Synthetic Myosin Filaments

Abstract. *A stable preparation of myosin filaments was formed in a medium at pH 8.0. The filament length varied from 0.2 to 0.5 micron. Most of the material sedimented at 21S, but there was a minor peak (due to monomer) at 6.8S. The filaments did not taper and had large bulbous irregularities at the ends.*

In an elegant investigation of muscle filaments, Huxley (*1*) described synthetic myosin filaments produced by lowering the ionic strength of the solution of myosin to 0.1 to 0.2 at *p*H 7.0. We now describe synthetic myosin filaments produced in a medium at *p*H 8.0.

Myosin was extracted from rabbit muscle, dissolved in 0.6*M* KCl (*2*), and cleared of actomyosin by dilution to an ionic strength of 0.3 and centrifugation (*3*). The procedure of clearing of actomyosin was repeated. The purified myosin dissolved in 0.6*M* KCl gave a single very sharp boundary in the ultracentrifuge. It was stored in 50 percent glycerol at −20°C. Samples of this stock solution were precipitated by dilution to an ionic strength of 0.05, and the precipitate was washed in 0.05*M* KCl and centrifuged. The pellet was then suspended and homogenized in 0.08*M* KCl and 0.025*M* tris buffer (*p*H 8.0). It appeared to go into "solution" and only a negligible amount precipitated on centrifugation at 30,000*g* for 15 minutes. The supernatant showed a marked Tyndall effect.

On ultracentrifugation there was a major sharp peak sedimenting at 21*S*; there was also a minor peak at 6.8*S* (Fig. 1). Synthetic filaments, which were observed electron microscopically (Fig. 2), would account for the major peak; the minor peak is presumably due to the presence of small amounts of myosin monomers. The preparations were relatively stable. Only minute amounts of precipitation occurred at 2- to 4-day intervals, and the supernatant showed no significant changes at 17 days. After 8 weeks, one preparation showed an increase in the concentration of the slower-moving component, and the sedimentation coefficient of the fast component increased to 53*S*. It is possible that an equilibrium exists between the two states of the myosin.

The filaments were fairly uniform,

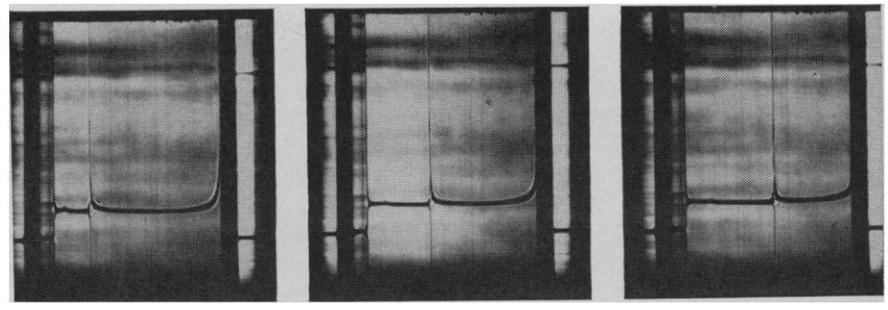

Fig. 1. Sedimentation patterns of myosin in 0.08*M* KCl, tris buffer 0.025*M*, *p*H 8.0. Protein concentration was 2.4 mg/ml. Examples of photographs (from left to right) taken at 6, 12, and 18 minutes after maximum speed of 59,780 rev/min was reached. The temperature was 21°C. The sedimentation coefficient of the peak is 21*S* and that of the minor one is 6.8*S* (corrected to 20°C).

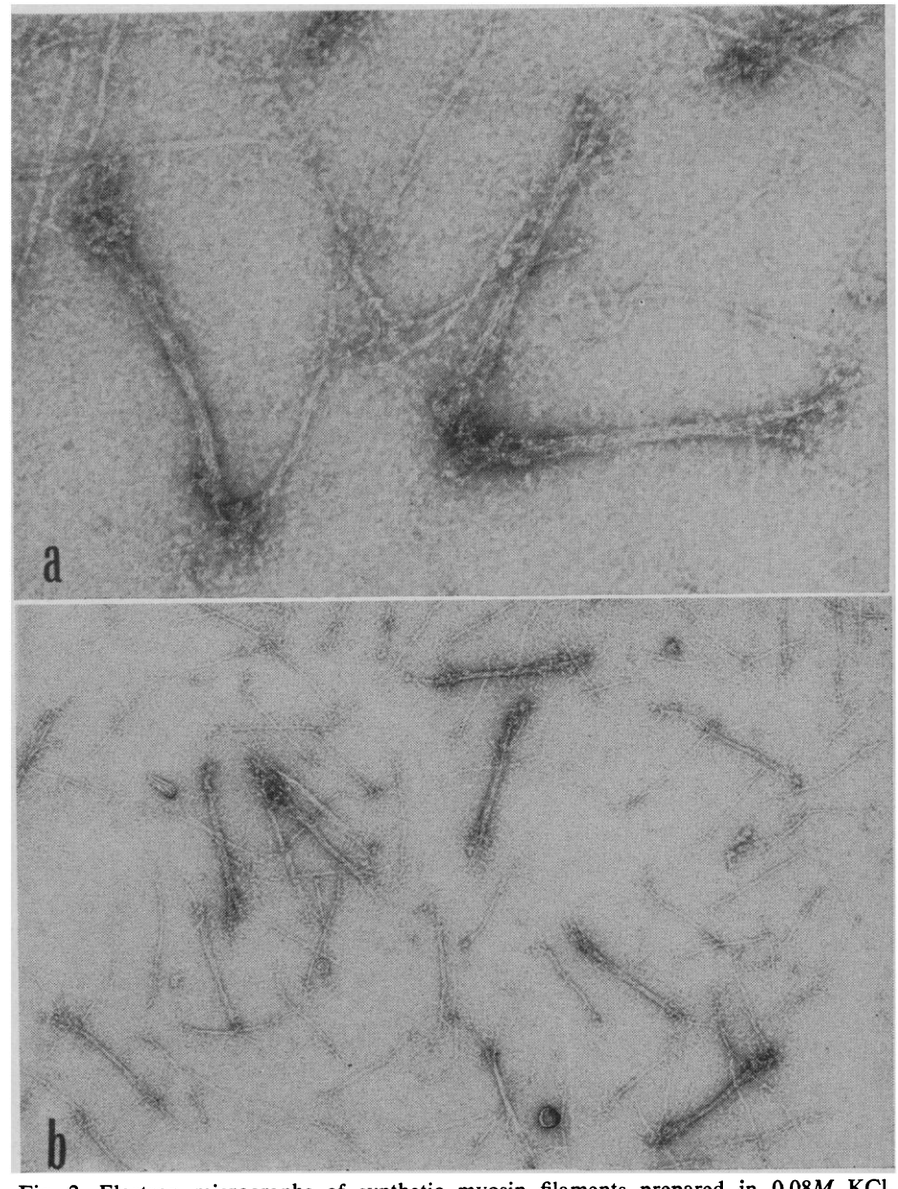

Fig. 2. Electron micrographs of synthetic myosin filaments prepared in 0.08*M* KCl, tris buffer 0.025*M*, *p*H 8.0. The preparation was negatively stained (*1*). Philips EM 200 with a 20-μ platinum objective aperture. (*a*) At × 132,200 there are large bulbous irregularities at the ends. (*b*) At × 48,185, the filaments do not appear to taper and the irregularities are clearly discernible.

varying from 0.2 to 0.5 μ (0.4 μ average) in length and 60 to 120 Å in diameter at the center. The end portions had large irregular projections similar to some examples illustrated by Huxley. The filaments, however, were not spindle-shaped, and the large irregularities were clearly discernible at low magnification (Fig. 2*b*). This is a consistently reproducible preparation, and it appears that the particular *p*H of the medium is a factor determining the size and characteristics of these filaments.

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References and Notes

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4. Supported by NIH grant GM-10383 and NSF grant G5836.

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Liquid Water in Frozen Tissue: Study by Nuclear Magnetic Resonance

Abstract. *Nuclear magnetic resonance (NMR) spectroscopy was used to examine the behavior and extent of liquid water in postrigor-frozen tissue of cod at temperatures below 0°C. A liquid-water phase persists in the tissue down to about −70°C; the extent of the phase decreases rapidly between 0° and −10°C and slowly at lower temperatures. That the NMR absorption peak of the liquid water increases in width, with decreasing temperature, suggests loss of mobility or structuring of the phase. A technique for introducing geometrically uniform cores of muscle into the probe of the high-resolution spectrometer permits quantitative determinations of liquid water.*

Information on the behavior of water in frozen tissue at temperatures below 0°C relates to understanding of the mechanisms of protein denaturation and tissue degradation during frozen storage, and may throw light on the complex role of water in the living cell. We now report results of nuclear magnetic resonance (NMR) spectroscopic examination (*1*) of postrigor-frozen muscle of cod (*Gadus morhua*).

Quantitative determinations of liquid water were made on geometrically uniform cores of tissue cut from fillets of dorsal muscle of fresh cod with a modified 5-mm Varian A-60 sample tube. The tube was modified (Fig. 1) by cutting-off the sealed end and by grinding a scalloped cutting edge on that end. When screwed into the fish fillet, the tube cut a core of tissue that remained within the tube; the lower end of the tube was then closed with a 5-mm plug of window putty. The tube, with core sample (at least 4 cm long), was inserted in the Varian A-60 probe to a depth of 3 mm above the temperature sensor, and the sample was rotated at 20 to 40 rev sec^{-1}. Spectra consisted of a single, broad, symmetrical peak whose position downfield of tetramethylsilane coincided with that of distilled water at the same temperature. If air bubbles or other gross inhomogeneities were introduced with the sample, spinning side bands appeared and peak shape was distorted. The core-cutting sample tube reduces "filling factor" variability, which hinders quantitative NMR measurements on nonliquid materials (*2*). Liquid-water content of tissue was obtained from NMR absorption-peak area by comparing the tissue-water peak area with the area of the water peak of a 23.3 percent NaCl (eutectic) solution at the same temperature. Weight-percentage of tissue water was calculated as follows:

$$\text{Liquid water (percent)} = (A_{tissue}/A_{eutectic})\ 0.766\ (\rho_{eutectic}/\rho_{tissue})$$

where A is the peak area (of tissue or eutectic solution), 0.766 is the weight in grams of liquid water per gram of eutectic solution, and ρ is the density (of tissue or eutectic solution) at 20°C. The validity of the calculation is shown by Fig. 2, which serves as a calibration chart and which plots known water contents of saline solutions of different concentrations (0.9, 3.0, 8.0, 16, and 23.3 percent NaCl) against the above function of peak-area ratio at temperatures between 38° and −21°C. Between 0° and −21.1°C the noneutectic solutions deposited varying amounts of pure ice. Their liquid-water contents depended on temperature and initial concentration and were computed from the NaCl-ice phase diagram; the points below 20 percent water in Fig. 2 represent such ice-salt solution mix-

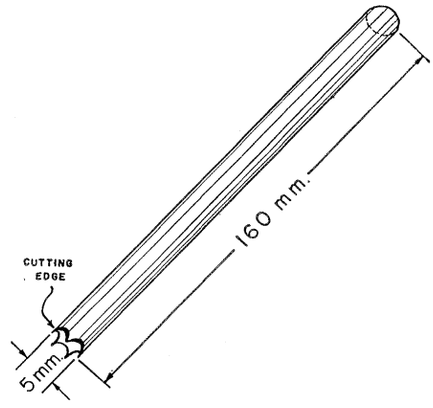

Fig. 1. Core-cutting sampling tube for NMR spectroscopy.

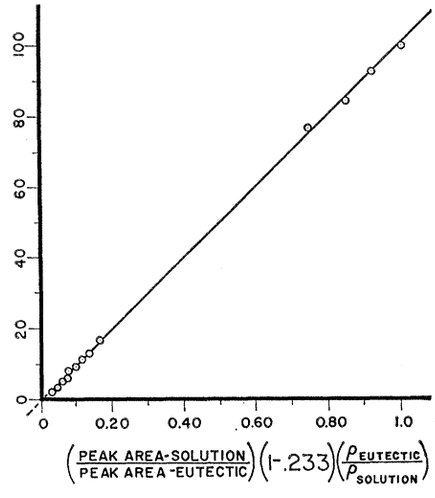

Fig. 2. Calibration curve: NMR peak-area ratio as a function of percentage by weight of liquid water (ordinate).

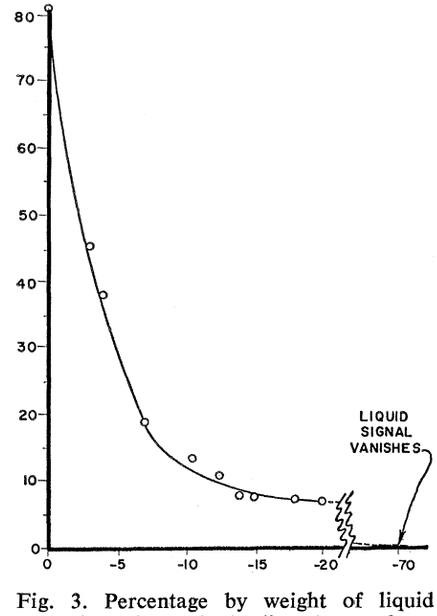

Fig. 3. Percentage by weight of liquid water in cod muscle (ordinate) as a function of temperature (Celsius, abscissa).