The influence of pressure on the self-assembly of the thick filament from the myosin of vertebrate skeletal muscle

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The thick-filament–monomeric-myosin equilibrium was prepared from pure myosin at pH 8.1. The application of hydrostatic pressure to the self-assembly equilibrium resulted in a biphasic dissociation curve in which a linear decrease in turbidity (a measure of weight added to or lost from the filament) was followed by a transition to a second pressure-insensitive phase. The first phase represents the effect of hydrostatic pressure on the growth or propagation phase of filament assembly. Here it was shown that hydrostatic pressure served to shorten the filaments in concert towards the bare zone whilst maintaining the narrow length distribution seen at atmospheric pressure; the filament concentration remained constant during the experiment. A more precise definition of the $\Delta\bar{v}$ for the assembly of monomer into filament was obtained than had hitherto been possible. The positioning of the bare zone at the centre of the filament seems to be one of the more obvious functions of the length-regulation mechanism. It also appears that all the basic structural elements of the native thick filament are potentially present in the pH 8.1 homopolymer; its length can be increased by physiological concentrations of $MgCl_2$ and decreased by pressure. The monodisperse native filament could then be formed by a fine tuning of the basic length-regulation mechanism of the homopolymer by the co-polymerization of the other thick-filament proteins.

A logical starting point from which to initiate a study of the self-assembly of the A-band thick filament is provided by a system *in vitro* in which purified myosin can be made to spontaneously self-assemble into a homopolymer with a structure similar to that of the native thick filament. There are a number of distinguishable classes of synthetic filament from which to choose (Josephs & Harrington, 1966). The type of filament found appears to primarily depend on the pH of the filamentogenic solvent. A pH decrease serves to increase filament stability; however, the increase in stability appears to be linked to a decrease in the structural integrity of the filament formed. The class of synthetic filament generated at a pH between 8.0 and 8.5 appears to be most closely related to the counterparts *in vivo*. Characteristically it has the appearance of a homogeneous population of polymers shorter in length than the native filaments (630 nm compared with 1550 nm), but is otherwise structurally similar with a clearly visible bare zone of length 180 nm and a filament diameter of 12.5 nm (Josephs & Harrington, 1968).

Experiments in the ultracentrifuge on the self-assembly of the pH 8.0–8.5 class of filaments showed that the assembled filament and monomer were in rapid equilibrium with each other (Josephs & Harrington, 1968). KCl concentration, pH and hydrostatic pressure were shown to have a marked effect on the equilibrium and led Josephs & Harrington (1968) to propose the following stoichiometry for the assembly reaction:

$$100\,(M–11\,KCl) + 67\,H^+ \rightleftharpoons (F–67\,H^+) + 1100\,KCl$$
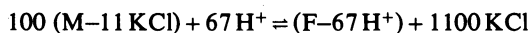
where M is monomer and F is assembled filament. The change in partial specific volume ($\Delta\bar{v}$) for the reaction was found to be $300 \pm 45\,cm^3$/mol of monomer assembled into filament, a particularly large value for a self-assembly reaction that reflects the marked sensitivity of the equilibrium to changes in hydrostatic pressure (Josephs & Harrington, 1968).

The pressure sensitivity of the self-assembly reaction led Davis & Gutfreund (1976) to examine the process in a pressure-jump apparatus. The instrument offers a number of advantages over

comparable rapid-reaction techniques when applied to the study of aggregates of delicate bipolymers (Davis & Gutfreund, 1976). The results from preliminary experiments, though difficult to interpret at the time, revealed an apparently simple thermodynamic and kinetic behaviour, an encouraging result in the light of the apparent structural complexity of the thick filaments.

The present paper, and the following paper on the kinetics of the reaction (Davis, 1981), examines the growth or propagation phase of the self-assembly process in detail. It is during this phase that the main structural and functional features of the bipolar filament are established. Pressure-jump studies on the equilibrium and thermodynamic behaviour of the self-assembly system have been linked to a technique whereby the physical structure of intermediates on the assembly pathway can be 'frozen' at pressure by chemical cross-linking for later examination in the electron microscope. The latter technique has recently been successfully applied to show the presence of a myosin dimer (Davis *et al.*, 1981) under conditions identical with those used in the present paper.

Experimental

Preparation of myosin

Myosin was prepared from the back and hind-leg muscles of a rabbit by the method of Starr & Offer (1971). The myosin was extracted from minced muscle with Guba–Straub solvent, precipitated by dilution into 16 vol. of cold water, fractionated by $(NH_4)_2SO_4$ precipitation and finally purified by column chromatography on DEAE-Sephadex A-50 (Richards *et al.*, 1967). The trailing edge of the myosin peak was used in the experiments. The myosin concentration was determined spectrophotometrically in 0.5 M-KCl; an $A^{1\%}_{1cm,280}$ value of 5.6 was used after a correction (less than 3% of the signal) had been made for turbidity.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the purified myosin in the presence of mercaptoethanol gave five distinct protein bands. Four of the bands arose from myosin; the fifth, the so called B-protein, was considered to be a contaminant (Starr & Offer, 1971). The B-protein band was later shown to be an artefact (Morimoto & Harrington, 1973). It seems reasonable to conclude that the myosin used in the experiments described is, for all intents and purposes, pure.

Preparation of filaments

The filaments were generated by a method similar to that used by Josephs & Harrington (1966).

The eluate from the DEAE-Sephadex A-50 column was diluted to the highest protein concentration required for the experiments. The dialysis medium contained 0.150 M-KCl and 0.005 M-Bicine [NN-bis-(2-hydroxyethyl)glycine] buffer at pH 8.1 at 5°C. The ionization of Bicine has a $\Delta \bar{v}$ close to zero (J. S. Davis, unpublished work). The solvent used for the first of the two 12-h dialyses contained 0.01 mM-NaN$_3$ as a preservative. pH was checked every half-hour at the start of the dialysis until stabilization occurred; the pH was adjusted with either 3 M-KOH or 5 M-HCl. The pH meter (PHM 64 Research pH meter; Radiometer, Copenhagen, Denmark) was calibrated in the cold-room with standard buffers. The effects of a deviation in the cold-room temperature from 5°C on the ionization of the Bicine buffer was taken into account when the pH of the dialysis medium was set. It is important that a lot of care is taken with the preparation and the handling of the filaments as the myosin self-assembly equilibrium is remarkably sensitive to small changes in salt concentration, the pH and the physical state of the myosin. The final filament preparation had the appearance of a homogeneous colloidal solution.

The pressure-jump apparatus

The design of the pressure-jump apparatus was similar to the instrument described by Davis & Gutfreund (1976). The light source used was a 12 V/55 W quartz-halogen bulb powered by a 12 V stabilized power supply (Coutant GP 12 V 10 A; Coutant Electronics, Reading RG1 8JR, Berks., U.K.). The high-radiance monochromator (Applied Photophysics, London W.1, U.K.) had its entrance slit set to 1.25 mm and the exit slit to a band-width of 5 nm. The light intensity was measured with an end-window photomultiplier (EMI 9592B; EMI Electronics, Hayes, Middx., U.K.) run from a 2 kV power supply (Brandenburg model 475R; Brandenburg, Thornton Heath, Surrey, U.K.). The signal from the photomultiplier passed through an amplifier with a 1 MHz band-width and was filtered through an adjustable set of time constants to optimize the signal-to-noise ratio. In the equilibrium experiments the amplifier output was simultaneously applied to a digital volt meter and to an XYt recorder. The approach to a new equilibrium position was followed on the recorder so that the equilibrium end-point of the reaction could be accurately established.

The upward pressure-jump experiments were carried out with an all-hydraulic pressurization device that could be fitted to the pressure-jump in place of the usual pressure-release valve (J. S. Davis, unpublished work).

Filament cross-linking

The method of Davis *et al.* (1981) was used to chemically cross-link the filaments under pressure with glutaraldehyde.

Materials

The AnalaR-grade chemicals used were generally obtained from BDH Chemials, Poole, Dorset, U.K. Bicine was obtained from the Sigma Chemical Company, St. Louis, MO, U.S.A. BDH standard buffers were used to calibrate the pH meter.

Results and discussion

Filament and monomer concentration at atmospheric pressure

It is essential to be able to quantitatively measure the amount of monomer being incorporated into, or dissociated from, the thick filament. To do this, a 'specific turbidity' has to be established to relate the amount of monomer subunit incorporated into the filament at atmospheric pressure to the turbidity of the solution.

A wavelength of 320 nm was selected as it represented the shortest wavelength that was free from significant interference from protein absorbance. The specific turbidity was obtained by measuring the turbidity of a solution of known myosin concentration in which all the monomer initially present had been polymerized into filament. The simplest way of achieving this is to select a salt concentration at which filamentogenesis is strongly favoured; such conditions were found to pertain at a salt concentration of 0.125 M-KCl. A linear relationship between turbidity and total myosin concentration is observed up to a total myosin concentration of 3 mg/ml. The slope of the 0.125 M-KCl dilution curve was used to calculate a specific turbidity coefficient for the assembly of monomer subunit into filament; an $A_{1cm,320}^{1\%}$ value of 1.49 at pH 8.1 and 5 °C was obtained. It should be noted that the 0.150 M-KCl dilution curve runs parallel to the 0.125 M-KCl dilution curve. The offset of the two curves on the concentration axis gives a rough measure of the amount of free monomer present in the 0.150 M-KCl sample. A more accurate measure of the free monomer concentration can be obtained by introducing a correction factor for the contribution from the monomer to the overall turbidity of the solution. The free monomer has an $A_{1cm,320}^{1\%}$ of 0.09 compared with an $A_{1cm,320}^{1\%}$ of 1.49 when assembled into the filament. The free monomer concentration is, as a result, some 6% greater than the value obtained from the intercept with the concentration axis. The presence of dimer along with monomer is not likely to cause a significant change in the turbidity concentration from non-filamentous myosin.

Measurement of filament and free monomer concentration at pressure

The specific-turbidity coefficients established at atmospheric pressure have to be extended to give a

measure of the quantity of monomer incorporated into, or dissociated from, filaments ranging in length from a short 180 nm structure, marginally longer than the central bare-zone, to the full 1550 nm length of the native filament.

The feasibility of such a task can best be assessed by recourse to a theoretical treatment of the light scattering of long rods (Berne, 1974). Certain conditions have to be met for the relationship to apply. The thickness of the polymer has to be much less than both the length of the shortest polymer and the wavelength of the incident light used. The thick-filament structure meets these requirements with a diameter of 15 nm and a minimum length of 180 nm. In addition the polymer has to be optically isotropic; this condition is probably met as the filament structure beyond the bare-zone appears to be built as a continuous array of subunits (Craig & Offer, 1976). With the above requirements met the mathematical relationship developed by Berne (1974) can be applied to find out whether the turbidity increment, seen as each monomer unit is added to the filament, remains constant and independent of length. The relationship is embodied in a parameter Q_0, which was calculated for an 180 nm-long filament dissolved in a solution with the refractive index of 0.150 M-KCl and illuminated with 320 nm light. A linear relationship between turbidity and weight added is established for Q_0 values greater than 2.5; the value calculated for the shortest possible filament was 3.33. The nett result was that the amount of monomer added to or lost from the filament can be obtained from the charge in turbidity.

A direct validation of the above conclusions comes from filament cross-linking experiments in which the turbidity of pressure-shortened filaments (Fig. 1) was shown to correlate directly with the mean filament length obtained from electron micrographs (Fig. 4).

Pressure on filament self-assembly

The application of hydrostatic pressure to the self-assembly equilibrium in 0.150 M-KCl/0.005 M-Bicine, pH 8.09, at 5 °C in a biphasic dissociation curve (Fig. 1). At first a linear decrease in turbidity with pressure occurs followed by a transition to a second linear phase in which an increase in pressure has virtually no effect on the turbidity of the solution. The second phase can best be described as a 'residual turbidity', the value finally reached being marginally greater than that found for salt-dissociated filament. The reaction is rapidly reversible over the first phase; it is much slower when the filaments are reconstituted from the second phase of the dissociation curve. The results suggested that the first phase represented the effect of pressure on the propagation or growth phase of the assembly
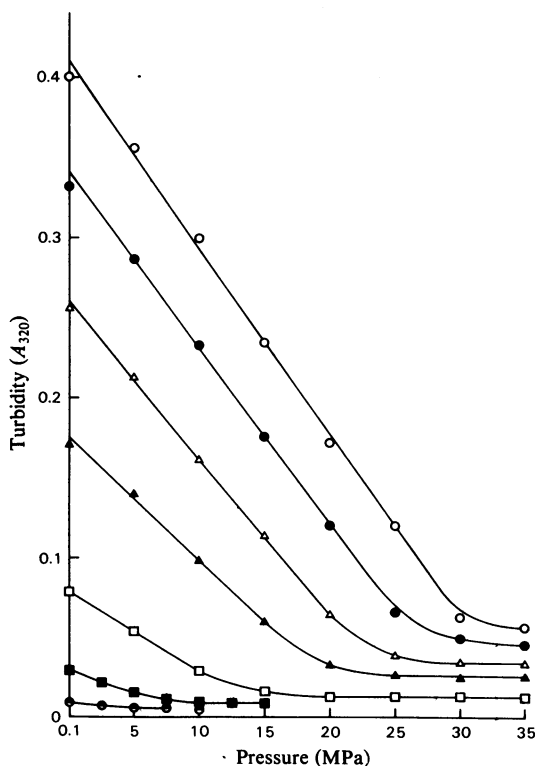
Fig. 1. *The effect of hydrostatic pressure on the turbidity of the myosin self-assembly equilibrium at different protein concentrations*

The total myosin concentrations were: O, 3.1 mg/ml; ●, 2.5 mg/ml; △, 1.9 mg/ml; ▲, 1.3 mg/ml; □, 0.6 mg/ml; ■, 0.3 mg/ml; ◓, 0.15 mg/ml. The reaction was carried out in 0.150 M-KCl/0.005 M-Bicine, pH 8.09, at 5°C.
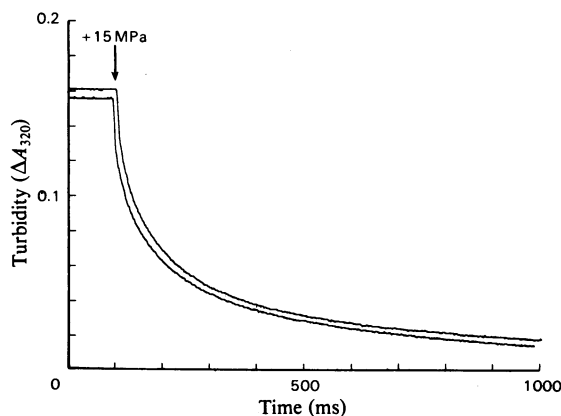

Fig. 2. *An upward-pressure-jump experiment on a fresh (after 24 h dialysis, lower curve) and a cycled (three pressurization-depressurization cycles, upper curve) filament equilibrium*

The total myosin concentration was 2.5 mg/ml.

reaction, the non-linear transition to the pressure-insensitive phase representing the approach to complete filament dissociation. The fact that the pressure-dependent linear phase extended over a remarkably large part of the total turbidity change (some 80% of the total) led to the idea that the filaments might well shorten the sequential loss of monomer from the filament ends whilst at the same time maintaining the narrow length-distribution seen at atmospheric pressure. If the system were heterodisperse, such a markedly uniform behaviour would not be expected as the non-linearity introduced by the dissociation of the bare-zone nucleus would appear at lower pressures.

The filament number concentration

The number of filaments present before and after repeated pressurization–depressurization cycles can be determined from the results of large-perturbation

upward-pressure-jump experiments. The kinetic rationale behind the method can best be explained by consideration of a simplified model for the kinetics of myosin-filament dissociation. The method requires that filament dissociation occurs by the sequential loss of monomer from the two filament ends. The dissociation reaction can then be depicted as a sequential first-order reaction.

$$F_i \xrightarrow{k_{-1}} F_{i-1} + M$$
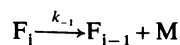
$$\frac{d[M]}{dt} = k_{-1}[F_i]$$

If the filament concentration $[F_i]$ remains constant the reaction kinetics simplify to the form of a pseudo-zero-order reaction. An increase or decrease in the initial velocity of the reaction after a number of assembly–disassembly cycles would indicate whether filaments had either been nucleated or lost from the solution. Such an experiment is shown in Fig. 2. The upward pressure-jump relaxation curve for myosin direct from the dialysis medium is shown, and above it and offset deliberately from it the relaxation curve for the same myosin filament sample passaged through three dissociation-association cycles. As can be seen, the two dissociation curves are virtually superimposable, a reasonable deduction being that the filament concentration has in fact remained constant. These experimental findings have been shown to hold for the full 20-fold range of myosin concentration studied.

The relationship between filament number concentration and turbidity

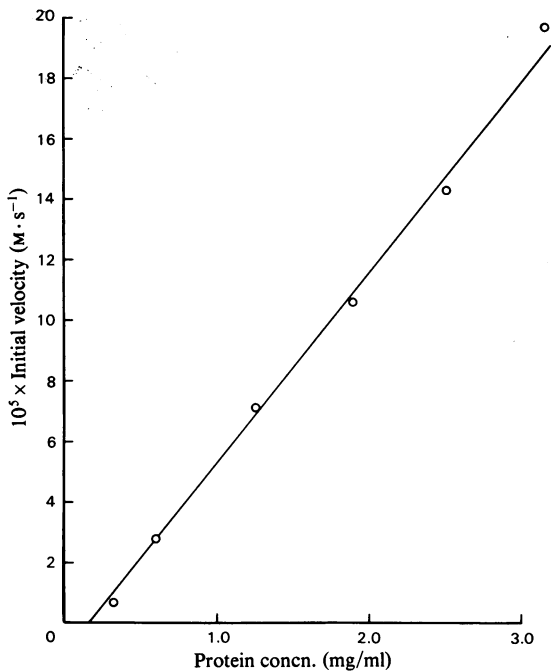The rationale developed in the previous section

Fig. 3. *The dependence of the initial velocity of filament dissociation on total protein concentration*
The initial velocities were obtained from 15 MPa upward-pressure-jump experiments.

Fig. 4. *The effect of hydrostatic pressure on the length of the thick filament*
The total myosin concentration was 1.26 mg/ml. Error bars represent $\pm$ s.d.

can be used to 'count' the number of filaments present at atmospheric pressure in any myosin filament preparation. A plot of the initial velocities of the dissociation reaction against total myosin concentration is shown in Fig. 3. A comparison of this curve with a plot of filament turbidity against total protein concentration at atmospheric pressure (Fig. 5) reveals a close similarity.

Therefore the turbidity of a filament preparation at atmospheric pressure is directly proportional to filament concentration, a valuable relationship for the interpretation of the equilibrium and kinetic data.

Filament length distribution under pressure

An analysis of the filament length distribution at various pressures was performed by 'freezing' the structures present by chemically cross-linking the polymer (see the Experimental section). The quenched filament solution was prepared for electron microscopy by the method of Huxley (1963); the sample was positively stained with 0.5% uranyl acetate. The length distribution and structure of the 'frozen' filaments were later determined from electron micrographs; 100 filaments were measured at each pressure. A plot of the mean length of the filament, together with their individual standard
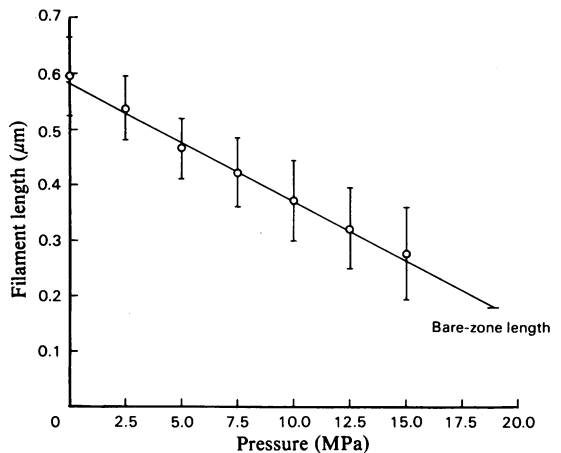
deviation error bars, against hydrostatic pressure is shown in Fig. 4. The linearity of the plot supports the thesis that the filaments shorten in concert with increasing hydrostatic pressure. The width of the filament length distribution (see s.d. bars in Fig. 4) increases somewhat at higher pressures. This is probably due to the presence of some full-length filaments, cross-linked before pressurization, and to the difficulty of accurately determining the number and length of very short filaments. The length–pressure graph (Fig. 4) can be compared directly with the turbidity–pressure graph (Fig. 1) for the same total myosin concentration (1.26 mg/ml). The two graphs are very similar, the bare-zone length being reached between 17.5 and 20 MPa in Fig. 4, and the transition from the pressure-sensitive to the pressure-insensitive phase (Fig. 1) occurring at much the same pressure. The parallel nature of the curves allows the percentage change in turbidity to be directly correlated with a particular filament length, a very useful relationship. The retention of the narrow length-distribution as the filaments shorten, and the extent of the linear phase of the filament dissociation curve both point to a high degree of co-operativity in the self-assembly of the thick filament.

The equilibrium constant for the thermodynamics of filament self-assembly

The overall equilibrium constant at various pressures and the change in $\Delta\bar{v}$ for the self-assembly reaction have been obtained from experiments in the ultracentrifuge over a 6 MPa pressure range (Josephs & Harrington, 1968). In a preliminary

study, Davis & Gutfreund (1976) discussed the rationale behind these experiments and obtained in pressure-jump experiments a $\Delta\bar{v}$ of $280\,cm^3/mol$ of monomer assembled into filament; the value concurred with the $\Delta\bar{v}$ of $300\pm45\,cm^3/mol$ obtained by Josephs & Harrington (1968).

The following findings make it now possible to carry out a more rigorous analysis of the equilibrium thermodynamic behaviour of the system. The filament concentration did not change under the experimental conditions used. The participation of a parallel dimer on the self-assembly pathway has been established in both kinetic and structural studies (Davis, 1981; Davis et al., 1981). It has also been shown that the dissociation rate and hence the equilibrium constant for the propagation reaction are length-dependent.

The best approach seems to be to apply the simplest model of the self-assembly reaction that gives a linear plot of $\ln K_{prop}$ against P in terms of the Planck equation. This is found for a two-state model with monomer in equilibrium with a fixed concentration of filament, the reaction being governed by an apparently length-independent equilibrium constant:

$$F_{i-1} + M \xrightleftharpoons{\;K_{prop}\;} F_i$$

$$K_{prop} = \frac{[F_i]}{[F_{i-1}][M]}$$
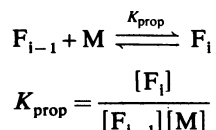
If

$$[F_i] = [F_{i-1}]$$

$$K_{prop} = \frac{1}{[M]}$$

The apparent monomer concentration is obtained from a re-plot of Fig. 1 as a graph of turbidity against total myosin concentration at different pressures (Fig. 5). Concentration is read from the intercept of the isobaric lines with the protein concentration axis, and corrected for the contribution to the turbidity signal made by monomer. A list of the free monomer concentrations and the propagation equilibrium constants at various pressures are given in Table 1. The slope of the graph of $\ln K_{prop}$ against pressure (Fig. 6) gave the $\Delta\bar{v}$ for the reaction as $240\,cm^3/mol$ of monomer incorporated into filament over a range from atmospheric pressure to 25 MPa.

The question now arises as to how the concentration axis intercept of Fig. 5, used for the determination of the pressure dependence of K_{prop}, relates to what should be a length-dependent K_{prop} that should decrease some 500-fold from the bare-zone out (Davis, 1981). The monomer concentration is, however, determined at a point where the filament concentration approaches zero. This
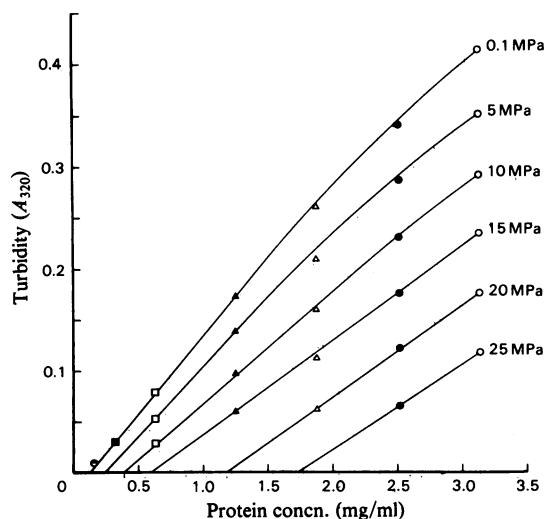

Fig. 5. *The effect of total myosin concentration on filament turbidity at various pressures*

The intercepts of the isobaric lines with the concentration axis are used to obtain the free myosin concentration in equilibrium with filament. The graph is essentially a re-plot of the first linear phase of Fig. 1 and the same symbols are used. Total myosin concentrations were: ○, 3.1 mg/ml; ●, 2.5 mg/ml; △, 1.9 mg/ml; ▲, 1.3 mg/ml; □, 0.6 mg/ml; ■, 0.3 mg/ml; ◒, 0.15 mg/ml.

Table 1. *The effect of hydrostatic pressure on the apparent propagation equilibrium constant*

The apparent monomer concentration is obtained from the intercept of the isobaric lines with the concentration axis in Fig. 5. K_{prop} is taken as the reciprocal of the free monomer concentration.

Pressure (MPa)	Apparent monomer concn. (mg/ml)	$10^{-6} \times K_{prop}$ (M^{-1})
0.1	0.131	3.84
5	0.254	1.83
10	0.403	1.15
15	0.625	0.744
20	1.24	0.375
25	1.84	0.252

conclusion stems from a previous finding that if the total myosin concentration was lowered at a fixed pressure the filaments would progressively shorten towards the bare-zone. The net result is that the monomer concentrations obtained by extrapolation to an *x*-axis intercept are all apparently in equilibrium with filament at the shortest length limit. As a result a correction factor is not needed to compensate for the different values of K_{prop}.
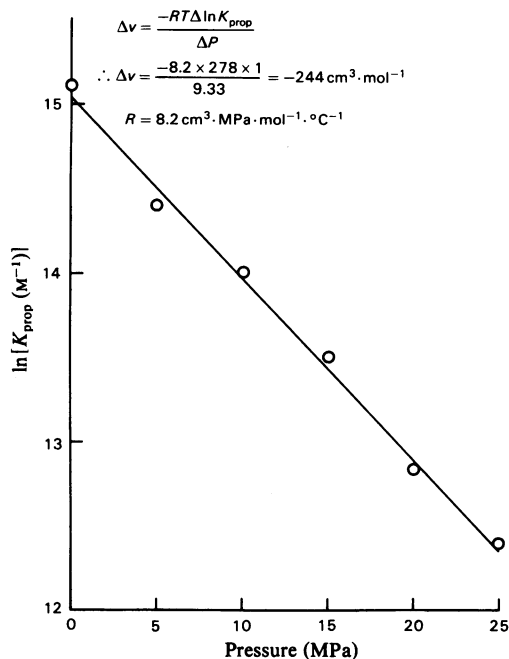
Fig. 6. *The effect of hydrostatic pressure on the natural logarithm of the apparent equilibrium constant for the propagation reaction*

The pressure-dependent values for the propagation equilibrium constant (K_{prop}) were calculated assuming a simple monomer–filament equilibrium. The values used are listed in Table 1. See the Results and discussion section for further details.

The choice of the two-state model for the self-assembly reaction is less justifiable in the light of the known participation of dimer in the reaction. A precise assignment of the $\Delta \bar{v}$ for the reaction has to await the time when a precise measure of the monomer and dimer concentrations can be made. However, the value of $\Delta \bar{v}$ for the incorporation of 1 mol of monomer into filament can be said to lie between two limits, a factor of two apart, the lower limit being set by a reaction in which dimer alone participates and the higher by a reaction in which monomer alone participates.

Conclusion

An important feature of the experiments on the self-assembly of the thick filament *in vitro* is that they have all been done with pure myosin. It therefore follows that results obtained and the conclusions drawn all relate to the intrinsic properties of myosin alone. The self-assembly of myosin monomer into filament is in all probability nucleated by an anti-parallel grouping of monomers to form the bare-zone (Huxley, 1963). The structure of the nucleus is later extended by what can conveniently be described as the propagation stage of the self-assembly reaction, giving rise as it does to the familiar structural features of the thick filament. In the experiments described, great care has been taken to ensure that the nucleation reaction does not interfere with the observations made on the propagation reaction.

The positioning of the bare-zone at the centre of the filament, regardless of filament length, appears to be one of the more obvious functions of the self-assembly mechanism. The length-regulation mechanism responsible for centring the bare-zone maintains the narrow length distribution amongst the filaments as they shorten from 630 nm to 275 nm. It is difficult to measure the length of the chemically cross-linked filaments much below 300 nm; however, the parallel nature of the graphs illustrating the pressure dependence of length and turbidity demonstrates that the former can be extrapolated to show that the filaments probably maintain their narrow length distribution down to the bare-zone.

Relationship between the narrow filament length distribution of the synthetic homopolymers of myosin and precise monodisperse 1550 nm length of the native heteropolymer is of interest. From a mechanistic point of view it is not too difficult to conceive of circumstances under which a homopolymer of the type studied in the present paper could be extended to give a mean filament length close to 1550 nm. An increase in pressure can decrease the length of the filament. The addition of $MgCl_2$ at physiological concentrations has been shown to have an opposite effect, extending the length of the filament from 630 nm to 940 nm *in vitro* (J. S. Davis & K. E. Elliott, unpublished work). It thus appears entirely feasible that the basic structural elements of the native thick filament are all potentially present in the pH 8.1 homopolymer. The construction of the monodisperse native filament would then occur by a fine-tuning of the basic length-regulation mechanism of the homopolymer by the copolymerization of other thick-filament proteins. The main objection to the above hypothesis is the rather unphysiological pH at which the filaments are generated *in vitro*.

An intriguing feature of the self-assembly equilibrium is its marked sensitivity to changes in hydrostatic pressure. The precise assignment of a $\Delta \bar{v}$ to a particular filament-assembly mechanism with a well defined initial and final state is not simple. Concepts developed for the non-co-operative assembly of monomer into a linear polymer do not transfer over easily to such patently co-operative processes as the self-assembly of the myosin filament. Experiments to determine the $\Delta \bar{v}$ in this, and in a previous

paper by Davis & Gutfreund (1976), were both performed in pressure-insensitive buffers. The two $\Delta\bar{v}$ values of 240 and 280 cm^3/mol of monomer assembled into filament are reasonably close; they are, however, somewhat lower than the value obtained by Josephs & Harrington (1968) from ultracentrifuge experiments. The main stumbling block lies in the assignment of the $\Delta\bar{v}$ values obtained to a particular reaction scheme. A discussion earlier in the present paper led to the $\Delta\bar{v}$ value obtained being assigned to a limiting case in which non-filamentous myosin is in equilibrium with a hypothetical first step in the propagation reaction. The problem of a length-dependent equilibrium constant for the propagation reaction can thus be avoided. The actual $\Delta\bar{v}$ value of 240 cm^3/mol of monomer incorporated into filament can be set between limits, a factor of two apart, one in which the non-filamentous myosin is present only as dimer and the other in which it is present only as monomer.

The Squire model for the structure of the thick filament showing a 3-fold rotation symmetry seems to have gained general acceptance (Squire, 1973). Squire, however, favoured an incomplete packing array for some distance to either side of the bare-zone. In the present set of experiments, no discontinuities were seen in the physical behaviour of the filaments as they were shortened over the area where incomplete packing was expected. This result lends support to the structural evidence from antibody staining experiments in which an apparently full cross-bridge array was seen to either side of the bare-zone (Craig & Offer, 1976). It also emphasizes the fact that the structure of the filament must be built up by a uniform sequential addition of subunit.

The experiments described established conditions of salt and pH under which the filament con-centration remains constant as they sequentially dissociate towards the bare-zone with increasing pressure. The mean length of the filaments at a particular pressure can be obtained from turbidity measured as a fraction of the turbidity signal for the full 630 nm filament at atmospheric pressure. All these findings form the essential basis for the interpretation of kinetic studies presented in the following paper (Davis, 1981).

References

Berne, B. J. (1974) *J. Mol. Biol.* **89**, 755–758

Craig, R. & Offer, G. (1976) *J. Mol. Biol.* **102**, 325–332

Davis, J. S. (1981) *Biochem. J.* **197**, 309–314

Davis, J. S. & Gutfreund, H. (1976) *FEBS Lett.* **72**, 199–207

Davis, J. S., Buck, J. & Greene, E. P. (1981) *FEBS Lett.* in the press

Huxley, H. E. (1963) *J. Mol. Biol.* **7**, 281–308

Josephs, R. & Harrington, W. F. (1966) *Biochemistry* **5**, 3474–3487

Josephs, R. & Harrington, W. F. (1968) *Biochemistry* **7**, 2834–2847

Morimoto, K. & Harrington, W. F. (1973) *J. Mol. Biol.* **77**, 165–175

Richards, E. G., Chung, C. S., Menzel, D. B. & Olcott, H. S. (1967) *Biochemistry* **6**, 528–540

Squire, J. M. (1973) *J. Mol. Biol.* **77**, 291–323

Starr, R. & Offer, G. (1971) *FEBS Lett.* **15**, 40–44