PHYSICAL AND CHEMICAL STUDIES OF MYXOMYOSIN, AN ATP-SENSITIVE PROTEIN IN CYTOPLASM*

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INTRODUCTION

Cytoplasmic extracts of the slime mold, *Physarum polycephalum*, are sensitive to additions of small amounts of ATP[1]. This sensitivity manifests itself in a rapid decrease in viscosity followed after a time by a spontaneous recovery. Since such phenomena, if operative within the cell, could affect and perhaps activate the process of protoplasmic streaming, it is of interest to isolate the responsible cytoplasmic material, to characterize it, and to study the above processes on a molecular level.

The active principle, referred to as myxomyosin, has been isolated in concentrates which contain 75% myxomyosin. The remainder of the material in these concentrates consists largely of degradation products of myxomyosin itself[2]. In the present work, the concentrate has been examined by a variety of physical chemical methods to establish the size and shape of the myxomyosin molecule. With the aid of this information, the action of ATP on the system has been studied.

EXPERIMENTAL

Materials

Slime mold plasmodia were grown by previously described procedures[1]. Only the myxomyosin concentrates designated solution V[2], were used. These solutions contained 0.2 μ K maleate at pH 7, a condition in which myxomyosin is stable[3]. This is also the pH of the unbuffered crude extract of plasmodia and the pH at which ATP has a maximum effect upon the viscosity of the crude extract[1].

Adenosine triphosphate (ATP) from Pabst Co. was used without further purification. The solution was neutralized with KOH to pH 7.0 before use. Yeast ribonucleic acid (RNA) from the California Foundation for Biochemical Research and crystalline pancreatic ribonuclease (RNase) from Armour Co. were used. All other chemicals were of reagent grade.

Methods

Concentration of the dialyzed myxomyosin solutions was determined by differential refractometry. The instrument used was that described by BRICE AND HALWER[4] and supplied by the Phoenix Instrument Co., Philadelphia, Pennsylvania. The RNA content was determined by the amount of TCA-precipitable phosphate as measured by a modification of the method of ALLEN[5].

The partial specific volume was determined from the difference in density of the solutions and the solvent[6].

Single and three-bulb Ostwald type viscometers were employed. Bulb volumes were 1.3 to 1.8 ml and maximum shear gradients for water were 90 to 700 sec^{-1}. The kinetic energy corrections

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for these viscometers are small and were neglected. All viscosity measurements were made at a temperature 24.42 ± 0.05 C.

Analytical ultracentrifugation was performed in the Model E Spinco ultracentrifuge equipped with a temperature control system. The direction of sedimentation in the ultracentrifuge pattern is from right to left. All runs were performed at the speed of 47,660 r.p.m. in the synthetic boundary cell[7,8] supplied by the Spinco Division, Beckman Instrument Corp.

Moving boundary electrophoresis patterns were obtained with the Perkin Elmer Model 38 apparatus and the 2 ml cell assembly. Prior to electrophoresis, samples were dialyzed with stirring for at least 10 hours. Buffer and solution conductances agreed to within one percent. In experiments with ATP appropriate amounts of cold, concentrated ATP solution were added just prior to the run both to a definite volume of dialyzed protein solution and the buffer.

The electron microscope used was the Model EMU-2A, Radio Corporation of America. The magnification was calibrated with carbon replicas of a grid containing 30,000 lines per inch supplied by Ernest F. Fullam, Inc. The myxomyosin preparation was sprayed immediately after dilution with cold 0.01 *M* KCl or 0.01 *M* ATP containing standard polystyrene latex, on to metal screens coated with collodion. The screens were shadowed at an angle of about 6:1 with thorium. The spherical polystyrene particles (2600 Å in diameter) serve as a check on the magnification as well as on the shadowing angle.

The birefrincenge instrument employed in this study was built at the California Institute of Technology according to the design of EDSALL, RICH AND GOLDSTEIN[9].

RESULTS AND DISCUSSION

The size and shape of myxomyosin

Even though the most extensively purified solutions contained substantial quantities, about 25%, of nondialysable impurity[2], it was possible to determine the mass and dimensions of myxomyosin because, as will be shown below, the molecules behave like rigid elongated rods. The impurities on the other hand are less asymmetric and are lower in molecular weight. The evidence for the elongated character of myxomyosin is found in the viscosity, electron microscope, birefringence, and sedimentation data presented below. The physical character of the impurity is indicated by the properties of the supernatant materials obtained during the differential centrifugation steps in the preparative, procedure, *cf.* Table II Fig. 2[2].

Viscosity

The viscosity of myxomyosin solutions has been measured over a concentration range, 0.1 to 0.8%, and at shear gradients from 70 to 1900 sec^{-1}. The data were extrapolated to zero gradient and zero concentration. Some uncertainty was involved in the extrapolation to zero gradient because of the non-linear character of the curves, which for the dilute solutions are shown in Fig. 1. The extrapolated relative viscosities at zero gradient obtained by drawing smooth curves through the data, give the values for the reduced viscosity plotted in Fig. 2a and 2b. Here the results are given for two preparations studied in two concentration ranges. On both systems the extrapolated value for the intrinsic viscosity at zero gradient is 300 ml/g. The uncertainty in this value is limited on the low side by the value of 250 obtained from results interpolated at 100 sec^{-1} (Fig. 2a, 2b). We estimate that the upper limit of error will not exceed 10%, and thus the intrinsic viscosity at zero gradient is 300 ± 30 ml/g.

The presence of slowly sedimenting impurities, estimated to be 25% by weight[2], introduces an error in the above evaluation of the intrinsic viscosity of myxomyosin. To correct this error, the viscosity of the supernatant solution from the first differential centrifugation was measured. This solution consists largely of the less viscous material. The reduced viscosity is independent of concentration, and gives a value

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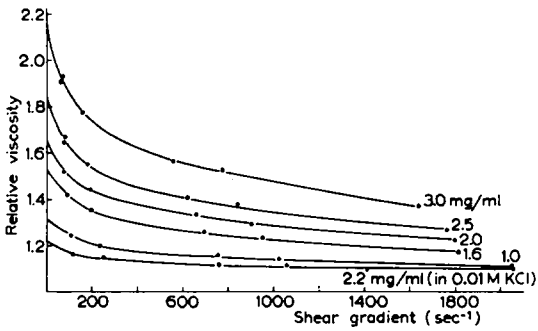

Fig. 1. The relative viscosity of dilute myxomyosin solutions *vs.* maximum shear gradient.

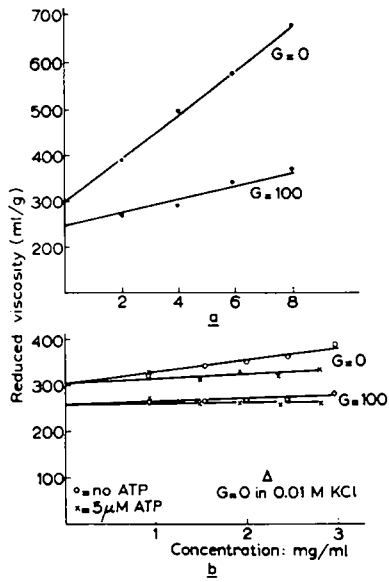

Fig. 2. The reduced viscosity of myxomyosin solutions *vs.* concentration at two levels of shear gradient. (a) Concentrated myxomyosin solutions; (b) dilute myxomyosin solutions with and without ATP.

of 63 ml/g for the intrinsic viscosity of the impurities. The corrected value for the intrinsic viscosity of myxomyosin then becomes 380 ml/g. The corrected viscosity increment based on a partial specific volume of 0.72 ml/g is 530. If the protein is assumed to be 25% hydrated, the viscosity increment becomes 396. This value gives an axial ratio of 80 ± 4* for a rigid prolate ellipsoid model. This model is regarded as equivalent to a rod. The foregoing data were used in Perrin's equation to obtain a frictional coefficient of 3.63. The assumed hydration contributes a further factor of 1.10 according to ONCLEY[10], so that the total frictional coefficient of myxomyosin is 4.0. This value together with sedimentation coefficient will be used in the calculation of the molecular weight. It should be noted that this value, calculated from the intrinsic viscosity of 380 ml/g, varies by about 4% as the assumed hydration value is changed from 0 to 50%.

Partial specific volume and refractive index increment

The partial specific volume as determined on two separate preparations was found to be 0.719 ± 0.004. It is recognized that this is an average value for all the non-dialyzable material. Since this non-dialyzable impurity consists largely of breakdown products[2], it may be expected to have essentially the same composition and partial specific volume as myxomyosin itself. The refractive index increment, again measured on the whole preparation, was found to be 0.185 ml/g.

* The value for the uncertainty in calculation of the axial ratio indicates the range of error involved in the extrapolation of the viscosity data. The extent of the ambiguity which arises from the assumed hydration value can be appreciated by noting that the axial ratio would be 90 for zero hydration and 70 for 50% hydration.

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Sedimentation and weight average molecular weight

A plot of $1/S_{20,w}$ *vs.* concentration is given in Fig. 3. The extrapolated value at zero concentration corresponds to a value of 30 ± 0.5 *S* for $S^{\circ}_{20,w}$. This value of *S*, the frictional coefficient of 4.0 derived from viscosity studies assuming a rod-like model, and a partial specific volume of 0.72, give a weight average molecular weight of 6 million with an experimental error estimated to be ± 10% based on uncertainties in the extrapolated sedimentation coefficient, the extrapolated reduced viscosity and partial specific volume measurement. The rod-shaped model is corroborated by measurements of flow birefringence and by electron microscopy. As indicated in the following sections the 30 *S* rods are not uniform in length; therefore, the value of 6 million corresponds to a weight average. It is not clear at present whether the material occurs in this size distribution in cytoplasm, or whether it suffers breakdown to this size distribution in the course of preparative procedure. In the fifteen or more preparations examined, high viscosity has always been associated with the presence of the 30 *S* component.

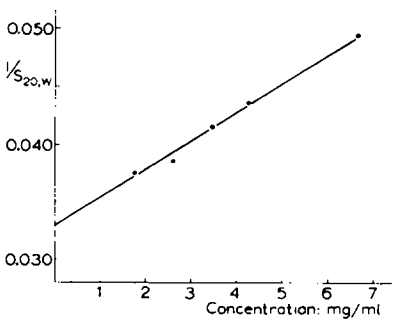

Fig. 3. $1/S_{20,w}$ of myxomyosin *vs.* concentration.

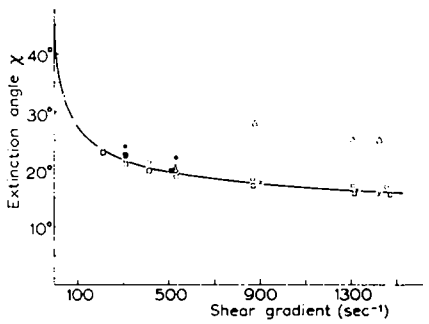

Fig. 4. Extinction angle, χ, of myxomyosin solutions *vs.* shear gradient.
○ — 2.7 mg/ml ● — 2.7 mg/ml (retested)
□ — 0.9 mg/ml ■ — 0.9 mg/ml (retested)
◇ — 0.45 mg/ml ◇ — 2.7 mg/ml in 0.01 *M* KCl

Flow birefringence

The extinction angle, χ, of myxomyosin solutions was measured to determine the length of the molecule. The results are given in Fig. 4 in which χ is plotted as a function of the shear gradient, *G*, for various concentrations of myxomyosin. Based on these data apparent rotary diffusion coefficients were evaluated. The corresponding molecular lengths were observed to vary with shear gradient from 6000 A at 1300 sec^{-1} to 11,000 A at 38 sec^{-1} indicating a heterogeneity in length. An axial ratio of 80, a relative viscosity of the medium of 1.02, and the experimental temperature, 25° C, were employed in the calculations[11,12]. The reproducibility of the calculated lengths among three separate preparations was in the range of 5%, or 300–500 A.

As a result of the shearing action during a set of measurements, χ increased 0.5 to 1.0 degree corresponding to a decrease in length of about 500 A (Fig. 4). This change was invariably observed upon repeating a set of measurements, and was more pronounced with concentrated solutions (0.3–0.4%) than with the dilute solutions (0.09–0.05%). The effect of shear appears to be related to the work softening effects which were observed during the determination of viscosity of concentrated solutions

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(0.8%). At these high concentrations, the relative viscosity dropped in successive measurements by 2 to 5%. This viscosity behavior, which is believed to result from the mechanical breakdown of molecular aggregates, is not found in the concentration range below 0.3%. The aggregates apparently dissociate spontaneously at higher dilution.

The values of χ measured with unworked solutions at several concentrations between 0.045 and 0.27% were independent of concentration. At these concentrations the molecules are free to rotate without interference from one another.

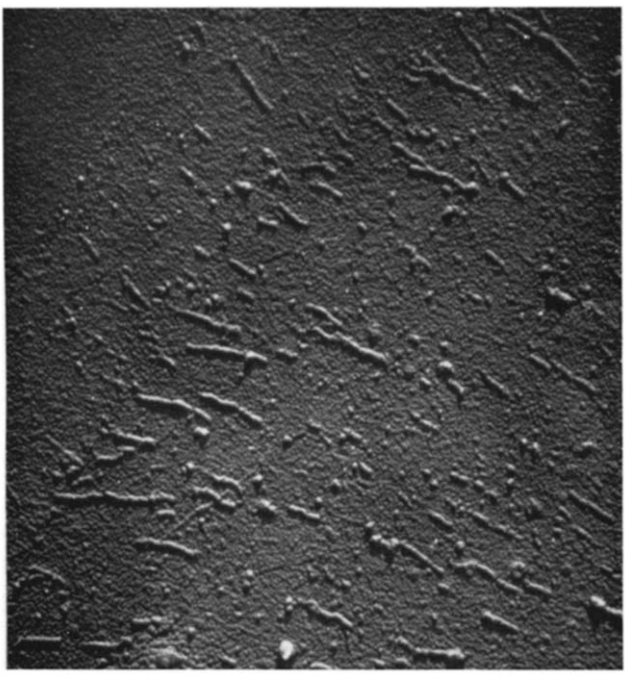

Fig. 5. Electron micrograph of myxomyosin. Thorium shadowing; Magnification 26,000 ×.

Electron microscopy

Electron micrographs of myxomyosin samples prepared by spraying and air-drying dilute solutions reveal the presence of elongated rod-like structures and some globular material (Fig. 5). The longest rods observed were about 7000 A with many falling within the range of 3500–4500 A. All of the rods possessed diameters between 60 to 80 A as measured from their shadows. Based on the value for the partial specific volume reported above, a rod 70 A in diameter and 7000 A in length should have a molecular weight of 20 million, while a rod of 60 A in diameter and 3500 A in length should have a molecular weight of 7 million. The smaller rods and perhaps the globular material appear to be breakdown products of myxomyosin. These could have been formed either during the purification of the active principle or in preparation of the screens for electron microscopy.

The dimensions by electron microscopy agree with those from viscosity data based upon the rigid model, but are smaller than those obtained from flow bire-

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fringence. The reasons for this discrepancy are not known, but may be related to the fact that birefringence of flow heavily weights the longest molecules in a distribution of rod-like molecules of varying length[13,11]. In the case of desoxyribonucleate, there is a similar disagreement in estimated length of the molecule as between light scattering and birefringence data[15].

Both the birefringence and electron microscopy results support the rigid rod model assumed in the calculation of axial ratio from the viscosity data and in the calculation of the molecular weight from viscosity and sedimentation data. In conclusion, these studies have revealed that myxomyosin is a large, asymmetric and stiff molecule which may possibly exist in a distribution of lengths. The weight and dimensions of myxomyosin reported here substantiate the suggestion[2] that it belongs in the category of structural proteins and closely resembles actomyosin in regard to its unique physical dimensions[16,17].

Degradation of myxomyosin at low ionic strength

After dialysis at 2° C overnight in 0.01 *M* KCl (pH 5) the reduced viscosity of the solution fell to about 100 ml/g, (Fig. 2b), one-third of the previous value in 0.2 μ ionic strength, and this viscosity was little dependent on shear gradient (Fig. 1). Both results suggest that the long rods had been degraded. In the ultracentrifuge this solution showed a very broad boundary which moved slowly (*ca.* 10 *S*) and spread rapidly until it almost disappeared during the run. Under the electron microscope, most of the molecules are shorter than 1000 A, although some rods, 2000–3000 A in length, are visible. Flow birefringence results confirm the view that the myxomyosin has been degraded. The extinction angle (Fig. 4) indicates an approximately 50% reduction in apparent length. The birefringence of the solution was also reduced. The effect of low ionic strength upon the viscosity was not reversed by addition of KCl to final concentrations of 0.2 and 0.5 *M* and incubation at 0° C or at room temperature for two hours.

The behavior at low ionic strength is unlike that of myosin or actomyosin which become insoluble, but similar to that of F-actin which transforms to G-actin. The observed degradation suggests that charged groups are involved in the stability of the giant molecule. Reversal of the degradation by other methods such as addition of Mg and Ca ions have not yet been tried.

The effect of ATP on myxomyosin

As is the case with actomyosin solutions, addition of ATP has a striking effect on the viscosity of myxomyosin solutions[1,2,3]. Physical-chemical measurements were made to determine the molecular basis for this decrease in viscosity and to gain an insight into the reaction between ATP and myxomyosin. The viscosity of a series of solutions was measured after addition of 5.0 μmole ATP/ml and before perceptible recovery was to be expected[3]. The lines of Fig. 2b are constructed from data obtained by interpolation to 100 sec^{-1} and by extrapolation to 0 sec^{-1}. It is apparent that at 100 sec^{-1} and at these low concentrations there is substantially no effect of ATP on the viscosity. This effect disappears gradually with increasing dilution. Thus ATP reduces the slope of the line, but does not change the intercept nor the intrinsic viscosity value of 300 ml/g. This observation suggests that the interaction of ATP and myxomyosin does not change the molecular size or shape of the giant molecule.

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It does not exclude, however, the less likely possibility that processes which increase viscosity such as aggregation and swelling, and those which decrease it such as disaggregation, dehydration and decrease in rigidity, occur concurrently and in such a way as to cancel. The effect of ATP on myxomyosin has been investigated further by electron microscopy and by ultracentrifugation.

Grids of myxomyosin diluted in 0.01 *M* ATP (pH 7.0) instead of 0.01 *M* KCl just 5 minutes before spraying were prepared for electron mycroscopy. Qualitatively, the myxomyosin rods were the same in the presence and in the absence of ATP with no distinctive difference in the distribution of width or length.

The ultracentrifuge patterns obtained under identical conditions except for the presence of ATP showed little difference in shape and relative area of the myxomyosin boundary. These runs were performed at temperatures of 5–8° C, conditions in which the viscosity does not recover[3]. The sedimentation coefficients, $S_{20,w}$, for 0.77% and 1% myxomyosin were 26.7 *S* and 25.4 *S* respectively and were 25.3 *S* and 24.6 *S* after addition of ATP. Under these conditions, however, the relative viscosity of these concentrated solutions was lowered 20–30% by ATP. The ultracentrifuge and electron microscope results support the conclusion from viscosity studies that the size and shape of myxomyosin are not noticeably affected by ATP.

Calculations based upon the measured dimensions of myxomyosin show that the molecule cannot rotate freely in solutions more concentrated than 0.2%, in substantial agreement with the results of the extinction angle measurements. It is to be expected then that in more concentrated solutions, strong interaction effects will be found. Recalling that ATP does not affect size and shape, we attribute the viscosity effect to a reduction of the tendency of myxomyosin to associate. This association diminishes with dilution as do the non-specific interactions. The equilibria involved in the association must be rapid since only small amounts of polymeric material are observed in the ultracentrifuge.

The drop in viscosity caused by ATP addition results from a process which is substantially independent of temperature[3]. It has been suggested that this corresponds to the binding of ATP to myxomyosin. Electrophoresis experiments have been performed to examine this suggestion.

The ascending pattern of myxomyosin concentrates[2] in 0.2 μ potassium maleate consists of three boundaries related to the active principle with about 20% of material which trails behind as impurities. The three boundaries have been described as[2], A, a nucleic acid ($14.5 \cdot 10^{-5}$ cm^2 volt^{-1} sec^{-1}); B, a nucleoprotein complex (8.1); and C, a nucleic acid-free myxomyosin (6.6). In addition to the impurities, the descending pattern contains a fast moving boundary, A, again the nucleic acid, ($13.6 \cdot 10^{-5}$) and a complex boundary of nucleic acid and myxomyosin, B + C (6.9) which does not resolve.

Neutral ATP solution was added in equal concentration (3 μmole/ml) to both myxomyosin and to the buffer against which the myxomyosin was run. Fig. 6 shows the effect on the original preparation and Fig. 7 shows the effect on a solution of low RNA content (see following section for RNA content). The descending patterns are essentially unaffected except that the resolution of the myxomyosin-nucleic acid complex boundary is slightly enhanced. In the ascending limb, however, the relative area of the faster moving B component is substantially increased with a concomitant decrease of the C component (Figs. 6 and 7). The extent of transformation of C to B

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depends upon the ATP:myxomyosin ratio. As this ratio was increased from 0.6 μmole/mg to 1 μmole/mg, the decrease in C changed from 25% to 45%. Since ATP does not change the frictional factor of myxomyosin, as shown by ultracentrifuge studies at low temperature, the increase in mobility of the myxomyosin toward the anode is interpreted as resulting from an increase in negative charge due to binding of ATP. It should be noted that additions of a much smaller amount of ATP (approximately 3%) are sufficient to give a maximum effect upon viscosity[3]. The binding appears to be specific. None is detectable by electrophoretic techniques when yeast nucleic acid, for example, is added to myxomyosin solutions (Fig. 7).

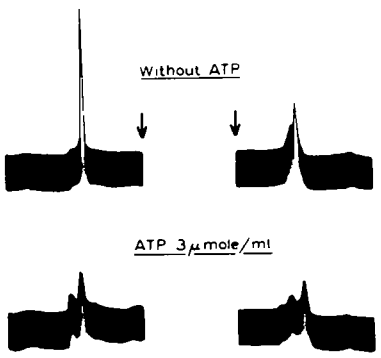

Fig. 6. Effect of ATP on the electrophoretic patterns of myxomyosin. Ascending pattern is on the left. Concentration: 6 mg/ml. Current: 6 mA. Time: 9,300 seconds. Medium: 0.2 μ potassium maleate, pH 7.0.

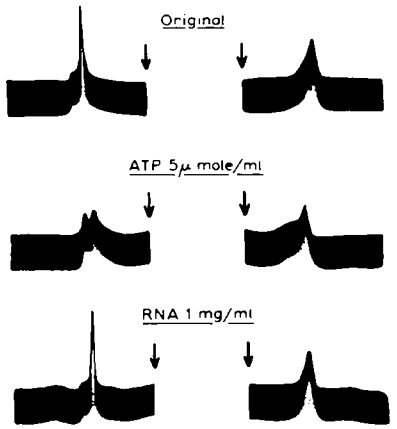

Fig. 7. Effect of ATP and yeast RNA on the RNA-poor myxomyosin solution prepared by enzyme treatment. Ascending pattern is on the left. Concentration: 5 mg/ml. Current: 6 mA. Time: 9000 seconds. Medium: 0.2 μ potassium maleate, pH 7.0.

The properties of myxomyosin in the presence of ATP have been studied by flow birefringence. The values obtained for the distribution of lengths were about 5% higher than obtained for samples without ATP. Since provisions were not available for the measurement of flow birefringence at low temperature or over short time periods, these data on the ATP effect must be regarded as dealing with myxomyosin in the refractory state[3], a condition in which the viscosity remains high even in the presence of ATP.

Removal of RNA from myxomyosin by ribonuclease

The myxomyosin examined in the above work contained about 9% RNA which was not separated from the protein (by salt fractionation and the two cycle differential centrifugation) in the preparative procedure[2]. In later experiments, however, it was found that the RNA could be removed by the action of pancreatic ribonuclease, and that the ATP-sensitive protein remained. In these experiments the resuspended myxomyosin concentrate from the pellet of the first cycle of differential centrifugation (solution IV)[2] was treated with 0.01% ribonuclease for one and a half hours at room temperature. In this period the solution increased in turbidity. The ribonuclease was removed with the supernatant in the second cycle of centrifugation (2 1/2 hours at

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100,000 *g*). The redissolved pellet is referred to here as the RNA-poor solution. The amount of RNA in this concentrate was about 1% as measured by TCA-precipitable phosphorus.

Ultracentrifuge analyses show that the myxomyosin content of the RNA-poor solution was 87.5% as compared with 75% in the original solution V preparation (Fig. 8). Neither the sedimentation coefficient nor the shape of the boundary changed as a result of removing the RNA. In electrophoresis experiments (Figs. 6 and 7), the fast moving A component disappeared, confirming the suggestion that the A boundary was nucleic acid[2]. The B component in the ascending pattern[2], thought to be a nucleoprotein complex, became smaller but did not totally disappear.

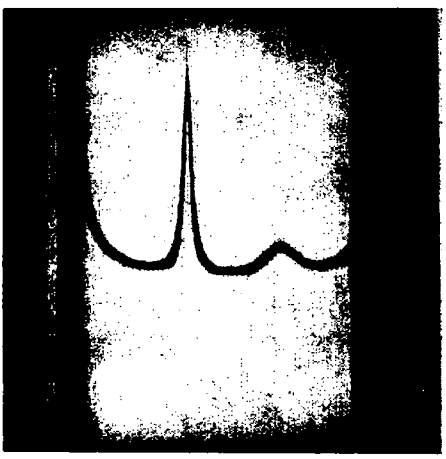

Fig. 8. The ultracentrifuge pattern of RNA-poor myxomyosin solution prepared by enzyme treatment. Concentration: 6.4 mg/ml. Medium: 0.2 μ potassium maleate, pH 7.0.

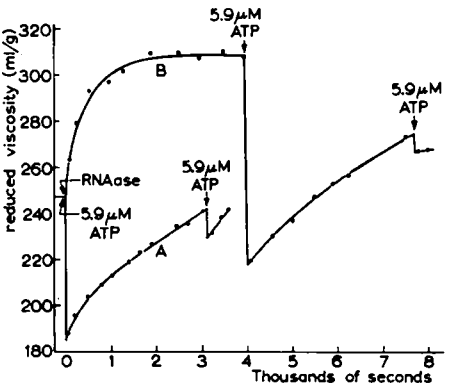

Fig. 9. The effect of RNase (0.25 mg/ml) on the viscosity of myxomyosin. Concentration: 5 mg/ml. Shear gradient: 600 sec^{-1}.

The viscosity of the RNA-poor solution was much higher than that of the original solution. At 0.5% for example, the reduced viscosity of the RNA-poor solution was 370 $\pm$ 10 compared with 240 $\pm$ 10. The above data were obtained at shear gradients of 500–700 sec^{-1}. This increase in viscosity is higher than can be accounted for by the increase in purity of the myxomyosin preparation. The RNA-poor solution exhibited a larger ATP response, 80 ml/g, than did the original solution 40–60 ml/g. Again, large amounts of ATP at room temperature caused the system to become refractory to ATP in the course of the recovery[3]. Addition of ribonuclease (0.03%) to a fresh RNA-poor solution did not affect the reduced viscosity nor the response to ATP, showing that either the ribonuclease does not aggregate with myxomyosin or, less likely, that the solution has been left saturated with respect to this reaction with the very small amount of ribonuclease remaining after the centrifugation process. Addition of commercial yeast RNA also did not affect the reduced viscosity nor the ATP response of the solution.

The effect of ribonuclease on viscosity of the original solution V, was also observed. After the addition of ribonuclease (0.01–0.03%), a rapid increase in viscosity of the solution occurred. This leveled off after *ca.* 30 minutes. Addition of ATP to

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such a ribonuclease treated preparation caused a large decrease in viscosity and a large amount of ATP induced the refractory state. A second addition of ribonuclease (0.01%) to a solution already treated with enzyme caused no further change in viscosity. When the original solution was first made refractory with large amounts of ATP[3], ribonuclease had no effect on its viscosity.

The above results show that the viscosity changes which occur upon enzymic removal of RNA, are not caused by complexing of myxomyosin with the enzyme. A plausible explanation, though still speculative, for the action of ribonuclease, is that the nucleic acid prevents the protein molecules from interacting with each other by forming a negatively charged complex with the protein. Removal of the RNA permits a more intensive interaction. As previously noted, this interaction can be reduced by ATP. Nucleic acid will thus act in the same way as ATP, except that it is less effective and not destroyed in the solution. Specificity appears to be involved since the addition of yeast RNA to the RNA-poor solution produced no decrease in viscosity. From the foregoing it is clear that RNA is not an indispensable part of myxomyosin as far as its reaction with ATP is concerned. Recent results by MIHALYI *et al.*[18] indicate that crystalline myosin also constantly contains a small amount of ribonucleic acid. This group of proteins because of their affinity to ATP may have a tendency to combine with polynucleotides.

It was of interest to observe the effect of ribonuclease on the crude extract from plasmodia since these extracts require activators before ATP shows a viscosity reducing effect[1]. It was found, however, that although ribonuclease lowered the RNA content of the crude extract by 90%, the viscosity of the treated solutions both before and after the activation by ATP was not affected, and response to ATP was unchanged. The mechanism of the activation of the crude extract is thus still unknown, but is probably not related to reactions involving RNA.

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SUMMARY

The size and shape of myxomyosin, an ATP-sensitive protein isolated from a myxomycete, have been investigated by viscosity, sedimentation, birefringence and electron microscope measurements. These studies show that the myxomyosin molecule is a long rigid rod which, in the electron micrographs, appears in a distribution of sizes whose most frequent dimensions are 4000–5000 Å in length by 70 Å in diameter. The molecular weight based on viscosity and sedimentation data is six million.

The myxomyosin molecule does not change in size or shape in the presence of ATP. The effect of ATP on the viscosity of the myxomyosin solution is best understood in terms of a reduction of a concentration-dependent interaction between myxomyosin molecules.

Myxomyosin, substantially free of RNA, can be prepared by including a treatment with pancreatic ribonuclease in the preparative procedure. The myxomyosin concentrates contain less impurity, are more viscous, and are more responsive to ATP than myxomyosin prepared without the enzyme. It is concluded that nucleic acid is not essential to the interaction of myxomyosin with ATP.

References p. 542.

REFERENCES

[1] P. O. P. Ts'o, J. Bonner, L. Eggman and J. Vinograd, *J. Gen. Physiol.*, 39 (1956) 325.
[2] P. O. P. Ts'o, L. Eggman and J. Vinograd, *J. Gen. Physiol.*, 39 (1956) 801.
[3] P. O. P. Ts'o, L. Eggman and J. Vinograd, *Arch. Biochem. Biophys.*, 66 (1957) 64.
[4] B. A. Brice and M. Halwer, *J. Optical Soc. Amer.*, 41 (1951) 1033.
[5] R. J. L. Allen, *Biochem. J.*, 34 (1940) 358.
[6] V. L. Koenig, *Arch. Biochem.*, 25 (1950) 241.
[7] G. Kegeles, *J. Am. Chem. Soc.*, 74 (1952) 5532.
[8] E. G. Pickels, W. F. Harrington, and H. K. Schachman, *Proc. Natl. Acad. Sci. U.S.*, 38 (1952) 943.
[9] J. T. Edsall, A. Rich and M. Goldstein, *Rev. Scientific Instrum.*, 23 (1952) 695.
[10] J. L. Oncley, *Ann. New York Acad. Sci.*, 41 (1941) 121.
[11] J. T. Edsall, *Size, Shape and Hydration of Protein Molecules*, in H. Neurath and K. Bailey, *The Proteins*, Vol. I, Part B, Academic Press, Inc., New York, 1954, p. 672.
[12] H. A. Scheraga, J. T. Edsall and J. O. Gadd, *J. Chem. Phys.*, 19 (1951) 1011.
[13] C. Sadron, *J. Phys. Radium*, 9 (1938) 381.
[14] J. B. Donnet, *J. Chim. Phys.*, 50 (1953) 377.
[15] M. Goldstein and M. E. Reichmann, *J. Am. Chem. Soc.*, 76 (1954) 3337.
[16] K. Bailey, *Structural proteins. II. Muscle*, in H. Neurath and K. Bailey, *The Proteins*, Vol. II, Part B, Academic Press, Inc., New York, 1954, p. 952.
[17] M. Dubuisson, *Muscular Contraction*, Charles C. Thomas, Springfield, Ill., 1954.
[18] E. Mihalyi, K. Laki and M. I. Knoller, *Arch. Biochem. Biophys.*, 68 (1957) 130.

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QUANTITATIVE INTERRELATIONSHIPS OF THE CHIEF COMPONENTS OF SOME CONNECTIVE TISSUES DURING FOETAL AND POST-NATAL DEVELOPMENT IN CATTLE*

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INTRODUCTION

In the course of its foetal and post-natal development the intercellular substance of connective tissue undergoes certain changes which are sometimes called maturation. Histological studies have been concerned chiefly with the morphology of the fibrous material, which as a rule has a network-like appearance in the early stages, the fine fibres having a strong affinity for complex silver salts (reticulin). This structure may change gradually, via several intermediate forms, into a coarser, stiffer, bundled structure, which is only very slightly argentophil (collagen). This maturation is not a uniform process that takes place in the same way in all connective tissues. Sometimes the reticular type is maintained (reticular connective tissue) and sometimes the

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