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Some Effects of Pressure Treatment on Actomyosin Systems

J. M. O'Shea, D. J. Horgan and J. J. Macfarlane

Meat Research Laboratory, Division of Food Research, CSIRO,
P.O. Box 12, Cannon Hill, Qld 4170.

Abstract

Natural actomyosin, actin and myosin, have been pressurized at up to 150 MN/m^2 for 1 h at 0°C and examined 3–5 h later.

Pressurization of myosin resulted in the formation of aggregates with a molecular weight approximately that expected for a dimer, whereas with F-actin depolymerization occurred. With actomyosin, a gel to sol transition was promoted. Viscosity and light-scattering measurements indicated that pressurization results in a large measure of disaggregation of actomyosin in solution.

Pressurization of actomyosin resulted in a greater decrease in the calcium-sensitive, than in the calcium-independent, Mg^{2+} ATPase activity. The Ca^{2+} and K^+-EDTA ATPase activities of myosin were inhibited to about the same extent.

Introduction

Although the application of high hydrostatic pressure has long been known to exert a great influence on proteins (see, for example, Johnson *et al.* 1954), there have been relatively few published reports on its effects on the myofibrillar proteins of muscle. Marsland and Brown (1942) reported that application of pressure to natural actomyosin systems induced a gel to sol transition. Ivanov *et al.* (1960) reported that actin has a high sensitivity to the action of pressure and undergoes depolymerization at 1000 atm (101 MN/m^2). However, they found that pressurization resulted in an increase in the molecular weight of myosin in 0·6 M KCl solution and suggested that under pressure actomyosin splits into its components. Ikkai and Ooi (1966) found that F-actin in the absence of ATP started to undergo irreversible denaturation at a pressure of *c.* 1500 kg/cm^2 (147 MN/m^2), and complete denaturation at 3000 kg/cm^2 (294 MN/m^2). They found evidence for the transformation of F- to G-actin under the influence of pressure. Ikkai and Ooi (1969) reported that turbid solutions of actomyosin and heavy actomeromyosin became transparent with increasing pressure, finally to give constant intensities of transmitted light at *c.* 2000 kg/cm^2 (196 MN/m^2). The effects of pressure on actomyosin systems were accounted for in terms of depolymerization or dissociation of protein components. Josephs and Harrington (1966, 1967, 1968) have studied the position of the monomer–polymer equilibrium for myosin in solutions of low ionic strength under the pressure developed in the ultracentrifuge, and they observed that the monomeric state was favoured by increase in pressure.

In a recent study Macfarlane (1974) showed that pressurization can promote the solubilization of proteins from homogenates of muscle in saline solution. Apparently the main action of pressurization was on the myofibrillar proteins. It was therefore

decided to investigate some of the effects of pressure on the main constituent proteins of the myofibril, to confirm and extend the relevant findings of the earlier investigators.

Because it was not possible in the present investigation to make measurements on solutions in the pressure cell, the results reported here are for those changes that persisted until samples were examined 3–5 h after pressure treatment.

Materials and Methods

Preparation of Proteins

All preparations were carried out in a laboratory temperature-controlled at 2°C. Natural actomyosin (hereinafter referred to as actomyosin), myosin and acetone-dried powder for actin extraction were prepared from the biceps femoris and semitendinosus muscles dissected from sheep immediately after slaughter, using methods described by Briskey and Fukazawa (1971). Actin was extracted from acetone-dried powder using the method described by Spudich and Watt (1971). The actin preparation appeared homogeneous as judged by electrophoresis on sodium dodecyl sulphate (SDS)–polyacrylamide gels. Examination of the myosin preparations by this method indicated the presence of traces of other proteins.

Buffers

For actomyosin and myosin the buffer used was 0·6 M KCl, 0·001 M EDTA, pH 6·5. For F-actin the buffer used was 0·002 M tris–HCl, 0·002 M ATP, 0·005 M 2-mercaptoethanol, 0·002 M $CaCl_2$, 0·6 M KCl, pH 8·0, at 25°C.

Protein Concentration Measurements

Protein concentrations were measured using the Biuret method (Gornall *et al.* 1949).

Pressure Treatment

Protein solutions were placed in glass vessels which were then fitted with liquid-tight stainless steel plungers with Neoprene seals. The vessels were placed in ice for at least 10 min before the beginning of an experiment and the solutions then exposed to the appropriate pressure in stainless steel pressure vessels in which water was the working fluid.

Light-scattering Measurements

Light-scattering measurements were carried out in a Brice Phoenix Light Scattering Photometer using a beam collimated to a width of 4 mm. The scattering cells used were cylindrical, with flat entrance and exit windows for the undeviated beam. The cells were calibrated with dilute fluorescein solutions to determine their angular dependence of scattering. Scattered intensities, using incident light of 436 nm, were measured at angles ranging from 50 to 130° relative to the undeviated beam. The specific refractive index increments were taken from published work (Gellert *et al.* 1959).

Buffers were made up using dust-free water and then further cleaned by several passages through Millipore filters of porosity 0·45 μm. Protein solutions were clarified by centrifugation in a Spinco Model L ultracentrifuge at 78 500 *g* and 5°C for 1 h. The centrifuged solutions were pipetted from the upper half of the solution in the centrifuge tubes, and transferred to the light-scattering cell. Solutions were examined visually and were rejected if floating dust particles or bubbles were observed. Dilutions were carried out by the addition of buffer to the solution in the light-scattering cell. The results are presented in the form of Zimm-type plots (Zimm 1946).

Viscosity Measurements

These were carried out using either an Ostwald dilution or a variable-shear viscometer. In the latter case measurements were carried out in a Cervantes low-shear viscometer (LS-100) with 15 rates of shear in the range 0–3·16 s^{-1}. Buffers for sample preparation were clarified by several passages through Millipore filters of porosity 0·45 μm. Protein solutions were clarified as for the light-scattering experiments. For each measurement 1·5 ml of sample solution was used; this was sufficient to cover the central cylinder completely and only the small-diameter torsion wire penetrated the surface of the solution. All measurements were carried out at 2°C. The measurement at a particular shear value was recorded only after the deflection reading remained steady for 30 s;

this generally occurred within 1 min from the start of shearing. Dilutions were carried out by the addition of buffer to the solutions. The log–log plot of relative viscosity (η/η_0), against shear rate for each concentration was extrapolated to a shear stress of 1×10^{-5} N/m^2. This procedure was adopted in view of the uncertainties involved in extrapolation to zero shear of the plot of these values, as discussed by Yang (1961).

Sol–Gel Determinations

Solubility determinations were carried out by centrifuging a known weight of solution for 1 h in a Spinco Model L ultracentrifuge at 78 500 *g* at 5°C. The supernatants were poured from the tubes and their protein content determined. The amount of gel remaining in the tube after 10 min draining time was weighed. All determinations were carried out in duplicate. Only in the experiments carried out at temperatures above 30°C where the gel tended to slide out of the tube was there marked variation between duplicates.

ATPase Activity Measurements

The magnesium-dependent ATPase activities of actomyosin preparations were measured in the presence and absence of calcium ions, by a slight modification of the coupled spectrophotometric assay described previously (Horgan 1974) for sarcoplasmic reticulum. The medium contained 20 mM KCl and 25 mM histidine, pH 7·4 (35°C).

The calcium-dependent and K^+-EDTA-dependent ATPase activities of myosin preparations were measured in a medium containing 5 mM ATP and 50 mM tris, pH 7·2 (25°C). The procedure used was as described previously (Newbold *et al.* 1973) except that the activating ions present were 5 mM Ca^{2+} and 0·6 M K^+ plus 1 mM EDTA respectively.

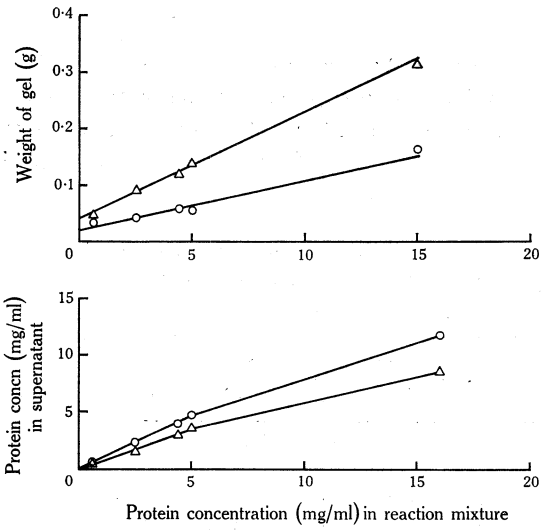

Fig. 1. Effect of actomyosin concentration in 0·6 M KCl solution on the yield of sol and gel fractions in control (△) and pressurized (○) (150 MN/m^2, 1 h, 0°C) samples.

Results

Gel–Sol Transition

Fig. 1 shows that the sol and gel yields from actomyosin in 0·6 M KCl solution were dependent on the concentration of protein present and that pressurization increased the amount present in the sol form. SDS–polyacrylamide gel electrophoresis of samples from the sol and the gel fractions of both pressurized and control samples did not reveal any differences in composition between these fractions.

From Fig. 2 it can be seen that in the range 0–30°C temperature had little effect on the sol and gel yields. However, at 45°C in control samples there was little actomyosin in the sol form although the values for the pressurized samples were similar to the values found at lower temperatures.

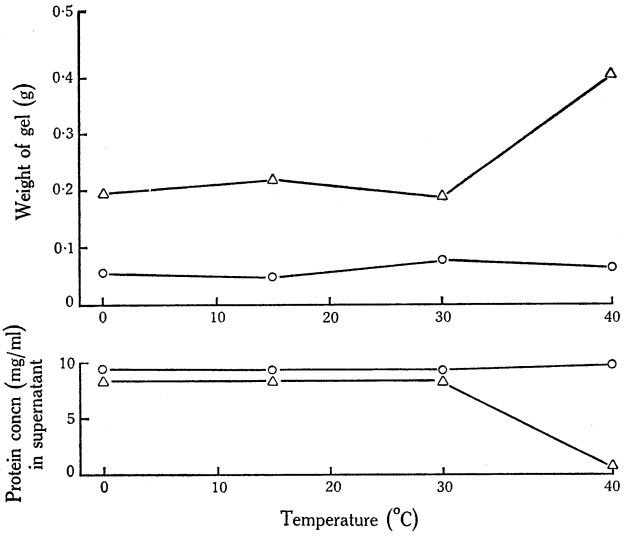

Fig. 2. Effect of temperature during pressurization (150 MN/m², 1 h, 0°C) on the yield of sol and gel fractions from actomyosin in 0·6 M KCl solution. △ Control. ○ Pressurized.

Viscosity Measurements

Table 1 shows that the intrinsic viscosity at low shear of the actomyosin solutions depends on the pressure to which they have been exposed. A major transition had occurred by 75 MN/m². The intrinsic viscosity at infinite shear of all these solutions was the same. After the relaxation of the shearing force, the viscometer reading promptly returned to zero.

Table 1. Intrinsic viscosity at low and infinite shear for natural actomyosin solutions exposed to various applied pressures for 1 h

Intrinsic viscosity, η, measured in decilitres per gram

Shear, G (N/m²)		Pressure (MN/m²)				
		0	50	75	100	150
1×10^{-5}	Sample 1	20	—	13	12	10
	Sample 2	18	10	8	7	6
∞	Sample 1	1·6	1·6	1·6	1·6	1·6
	Sample 2	1·6	1·6	1·6	1·6	1·6

Fig. 3 shows the plot of relative viscosity against shearing stress at equivalent concentration for control and pressurized samples of actin and actomyosin. The shear dependence of the pressurized sample was less than that of the control samples.

The plots of reduced viscosity, $(\eta-\eta_0)/\eta_0 C$, against C conform to the equation of Martin (1942)

$$\ln(\eta \text{ red}) = \ln(\eta) + k(\eta)C. \qquad (1)$$

The values of the slope constant, k, calculated using this equation for pressurized

(150 MN/m^2, 1 h, 0°C) and control actomyosin are shown in the following tabulation:

Rate of shear (s^{-1})	Slope constant k for Untreated actomyosin	Pressurized actomyosin
0·0734	8·48	2·05
0·130	6·93	1·84
1·484	5·44	1·68
3·16	5·44	1·64

There was a decrease in the value of k with increasing rate of shear.

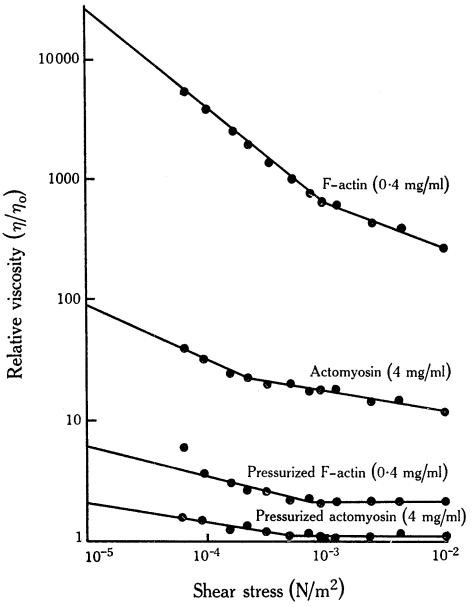

Fig. 3. Relative viscosity *v.* shear stress for control and pressurized (150 MN/m^2, 1 h, 0°C) actin and actomyosin.

Fig. 4 shows the result recorded for control and pressurized actomyosin solutions when viscometry was carried out using an Ostwald dilution viscometer (flow time for water at 25°C was 60 s). In all cases, the plot of ln (η red) against C was linear and extrapolated to 1·6. Values obtained for k are as indicated in Fig. 4. These ranged from $k = 4$ for control actomyosin to $k = 1{\cdot}02$ for actomyosin pressure-treated at 150 MN/m^2. Exposure of F-actin to pressure resulted in a drastic decrease in relative viscosity (Fig. 3).

The intrinsic viscosity of myosin was found to be 2·9 dl/g. This is somewhat higher than the values reported by other workers, possibly because of the presence of a high-molecular-weight polymer (Herbert and Carlson 1971). Another possible cause of a high intrinsic viscosity is the presence of actomyosin in the sample (Brahms and Brezner 1961).

Light-scattering Measurements

Fig. 5 shows the angular scattering envelope (Zimm 1946) of control and pressurized actomyosin. The average radius of gyration, $\langle \rho^2 \rangle^{\frac{1}{2}}$, was determined from the initial slope of the $KC/R\theta$ *v.* $\sin^2 \theta/2$ plot (Zimm 1946). The Zimm diagram for control actomyosin is shown in Fig. 6. In constructing this diagram most weight was assigned

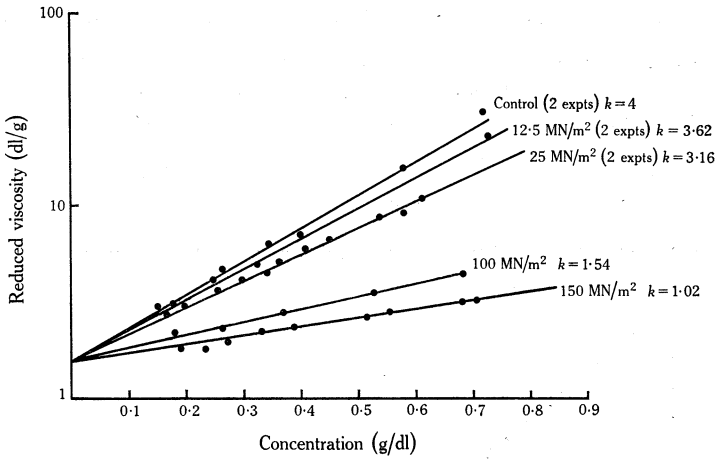

Fig. 4. Concentration dependence of $\log(\eta \text{ red})/C$ for actomyosin samples exposed to various pressures for 1 h, and showing the corresponding values of k where $\ln(\eta \text{ red}) = \ln(\eta) + k(\eta)C$.

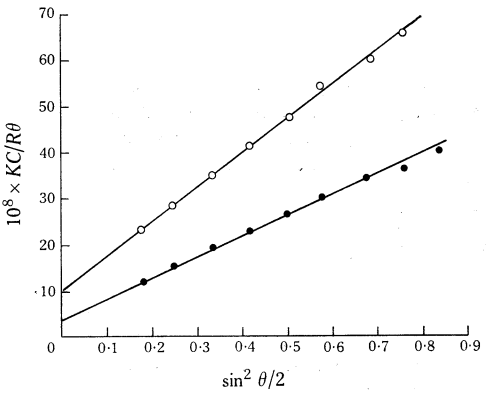

Fig. 5. Angular scattering envelope for control (●) and pressurized (○) (150 MN/m², 1 h, 0°C) actomyosin. Control actomyosin molecular weight = 25×10^6, $\langle \rho^2 \rangle^{\frac{1}{2}} = 163$ nm. Pressurized actomyosin molecular weight = $10{\cdot}9 \times 10^6$, $\langle \rho^2 \rangle^{\frac{1}{2}} = 134$ nm.

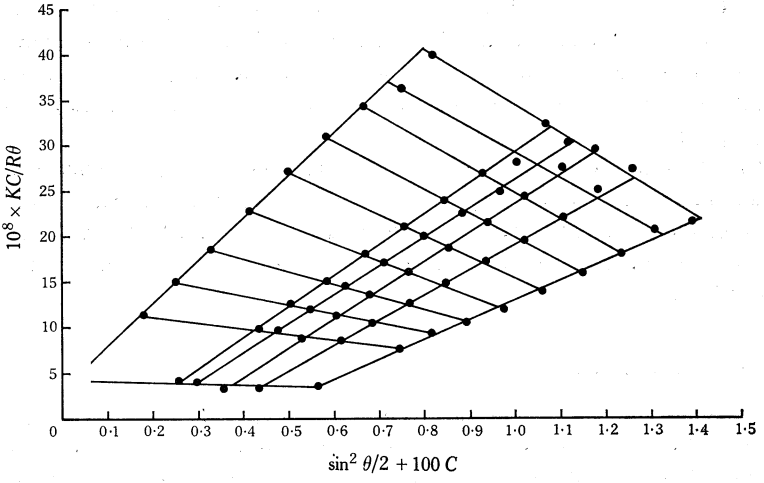

Fig. 6. Zimm diagram for actomyosin.

to the points at intermediate angles (Gellert and Englander 1963). The arcing over of the curves at higher angles has been previously discussed in the literature (Holtzer *et al.* 1962). The zero or negative value of the $\theta = 0$ plot has been previously observed with myosin (Holtzer and Lowey 1959) and actomyosin (Holtzer *et al.* 1962). It has been attributed to a poor solvent and the approaching phase separation (Flory 1942).

The value of 25×10^6 for the molecular weight of the control actomyosin is of the same order as that reported by Gergely (1956), but is less than that of Gellert *et al.* (1959) who reported values of 40×10^6 to 50×10^6 for material prepared by similar methods. The difference is thought to arise from the fact that Gellert *et al.* (1959) used a low-angle extrapolation, which was not possible in this work.

It can be seen from Fig. 5 that the effect of pressure on actomyosin was to decrease the molecular weight and the radius of gyration.

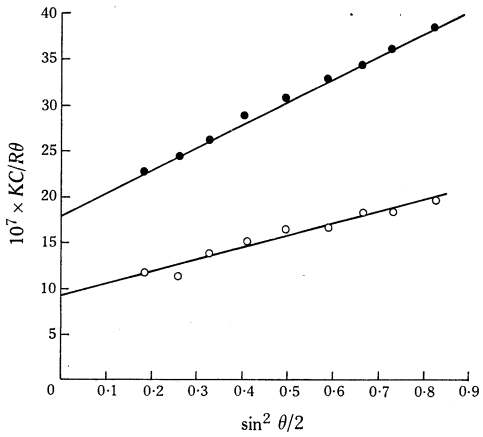

Fig. 7. Angular scattering envelopes for control (●) and pressurized (○) (150 MN/m², 1 h, 0°C) myosin. Control myosin molecular weight = 56×10^4, $\langle \rho^2 \rangle^{\frac{1}{2}} = 53$ nm. Pressurized myosin molecular weight = 11×10^5, $\langle \rho^2 \rangle^{\frac{1}{2}} = 53$ nm.

Fig. 7 shows the angular scattering envelope of control and pressurized myosin. The molecular weight and radius of gyration found here for the control samples are in reasonable agreement with those earlier reported (Lowey and Holtzer 1959), although the preparations show evidence of higher aggregates being present. The effect of pressure on the myosin was to increase its molecular weight. A similar observation has also been made by Ivanov *et al.* (1960). The radius of gyration, however, was not significantly changed.

ATPase Measurements

Fig. 8 shows the effect of increasing pressures on the Mg^{2+} ATPase activities of actomyosin preparations. There appeared to be two effects. Firstly, there was a gradual and reasonably linear decrease in the Mg^{2+} ATPase activity measured in the absence of calcium ions as the pressure was increased. Secondly, there was a decline in the calcium stimulation of the Mg^{2+} ATPase as the pressure was increased. This decline was rapid at first and then slowed down at high pressure.

The results presented in the following tabulation show that the Ca^{2+} ATPase and K^+-EDTA ATPase activities of myosin were both decreased to approximately 40% of their original values by the pressurization treatment (150 MN/m², 1 h, 0°C),

and there was no selective destruction of either activity:

Myosin	Specific activities [μmol min^{-1} (mg protein)$^{-1}$]	
	K^+-EDTA ATPase	Ca^{2+} ATPase
Control	0·141	0·037
Pressurized	0·057	0·014

Discussion

Changes in the degree of ionization of buffer systems will result in change of pH, the magnitude of which will depend on the buffer system used. In the present study it was not possible to measure the pH of solutions while under pressure. However, similar results for viscosity changes were obtained regardless of whether the protein was in an unbuffered solution or one buffered with phosphate. Therefore, providing that the pressure-induced pH changes in the two systems are dissimilar, this result suggests that for the reactions studied here pressure-induced pH changes were not important.

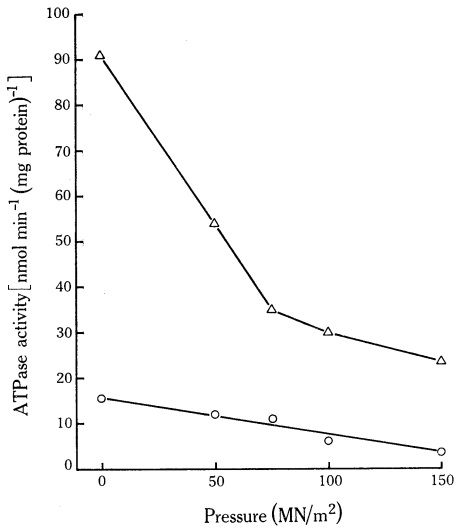

Fig. 8. Effect of pressurization for 1 h at 0°C on actomyosin ATPase activity. △ Activity in presence of Ca^{2+}. ○ Activity in absence of Ca^{2+}.

From the results presented in Fig. 1, it is seen that an effect of pressurization is to promote the transition to a sol of the gel present in actomyosin solutions. The pressure-induced transition is not markedly affected by temperature over the range 0–30°C. However, at 45°C, pressurization opposes the irreversible denaturation and insolubilization of actomyosin that is normally associated with heating to this temperature. To be effective in this respect samples have to be pressurized before heat-induced gelation takes place. This can be seen from the investigations reported by Marsland and Brown (1942), who also found that exposure of actomyosin to pressure fosters solation, but that at 37°C the actomyosin gradually formed a gel which could not be solated by increase in pressure.

The remaining experiments on actomyosin discussed here were carried out on solutions from which gel was removed by centrifuging and decanting the supernatant liquid.

Pressurization of myosin in solution was found to promote formation of aggregates with a molecular weight approximating that expected for a dimer. Ivanov *et al.* (1960) found that the molecular weight of myosin after the action of 405 MN/m^2 pressure increased 1·5–2 times. However, Josephs and Harrington (1968) found a dependence of the equilibrium constant on the hydrostatic pressure generated in high-speed sedimentation experiments and estimated a positive volume change for the association process of 380 cc/mol of monomer. Therefore under pressure the monomer form of myosin would be expected to be favoured. To account for the experimental observation it is assumed that the pressure-treated myosin rapidly aggregates following the release of pressure. These aggregates are probably mostly overlapping dimers because the light scattering measurement reveals a doubling of molecular weight with no change in radius of gyration.

The large decrease in the relative viscosity of actin as a result of pressurization is consistent with a depolymerization of F-actin as has been observed by other workers (Ikkai and Ooi 1966). Therefore both the major constituent proteins of actomyosin probably disaggregate under pressure, although as mentioned above myosin presumably aggregated following release of pressure.

Viscometry and light-scattering measurements indicate that pressurization results in a large measure of disaggregation of actomyosin in solution. This could be a consequence of destruction of interactions between myosin molecules, as mentioned above, or of actin molecules, or as suggested by Ivanov *et al.* (1960), by the splitting of actomyosin into actin and myosin components. However, this splitting does not seem likely to occur; Ikkai and Ooi (1969), from studies on synthetic heavy actomeromyosin, suggest that this material does not simply dissociate under pressure into actin and heavy meromyosin.

Little can be inferred about the nature of the changes responsible for the pressure-induced decrease in aggregation of actomyosin from the light-scattering and the viscometry measurements. Moore (1967) suggests that the decrease with increasing rate of shear in the value of the slope constant k of equation (1) occurs in systems in which chain entanglement effects are significant. He also points out that values of $k > 0{\cdot}5$ (Fig. 4) are associated with a poor solvent system, and that higher values may reflect aggregation. On this basis the effect of pressure on actomyosin is to improve solvent–solute interaction and to decrease aggregation. Also Simha (1949) has pointed out that the value of k is dependent on the shape of the molecule and is increased by branching and again the results are consistent with pressurization decreasing the degree of branching or crosslinking.

With actomyosin preparations the ATPase activity measurements indicate that pressurization has two effects. The steady decrease in the calcium-independent Mg^{2+} ATPase activity (Fig. 8) could be due to either an effect on the myosin component or on the association between actin and myosin (for fuller discussion, see for example Perry 1965). The more pronounced effect on the calcium-sensitive Mg^{2+} ATPase could be due to an effect on the troponin component of the natural actomyosin (Hartshorne *et al.* 1967; Schaub and Perry 1971).

Pressurization does affect the ATPase activity of the myosin molecule (see tabulation on p. 204). The equal inhibition of Ca^{2+} ATPase and K^{+}-EDTA ATPase activities shows that the pressure effect is not due to the selective destruction of one of the two reactive sulphydryl groups located at or near the active site of myosin

(Sekine and Kielley 1964). If, as reported by Reisler *et al.* (1974), the SH_1 group is more exposed than the SH_2 group in the absence of nucleotide, a selective destruction might be expected if pressure was affecting this area of the molecule. The absence of such an effect indicates that pressure could be destroying the ATPase activity by an effect on some other part of the molecule, possibly the light chains.

Acknowledgments

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References

Brahms, J., and Brezner, J. (1961). Interaction of myosin A with ions. *Arch. Biochem. Biophys.* **95**, 219.

Briskey, E. J., and Fukazawa, T. (1971). Myofibrillar proteins of skeletal muscle. *Adv. Food Res.* **19**, 279.

Flory, P. J. (1942). Thermodynamics of high polymer solutions. *J. Chem. Phys.* **10**, 51.

Gellert, M. F., von Hippel, P. H., Schachman, H. K., and Morales, M. F. (1959). Studies on the contractile proteins of muscle. 1. The ATP-myosin B interaction. *J. Am. Chem. Soc.* **81**, 1384.

Gellert, M. F., and Englander, S. W. (1963). The molecular weight of rabbit myosin A by light scattering. *Biochemistry* **2**, 39.

Gergely, J. (1956). The interaction between actomyosin and adenosinetriphosphate. Light-scattering studies. *J. Biol. Chem.* **220**, 917.

Gornall, A. G., Bardawill, G. J., and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**, 751.

Hartshorne, D. J., Perry, S. V., and Schaub, M. C. (1967). A protein factor inhibiting the magnesium-activated adenosine triphosphatase of desensitized actomyosin. *Biochem. J.* **104**, 907.

Herbert, T. J., and Carlson, F. D. (1971). Spectroscopic study of the self-association of myosin. *Biopolymers* **10**, 2231.

Holtzer, A., and Lowey, S. (1959). The molecular weight, size and shape of the myosin molecule. *J. Am. Chem. Soc.* **81**, 1370.

Holtzer, A., Lowey, S., and Schuster, T. M. (1962). In 'The Molecular Basis of Neoplasia'. p. 259. (University of Texas Press: Austin, Texas.)

Horgan, D. J. (1974). Modification of sarcoplasmic reticulum adenosine triphosphatase by adenosine triphosphate and magnesium. *Arch. Biochem. Biophys.* **162**, 6.

Ikkai, T., and Ooi, T. (1966). The effects of pressure on F–G transformation of actin. *Biochemistry* **5**, 1551.

Ikkai, T., and Ooi, T. (1969). The effects of pressure on actomyosin systems. *Biochemistry* **8**, 2615.

Ivanov, I. I., Bert, Yu. N., and Lebedeva, N. A. (1960). Changes in some properties of myosin, actomyosin, and actin under the influence of high pressure. *Biokhimiya* **25**, 505.

Johnson, F. H., Eyring, H., and Polissar, M. J. (1954). 'The Kinetic Basis of Molecular Biology'. (John Wiley and Sons: New York.)

Josephs, R., and Harrington, W. F. (1966). Studies on the formation and physical chemical properties of synthetic myosin filaments. *Biochemistry* **5**, 3474.

Josephs, R., and Harrington, W. F. (1967). An unusual pressure dependence for a reversibly associating protein system; sedimentation studies on myosin. *Proc. Nat. Acad. Sci. U.S.A.* **58**, 1587.

Josephs, R., and Harrington, W. F. (1968). On the stability of myosin filaments. *Biochemistry* **7**, 2834.

Lowey, S., and Holtzer, A. (1959). The aggregation of myosin. *J. Am. Chem. Soc.* **81**, 1378.

Macfarlane, J. J. (1974). Pressure-induced solubilization of meat proteins in saline solution. *J. Food Sci.* **39**, 542.

Marsland, D. A., and Brown, D. E. S. (1942). The effects of pressure on sol–gel equilibria, with special reference to myosin and other protoplasmic gels. *J. Cell. Comp. Physiol.* **20**, 295.

Martin, A. F. (1942). Abstr. 103rd Am. Chem. Soc. Meeting. p. 1-C, in Yang, J. T. (1961). *Adv. Protein Chem.* **16**, 323.

Moore, W. R. (1967). Viscosities of dilute polymer solutions. *Prog. Polym. Sci.* **1**, 1.

Newbold, R. P., Tume, R. K., and Horgan, D. J. (1973). Effect of feeding a protected safflower oil supplement on the composition and properties of the sarcoplasmic reticulum and on postmortem changes in bovine skeletal muscle. *J. Food Sci.* **38**, 821.

Perry, S. V. (1965). Muscle proteins in contraction. In 'Muscle'. (Eds W. M. Paul, E. E. Daniel, C. M. Kay and G. Monckton.) p. 29. (Pergamon Press: Oxford.)

Reisler, E., Burke, M., and Harrington, W. F. (1974). Cooperative role of two sulfhydryl groups in myosin adenosine triphosphatase. *Biochemistry* **13**, 2014.

Schaub, M. C., and Perry, S. V. (1971). The regulatory proteins of the myofibril. Characterization and properties of the inhibitory factory (troponin B). *Biochem. J.* **123**, 367.

Sekine, T., and Kielley, W. W. (1964). The enzymic properties of n-ethylmaleimide-modified myosin. *Biochim. Biophys. Acta* **81**, 336.

Simha, R. (1949). Effect of concentration on the viscosity of dilute solutions. *J. Res. Nat. Bur. Stand.* **42**, 409.

Spudich, J. A., and Watt, S. (1971). The regulation of rabbit skeletal muscle contraction 1. Biochemical studies of the interaction of the tropomyosin–troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* **246**, 4866.

Yang, J. T. (1961). The viscosity of macromolecules in relation to molecular conformation. *Adv. Protein Chem.* **16**, 323.

Zimm, B. H. (1946). Application of the methods of molecular distribution to solutions of large molecules. *J. Chem. Phys.* **14**, 164.

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