYÖRGYI, *Studies Inst. Med. Chem.*, Univ. Szeged, Vol. III, (1943) 76.
A. SZENT-GYÖRGYI, *Acta Physiol. Scand.*, Vol. 9, suppl. 25, (1944).
[6] I. BANGA AND A. SZENT-GYÖRGYI, *Studies Inst. of Med. Chem.*, Univ. Szeged, Vol. I, 5 (1941–42).

Received June 17th, 1948