LASER RAMAN LIGHT-SCATTERING OBSERVATIONS OF CONFORMATIONAL CHANGES IN MYOSIN INDUCED BY INORGANIC SALTS

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ABSTRACT The Raman spectra of aqueous solutions of myosin and mixtures of myosin in solutions of the salts $CaCl_2$, $MgCl_2$, and LiBr have been taken. The spectrum of the solvent background has been subtracted by means of a computer, leaving only the Raman peaks of the protein. From an analysis of the Raman bands in the regions at 900, 940, 1,240–1,300, and 1,650–1,670 cm^{-1}, it seems likely that $CaCl_2$ effects an α-to β-transition in myosin, probably owing to the interaction of the Ca^{2+} ion, LiBr appears to denature the protein leading to increased random coil structure, and $MgCl_2$ appears to have an effect intermediate between the two other salts. These results are reported for concentrations as low as 10^{-5} M of $CaCl_2$ and $MgCl_2$.

This investigation indicates the usefulness of the Raman light-scattering technique for the study of protein conformational changes.

INTRODUCTION

Raman spectroscopy is now well established as a useful means of estimating, at least qualitatively, the secondary structure of proteins and polypeptides (1–9). The effect of salts on polypeptide conformation in aqueous solution has recently been studied by this technique (8). An attempt to obtain quantitative measurements of the secondary structure of proteins has also been made recently (9), but we will not attempt to do this here. The feasibility of such a study on myosin has already been discussed by Carew et al. (10) in a pioneering paper in which the Raman bands of myosin were assigned.

Myosin performs three important physiological functions: (*a*) formation of filaments through aggregation; (*b*) hydrolysis of ATP to Pi and ADP (ATPase); and (*c*) combination with actin. There is also evidence concerning a fourth function, related to the three stated but of more fundamental importance, namely: the provision of a driving force for muscle contraction resulting from conformational changes at the position of its intersection with actin (11–13).

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The ATPase activity of the myosin-actin complex is controlled by the presence of the inorganic ions Ca^{2+} and Mg^{2+}. For example, Szent-Györgyi (14) observed 30 years ago that the addition of ATP to a suspension of actomyosin at low ionic strength caused sudden precipitation ("super-precipitation") in the presence of Mg^{2+}. Indeed, Mg^{2+}-ATP is now thought to be the physiological substrate for actomyosin ATPase activity (15). In contrast, the role of Ca^{2+} ion is one of regulation; it permits interaction of actin and myosin. In vertebrate striated muscle, this occurs as follows. Excitation of the plasma membrane of muscle induces discharge of Ca^{2+} from the sarcoplasmic reticulum, which then diffuses to the contractile system to be bound to the troponin C component and is thus accepted into a complex composed of tropomyosin and three components of troponin located on the thin filament. When Ca^{2+} binds with troponin, a structural change is transmitted to the tropomyosin complexed with the troponin, and this structural change is further transmitted from tropomyosin to the F-actin with which it is combined. Myosin then binds with actin; actin-activated Mg^{2+}-dependent actomyosin ATPase occurs, and contraction ensues. During muscle relaxation, Ca^{2+} is removed from the contractile system by absorption back into the sarcoplasmic reticulum; the binding between actin and myosin is inhibited, and the ATPase activity of myosin, now unmodified by actin, is greatly reduced.

In this contractile process, the reaction of Ca^{2+} with myosin is probably not as important as the reaction of this ion with tropomyosin and troponin, since it has been established that vertebrate striated myosin does not bind reversibly in the range of physiological Ca^{2+} concentrations (16,17). However, calcium ions, at concentrations about 10 times higher than the range of calcium control required to activate muscle, do induce changes in vertebrate striated myosin (18), and it is difficult to exclude myosin control in vertebrate muscles based on in vitro evidence (13). In molluscan muscle, the factors that regulate contraction by interaction with Ca^{2+} are associated with myosin (18,19). Thus, in molluscan muscle, and in many other invertebrate muscles, many of which contain both actin- and myosin-linked control systems (20), Ca^{2+} ions do interact directly with myosin.

As both the Ca^{2+} and Mg^{2+} ions are present with the contractile apparatus in vivo, their presence in a study of conformational changes in myosin is apparent; but the inclusion of a third ion, Li^{+}, requires some explanation. Tonomura et al. (21) reported the effect of LiBr on the optical rotatory dispersion (ORD) of myosin A and expressed their results in terms of the equation of Moffitt and Yang (22). Their conclusion, that LiBr (and also LiCl, KI, and KSCN) induces a helix-to-coil transition in myosin, is based on the two-compartment model for protein conformation offered by this equation of helix and nonhelix. Although the helical content of a protein may be calculated by this model (23), the conclusion that the absence of helical content indicates the presence of a random coil conformation is not necessarily valid, and positive identification of the nonhelical content is required (24–26). As laser Raman spectroscopy offers a probe for α-helix, β-sheet, and random coil configurations in protein structure (1–9), the effect of LiBr was reinvestigated by the inelastic light-scattering technique.

METHODS

The myosin samples were prepared from rabbit skeletal muscle using either the technique described in Arakawa et al. (27) (giving a 2% solution) or the technique described in Hammond and Goll (28) (giving a 3.7% solution). Since ammonium sulphate was used to salt out the protein from a chromatographic column in the latter procedure (28), a sharp band at 980 cm^{-1} owing to SO_4^{-2} appears in the Raman spectrum for the 3.7% samples. Chen and Lord (29) have recently shown how this band may be taken as an indicator of ion binding and conformational change, and so it was used in this study. All 3.7% samples also contained 500 mM KCl, 1 mM $KHCO_3$, 0.1 mM DTE (dithioerythritol), and 1 mM NaN_3. All 2% samples contained 500 mM KCl and 1 mM NaN_3. The Raman spectra for myosin prepared by both procedures were, however, similar.

The Raman spectra were taken using the 514.5-mm line of an argon laser as the exciting source. The Raman apparatus in one series of experiments consisted of a Spex 1301 double monochromator equipped with a stepping motor (Spex Industries, Inc., Metuchen, N.J.). Both the stepping motor and the output of the photomultiplier were connected to a Varian 620/i dedicated computer Varian Data Machines, Irvine, Calif.). The data acquisition is performed by counting the photoelectron pulses emitted by the photomultiplier and subsequently amplified and standardized by means of an Ortec amplifier-discriminator (Ortec Inc., Oak Ridge, Tenn.). To suppress errors due to slow drifts in the background owing to extraneous luminescence, such as impurity fluorescence, a series of scans was taken beginning at 550 cm^{-1} from the incident light frequency and stepping one wave number each second to 1,750 cm^{-1}. Generally about four to eight scans were taken and added together. The Raman spectra of the corresponding salt solutions were also taken and computer subtracted from the myosin spectrum. This allowed the analysis of the amide I band without the use of deuteration to remove the conflicting H_2O band. It seems likely that this procedure will permit the use of the amide I band and the amide III band for the quantitative determination of the secondary structure of proteins by a method similar to that of Lippert et al. (9) without the problems involved in the deuteration procedure.

In a second series of experiments, the Raman apparatus consisted of an argon laser and a Cary 81 double monochromator (Varian Associates, Instruments Div., Palo Alto, Calif.) adapted for photodetection, which was tuned to the 5145 Å line.

RESULTS AND DISCUSSION

The Raman spectra of the myosin in aqueous solution and in salt solutions are shown in Fig. 1. In each spectrum, the solvent Raman spectrum has been subtracted by means of a computer, as described previously. After water subtraction, we expected that the troughs in the spectrum would correspond to a number of photon counts which is very low compared to the number of counts at the peaks. However, we found that this is not the case. Superimposed upon the Raman spectrum is a broad luminescence of unknown origin. The criterion for the exact amount of water subtraction has been taken as a flat base line in the region 1,750- to 1,800 cm^{-1}. The HOH-bending vibration at 1,660 cm^{-1} extends to this region, and there is no competition from any of the protein bands, including the amide I. Thus, if the solvent spectrum is too intense, one obtains a negative trough in this region, whereas if the solvent water spectrum is too weak, what appears as a positive extension of the amide I band is obtained. Some slight remnants of these effects can be seen in Fig. 1. The most accurate solvent sub-

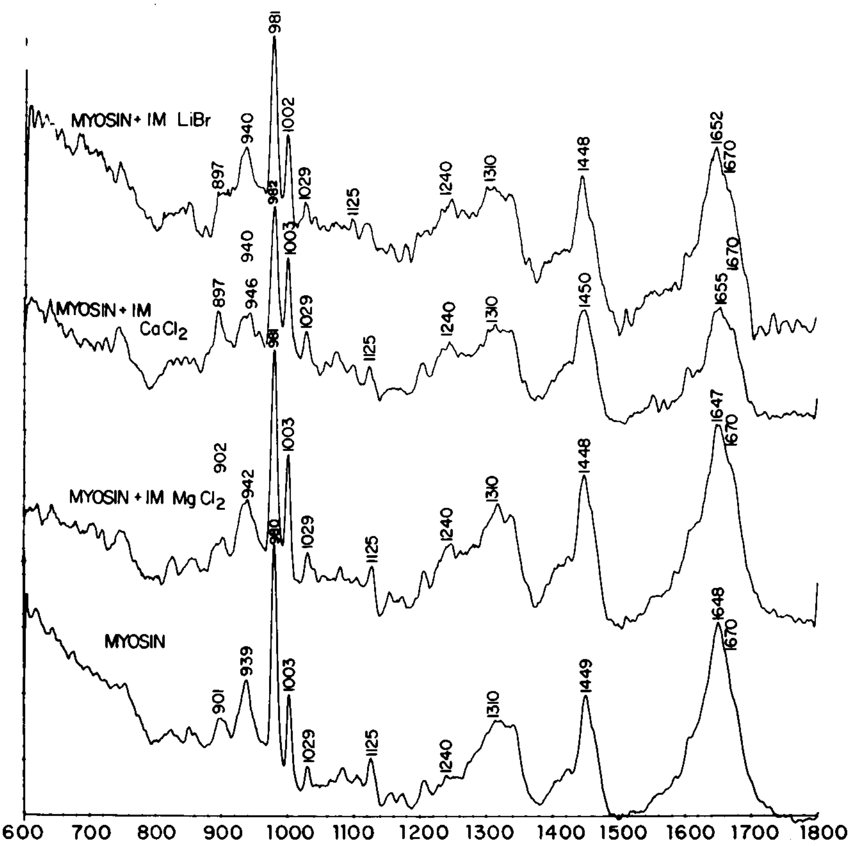

FIGURE 1 Solvent subtracted Raman spectra of aqueous solutions of 3.7% myosin.

traction was obtained in the myosin + $CaCl_2$ spectrum, where the base line is completely flat in the 1,750- to 1,800-cm^{-1} region. The myosin + $MgCl_2$ spectrum still has a slight HOH spectrum since in this region the Raman intensity is still decreasing to the right. The myosin + LiBr is noisy, but appears to increase slightly to the right in the 1,750- to 1,800-cm^{-1} region, showing that perhaps a little too much solvent spectrum was subtracted. However, in each case, the amount of solvent spectrum remaining is extremely small and does not affect the measured frequencies or intensities of the amide I band more than a few percent. This has been established by making the measurements with slightly too little and too large amounts of water being subtracted. In each case, the measured peak heights were the same within experimental error.

Thus, instead of using the actual net number of photoelectron counts at a given peak height or the total number of counts under a given Raman band, we used the method of estimating peak intensities suggested by Lippert et al. (9) and measuring the heights from a line drawn between two troughs on either side of the peak. Since this procedure is already described elsewhere (9), we will not discuss it here.

The intensity of each of the conformationally sensitive bands of interest are listed in

TABLE I

INTENSITIES OF SENSITIVE BANDS IN 3.7% SOLUTION

	Myosin		Myosin + 1 M $CaCl_2$	Myosin + 1 M $MgCl_2$	Myosin + 1 M LiBr
	cm^{-1}				
C—C residue stretch	902	0.30	0.60	0.19	0.036
C—C residue stretch	940	0.75	0.37	0.48	0.68
SO_4^{-2}	980	2.6	1.7	1.9	2.2
C—C ring stretch	1,004	1.0	1.0	1.0	1.0
Amide III β-chain and random coil	1,240	0.40	0.63	0.58	0.84
Amide III α-helix, CH bend, CH twist	1,310	1.0	0.88	0.88	1.2
CH_3 antisymmetric, CH_2, CH bend	1,450	1.5	1.3	1.5	1.6
	1,645	2.3	1.3	1.9	2.4
Amide I α-helix	1,650	2.5	1.3	1.9	2.5
	1,655	2.3	1.4	1.9	2.5
β-chain, random coil	1,670	1.6	1.1	1.5	2.0

Table I. The listed intensities are the ratio of the peak heights at the given frequency to the peak height at 1,003 cm^{-1}. This latter band is due to phenylalanine, and it is assumed not to change with conformation. Another band also considered to be independent of conformation for any given protein is the CH_2-bending mode at 1,450 cm^{-1}. As can be seen from Table I, the ratio of this band to that at 1,003 cm^{-1} remains remarkably constant.

To determine the changes in conformation induced by the salts, we have tabulated the ratio of the band heights at 1,300 cm^{-1}/1,240 cm^{-1} for the amide III region and the ratio at 1,650 cm^{-1}/1,670 cm^{-1} for the amide I region. Although these ratios would not be meaningful from one protein to another, for the same protein they give the change in the amount of α-helix. The larger the number, the larger the amount of α-helix. These ratios are tabulated in Table II, and it is immediately obvious that in each case the salt at a concentration of 1 M greatly reduces the amount of α-helical content of the myosin. The effect is present at far lower concentrations, and Fig. 2 demonstrates the concentration dependence of these changes for $CaCl_2$ and $MgCl_2$.

The question of whether the decrease in the α-helical content is due to an increase in the amount of β-structure or random coil requires a very careful analysis of these

TABLE II

INTENSITY RATIOS INDICATING RATIOS OF α TO (β + RANDOM COIL) CONFORMATIONS (3.7% SOLUTION)

	Myosin	Myosin + 1 M $CaCl_2$	Myosin + 1 M $MgCl_2$	Myosin + 1 M LiBr
$I_{1,310}/I_{1,240}$	2.5	1.4	1.5	1.4
$I_{1,650}/I_{1,670}$	1.6	1.2	1.3	1.2

bands, which is still underway using known proteins as models. This is because the β- and the random coil bands overlap so that the intensity in the amide III region at 1,240 and at 1,670 cm^{-1} in the amide I region are due to contributions from both random coil and β-structure (4, 9). However, we propose another way of looking at these Raman data using the C—C stretching vibrations at ~900 and 940 cm^{-1}. These vibrations are known both from work on model polypeptides and proteins to be sensitive

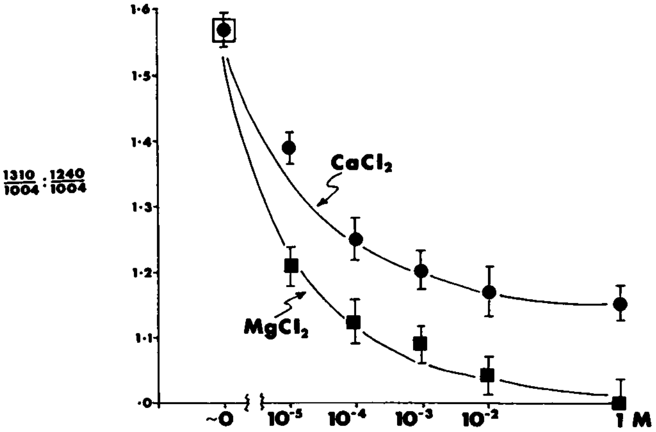

FIGURE 2

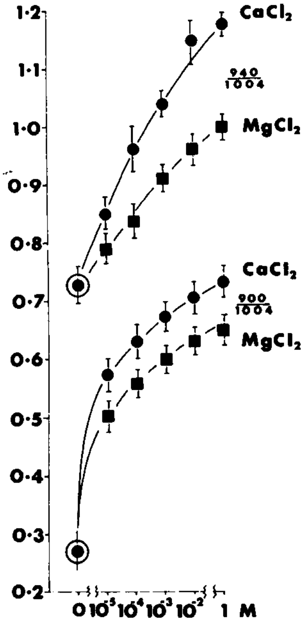

FIGURE 3

FIGURE 2 Concentration dependent changes in the amide III band ratios of the myosin bands 1,240 and 1,310 cm^{-1} normalized to the band 1,004 cm^{-1} induced by $CaCl_2$ (●) and $MgCl_2$ (■) for aqueous solutions of 2.3% and at salt concentrations of 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, and 1 M.

FIGURE 3 Concentration-dependent changes in the ratios of the myosin bands 940 cm^{-1} (upper pair) and 904 cm^{-1} (lower pair) normalized to the band at 1,004 cm^{-1} induced by $CaCl_2$ (●) and $MgCl_2$ (■) for aqueous solutions of 2.3% and at salt concentrations of 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, and 1 M.

to the conformation of the polypeptide backbone (4–7). Generally, upon denaturation the bands broaden and weaken in intensity (6, 7). However, in going from α- to β-structures in poly-L-lysine, there is actually an increase in the 900-cm^{-1} band and a change in the location of the 940-cm^{-1} band (4).

In the case of changes in the intensity of the 900-cm^{-1} band: Fig. 1 shows that there is a considerable increase in the band at 900 cm^{-1} when $CaCl_2$ is the added salt, but a decrease when $MgCl_2$ or LiBr is the added salt. The poly-L-lysine spectra show a change similar to this in going from α- to the β-form. This can be seen in Fig. 1 of ref. 4. On the basis of this evidence we therefore assign the increase in the 900-cm^{-1} band of myosin after $CaCl_2$ addition to the formation of increased β-structure.

In the case of changes in wave number of the 940-cm^{-1} band: There is a discrepancy between the results obtained from the 3.7% samples and those obtained from the 2.0% samples. With the 3.7% samples and at a salt concentration of 1 M, the 940-cm^{-1} band diminished for all three salts (Table I). On the other hand, with the 2.0% samples, the addition of $CaCl_2$ and $MgCl_2$ increased the 940-cm^{-1} band (Fig. 3). This discrepancy may be accounted for by the observation of Yu et al. (ref. 4, page 2166) that the C—C stretch of poly-L-lysine in H_2O shifts from 945 cm^{-1} in the α-form to ~ 1,002 cm^{-1} in the β-form. We assume, therefore, that the diminishing of the 940-cm^{-1} band in the 3.7% samples indicates a shift of increasing vibrational modes to the 1,002-cm^{-1} band, rather than a diminishing of the C—C stretch. This increase in the C—C stretch vibrations remains at 940 cm^{-1} in the 2.0% samples as a concentration effect.

An increase in β-structure would necessarily mean an increase in interchain hydrogen bonding. Since such binding could occur between different myosin molecules, our observations are compatible with those of Margossian and Lowey (30), who have reported that 0.1 mM Ca^{2+} induced a reversible aggregation of myosin heads, as well as a diminished binding of myosin to actin, and with Morimoto and Harrington (31), who found changes in sedimentation velocity and viscosity measurements attributable to conformational transitions within the thick filament when the Ca^{2+} concentration is altered from 10^{-7} to 10^{-5} M in an ionic medium comparable to that of living muscle. These authors and others (32) also report that the structural changes observed in the thick filament very likely result from direct association of Ca^{2+} with the DTNB light chains of myosin.[1] Furthermore, as it is reported that the infrared spectra of films of both light and heavy meromyosin indicate conformations and conformational changes similar to those observed in myosin (33), it is interesting that β-structure was observed in both heavy and light meromyosin with the films were prepared under acidic conditions (33).

The presence of 500 mM KCl in all samples may have influenced the result obtained, since Ca^{2+} has a maximum quenching effect on the fluorescence of a bound probe,

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[1] DTNB light chains are the light chains removed from myosin by treatment with 5,5′-dithiobis (2-nitrobenzoic acid).

1-anilino-8-naphthalenesulfonate (ANS), in KCl (34). In the presence of K^+, Ca^{2+} induces a larger change in the structure of the ANS-binding site of myosin than in LiCl or NaCl (34).

The changes in the sulphate marker are rather difficult to interpret. The SO_4^{-2} band appears to decrease with added salt. If one follows the previous interpretation (29) of this effect, one must conclude that the SO_4^{-2} ion is more in the HSO_4^- form when salt is added. One possibility is that the new conformation of the protein allows more SO_4^{-2} binding to the protein.

As a comparison experiment for this study of myosin, the effects of changes in concentration in Ca^{2+} and Mg^{2+} were investigated in the case of another muscle protein, tropomyosin. The results did not demonstrate a change. Therefore, the salt-induced changes in myosin structure reported here do not stem from the nonspecific binding of metal ions to a polypeptide chain; i.e., they do not reflect nonspecific changes in α-helical content that would be found in any protein possessing α-helical structure.

Nevertheless, to detect the exact site of salt binding, further studies must be separately made on the myosin rod and subfragment I. The thrust of the present study thus lies in indicating the usefulness of the Raman light-scattering technique in investigating conformational changes in proteins.

The demonstration in the present paper of the direct effect of Ca^{2+} on myosin is probably not to be interpreted as indicating the major influence on myosin in muscle contraction. The major effect of Ca^{2+} on myosin is more probably in the regulation of the level of phosphorylation of myosin (35, 36) through activation of a Ca^{2+}-sensitive protein kinase (37). The direct effect of Ca^{2+} on myosin demonstrated here and elsewhere (13, 18) may be regarded as a subsidiary effect at the present time.

CONCLUSIONS

The Raman spectra of aqueous solutions of myosin change with salt concentrations as low as 10^{-5} M. In all cases, the amount of α-helix is reduced whereas the effect on the C—C stretching vibration is such that one can conclude that there is an increase in the β-structure when Ca^{2+} ions are added. The investigation indicates the usefulness of the Raman light-scattering technique for the study of protein conformational changes.

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REFERENCES

1. Frushour, B. G., and J. L. Koenig. 1975. Raman spectroscopy of proteins. *In Advances in Infrared and Raman Spectroscopy.* R. J. H. Clark, and R. E. Hester, editors. Heyden, London. **1**:35–97.
2. Peticolas, W. L. 1975. Applications of Raman spectroscopy to biological macromolecules. *Biochimie (Paris).* **57**:417–428.

3. Yu, N-T. 1977. Raman spectroscopy: a conformational probe in biochemistry. *CRC Crit. Rev. Biochem.* **4**:229-280.
4. Yu, T.-J., J. L. Lippert, and W. L. Peticolas. 1973. Laser Raman studies of conformational variations of poly-L-lysine. *Biopolymers.* **12**:2161-2176.
5. Chen, M. C., R. C. Lord, and R. Mendelsohn. 1973. Laser-excited Raman spectroscopy of biomolecules. IV. Thermal denaturation of aqueous lysozyme. *Biochim. Biophys. Acta.* **328**:252-260.
6. Chen, M. C., R. C. Lord, and R. Mendelsohn. 1974. Laser-excited Raman spectroscopy of biomolecules. V. Conformational changes associated with the chemical denaturation of lysozyme. *J. Am. Chem. Soc.* **96**:3038-3042.
7. Kuck, J. F. R., Jr., E. J. East, and N-T. Yu. 1976. Prevalence of α-helical form in avial lens protein. *Exp. Eye Res.* **23**:9-14.
8. Painter, P. C., and J. L. Koenig. 1976. The solution conformation of poly(L-lysine). A Raman and infrared spectroscopic study. *Biopolymers.* **15**:229-240.
9. Lippert, J. L., D. Tyminski, and P. Desmeules. 1976. Determination of the secondary structure of proteins by laser Raman spectroscopy. *J. Am. Chem. Soc.* **98**:7075-7080.
10. Carew, E. B., I. M. Asher, and H. E. Stanley. 1975. Laser Raman spectroscopy—a new probe of myosin substructure. *Science (Wash. D.C.).* **188**:933-936.
11. Huxley, H. E. 1969. The mechanism of muscle contraction. *Science (Wash. D.C.).* **164**:1356-1366.
12. Tonomura, Y. 1973. *Muscle Proteins, Muscle Contraction and Cation Transport.* University Park Press, Baltimore. 433 pp.
13. Szent-Györgyi, A. 1975. Calcium regulation of muscle contraction. *Biophys. J.* **15**:707-723.
14. Szent-Györgyi, A. 1947. *Chemistry of Muscle Contraction.* 1st edition. Academic Press, Inc., New York. 150 pp.
15. Mandelkow, E-M., and E. Mandelkow. 1973. Fluorimetric studies on the influence of metal ions and chelators on the interaction between myosin and ATP. *FEBS Lett. (Fed. Eur. Biochem. Soc.)* **33**:161-166.
16. Ebashi, S., and M. Endo. 1968. Calcium ion and muscle contraction. *Prog. Biophys. Mol. Biol.* **18**: 123-183.
17. Bremel, R. D., and A. Weber. 1975. Calcium binding to rabbit skeletal myosin under physiological conditions. *Biochim. Biophys. Acta.* **376**:366-374.
18. Kendrick-Jones, J., E. M. Szentkiralyi, and Szent-Györgyi, A. G. 1976. Regulatory light chains in myosin. *J. Mol. Biol.* **104**:747-776.
19. Kendrick-Jones, J., W. Lehman, and A. G. Szent-Györgyi. 1970. Regulation in molluscan muscles. *J. Mol. Biol.* **54**:313-326.
20. Lehman, W., and A. G. Szent-Györgyi. 1975. Regulation of muscle contraction: distribution of actin control and myosin control in animal kingdom. *J. Gen. Physiol.* **66**:1-30.
21. Tonomura, Y., K. Sekiya, and K. Imamura. 1962. The optical rotatory dispersion of myosin A. I. Effect of inorganic salt. *J. Biol. Chem.* **237**:3110-3115.
22. Moffitt, W., and J. J. Yang. 1956. The optical rotatory dispersion of simple polypeptides I. *Proc. Natl. Acad. Sci. U.S.A.* **42**:596-603.
23. Lowey, S., H. S. Slayter, A. G. Weeds, and H. Baker. 1969. Substructure of the myosin molecule. I. Subfragments of myosin by enzymic degradation. *J. Mol. Biol.* **42**:1-29.
24. Chen, Y-H., J. T. Yang, and K. H. Chau. 1974. Determination of the helix and β form of proteins in aqueous solution by circular dichroism. *Biochemistry.* **13**:3350-3359.
25. Chen, Y-H., J. T. Yang, and H. M. Martinez. 1972. Determination of the secondary structure of proteins by circular dichroism and optical rotatory dispersion. *Biochemistry.* **11**:4120-4131.
26. Baker, C. C., and I. Isenberg. 1976. An analysis of the circular dichroic spectra of proteins. *Biochemistry.* **15**:629-634.
27. Arakawa, N., R. M. Robson, and D. E. Goll. 1970. An improved method for the preparation of α-actinin from rabbit striated muscle. *Biochim. Biophys. Acta.* **200**:284-295.
28. Hammond, K. S., and D. E. Goll. 1975. Purification of insect myosin and α-actinin. *Biochem. J.* **151**: 189-192.
29. Chen, M. C., and R. C. Lord. 1976. Laser Raman spectroscopic studies of the thermal unfolding of ribonuclease A. *Biochemistry.* **15**:1889-1897.

30. MARGOSSIAN, S. S., and S. LOWEY. 1977. Calcium induced aggregation of myosin subfragments. *Biophys. J.* **17**:37a. (Abstr.)

31. MORIMOTO, K., and W. S. HARRINGTON. 1974. Evidence for structural changes in vertebrate thick filaments induced by calcium. *J. Mol. Biol.* **88**:693–709.

32. HOLT, J. C., and S. LOWEY. 1975. An immunological approach to the role of the low molecular weight subunits in myosin. II. Interaction of myosin and its subfragments with antibodies to the light chains. *Biochemistry.* **14**:4609–4620.

33. JACOBSON, A. L., V. VEJDOVSKY, and P. J. KRUEGER. 1974. Infrared spectra of light and heavy meromyosin. *Int. J. Biochem.* **5**:547–553.

34. CHEUNG, H. C., and R. COOKE. 1971. Effects of alkali ions on myosin conformation. *Biopolymers.* **10**: 523–529.

35. BÁRÁNY, K., and M. BÁRÁNY. 1977. Phosphorylation of the 18,000-Dalton light chain of myosin during a single tetanus of frog muscle. *J. Biol. Chem.* **252**:4752–4754.

36. GREENGARD, P. 1977. Phosphorylated proteins as physiological effectors. *Science (Wash. D.C.).* **199**: 146–152.

37. FREARSON, N., R. J. SOLARO, and S. V. PERRY. 1976. Changes in phosphorylation of P light chain of myosin in perfused rabbit heart. *Nature.* **264**:801–802.