Hydrodynamic and Optical Properties of Troponin A. Demonstration of a Conformational Change upon Binding Calcium Ion†

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ABSTRACT: Comparative spectral, sodium dodecyl sulfate–polyacrylamide gel, and sedimentation equilibrium data are presented for the Ca^{2+}-sensitizing factor, troponin A, prepared by either the Hartshorne–Mueller method or by DEAE-Sephadex A-25 column chromatography in 6 M urea. The Hartshorne–Mueller material appears to be 90–95% homogeneous with respect to size on sodium dodecyl sulfate–polyacrylamide gels and is probably contaminated with some nucleotide material in view of its high $E^{1\%}_{1\ cm,\ 260\ m\mu}$ value. The material prepared by Sephadex chromatography in 6 M urea appears to be completely homogeneous on sodium dodecyl sulfate–polyacrylamide gels and possesses an absorption spectrum typical for a protein with a high Phe:Tyr ratio. The minimum molecular weight of either preparation of troponin A is 22,200. In 50 mM Tris little aggregation occurs below free Ca^{2+} concentrations of 5×10^{-5} M. Above 10^{-4} M free Ca^{2+}, more extensive aggregation is obvious. Circular dichroism studies indicate that a conformational change corresponding to an increase in apparent α-helix occurs as total Ca^{2+} levels are raised from 10^{-6} to 5×10^{-4} M, or free Ca^{2+} levels are raised from 10^{-8} to 5×10^{-6} M. This conformational change is also reflected by an increase in sedimentation constant with no alteration in molecular weight, which implies a transformation to a more symmetric molecular shape.

The troponin system of skeletal muscle is composed of several proteins (Drabikowski *et al.*, 1971; Ebashi *et al.*, 1971; Greaser and Gergely, 1971; Murray and Kay, 1971). Two of these, the inhibitory factor and troponin A, appear to be essential to the regulation of the ATPase activity of actomyosin. Inhibitory factor, acting with tropomyosin, inhibits the ATPase activity of synthetic actomyosin in the presence or in the absence of Ca^{2+}. Addition of troponin A (Ca^{2+}-sensitizing factor) to the system has little effect in the absence of Ca^{2+}, but in the presence of Ca^{2+} it overcomes the effect of inhibitory factor. At least two other factors exist (Greaser and Gergely, 1971) but their functions are not obvious.

Troponin A has the ability to bind Ca^{2+} very strongly (Fuchs, 1971). The binding constant is about 10^6 M^{-1} (Ebashi *et al.*, 1968; Hartshorne and Pyun, 1971). This binding appears to facilitate a conformational change somewhere in the contractile apparatus (Han and Benson, 1970), probably in the troponin A moiety itself. Ahmed *et al.* (1970) have found that tropocalcin, a protein obtained from troponin by Ca^{2+} precipitation, exhibits a coil to helix transition upon increasing Ca^{2+} levels to 10^{-5} or 10^{-4} M.

Basically two approaches have been useful in the preparation of troponin A. That of Hartshorne and Mueller (1968) involves a precipitation at high ionic strength and low pH. The other approach involves ion exchange chromatography in 4–6 M urea (Drabikowski *et al.*, 1971; Schaub *et al.*, 1972). This paper presents absorption and circular dichroic spectra, and sedimentation equilibrium data on troponin A prepared by one or both of these methods. Direct evidence from circular dichroism and sedimentation velocity for a conformational change in troponin A, due to an increase in Ca^{2+} concentration, is presented.

Materials and Methods

Isolation and Purification of Troponin A. Troponin A was prepared from rabbit skeletal muscle as described previously (Murray and Kay, 1971) using the method of Hartshorne and Mueller (1968), except that the pH 1 precipitation step was carried out three times.

Crude troponin A was also prepared as described previously (Murray and Kay, 1971) with one modification. The preparation of muscle powder using the Bailey sequence of organic solvents was replaced by the treatment of wet muscle residue as indicated by Ebashi *et al.* (1971). The resulting crude troponin A was chromatographed on a column of DEAE-Sephadex A-25 at 4° in a solvent system consisting of 50 mM Tris buffer at pH 8.0, 6 M urea, and 0.5 mM dithiothreitol. The protein was eluted with the same buffer containing a linear gradient to 0.6 M KCl.

Sodium Dodecyl Sulfate–Polyacrylamide Gels. Sodium dodecyl sulfate–polyacrylamide gels were prepared using methodology similar to that of Shapiro *et al.* (1967). Protein samples were heated at 80° for 10 min in 1% sodium dodecyl sulfate, 1 mM dithiothreitol, electrophoresed on 10% gels for 3 hr at 6.5 mA/tube, stained with Coomassie Blue for 3–4 hr, and destained with a Canalco horizontal destainer. Gels were scanned in a Gilford 240 spectrophotometer at 550 mμ.

Amino Acid Analysis. Amino acid analysis was carried out on a Beckman 120B amino acid analyzer. Results are averages of duplicate hydrolyses at 24, 48, 72, and 96 hr.

Protein Concentration and Absorption Spectra. Protein concentrations were determined by synthetic boundary runs on a Beckman Model E ultracentrifuge with interference optics. A dn/dc value of 0.185 was assumed. Protein samples were dialyzed for 2 days in the cold prior to concentration determinations. Absorption spectra were obtained using a Cary 15 spectrophotometer.

Ultracentrifugation. Molecular weight studies were carried

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out on a Beckman Model E ultracentrifuge employing the Rayleigh interference optical system. For low-speed experiments the methodology of Richards *et al.* (1968) was used and for high-speed runs that of Yphantis (1964) was employed. Speeds for low-speed runs were 14,000–20,000 rpm and those for the high-speed ones were 40,000–44,000 rpm. The temperature for all sedimentation equilibrium experiments was 20°. Molecular weights were computed using an IBM 360 computer with programs made available to us by Mr. W. T. Wolodko. A value of 0.73 ml/g for the partial specific volume of the protein was calculated from the amino acid analysis. Sedimentation velocity experiments were carried out at 60,000 rpm and at 20° in two 2° Kel F cells, one with a wedge window in the usual manner.

Circular Dichroism. The circular dichroism measurements were made on a Cary Model 6001 circular dichroism attachment to a Cary 60 recording spectropolarimeter equipped with a water-cooled lamp housing maintained at 27°. The instrument was calibrated with an aqueous solution of *d*-10-camphorsulfonic acid with a difference in molecular extinction ($E_l - E_r$) of 2.16. Constant nitrogen flushing was employed. Cells of 0.5-mm path length were used over the range of 185–250 mμ with protein concentrations of approximately 0.1%. For the near-ultraviolet region (250–320 mμ), 1-cm cells were used with protein concentrations of about 0.3%. The mean residue ellipticity obtained is given by: $[\theta] = \theta^\circ M/100lc$, where M is the mean residue molecular weight (taken as 115 for troponin A), θ° is the observed ellipticity in degrees, l is cell path length in dm, and c is the protein concentration in g/cm³. The units of $[\theta]$ are (deg cm²)/dmole.

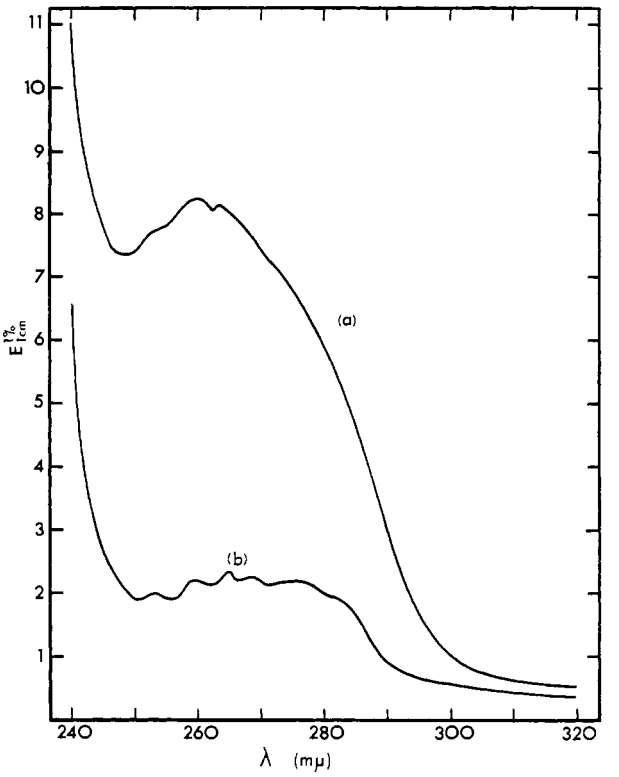

FIGURE 1: Absorption spectra of (a) troponin A prepared by the Hartshorne and Mueller (1968) approach, and (b) troponin A prepared by ion-exchange chromatography in urea. In both cases the solvent was 50 mM Tris (pH 7.6).

Results and Discussion

Sodium Dodecyl Sulfate–Polyacrylamide Gel and Spectral Studies of Troponin A Preparations. Troponin A prepared using the Hartshorne and Mueller approach was found from densitometric scans of sodium dodecyl sulfate–polyacrylamide gels to be 90–95% homogeneous with respect to size. Gels were similar to those shown previously (Murray and Kay, 1971). The molecular weight estimated from these gels was 22,000. The absorption spectrum of this protein is shown in Figure 1. The high $E^{1\%}_{1\,cm,\,260\,m\mu}$ probably reflects the presence of some nucleotide-containing material.

Troponin A was also prepared by ion-exchange chromatography in 6 M urea (Figure 2). The troponin A peak represents a small portion of the total $OD_{278\,m\mu}$ but the yield by weight was about one-third of the material applied to the column. The material eluting after troponin A appeared to be nucleotide rich, since its $OD_{278\,m\mu}$:$OD_{260\,m\mu}$ was very low. This troponin A preparation was run on sodium dodecyl sulfate–polyacrylamide gels (Figure 3). Contamination by any one species of different size was less than 1%. The molecular weight as estimated from the gels was 19,000. The absorption spectrum (Figure 1) of this preparation of troponin A is rather unusual in that the $E^{1\%}_{1\,cm}$ is very low (<2.5) in the 250- to 280-mμ region and five clearly defined maxima are obvious. This stems from the fact that this protein has a high Phe:Tyr ratio (Table I) and the contribution of the Phe appears to be predominant, as also reflected in the aromatic CD analysis of this material (see below).

A comparison of the amino acid analysis of one of our troponin A preparations with that produced by other workers reveals that there appears to be general agreement in the amino acid composition. However small discrepancies do arise, the most notable of which is the higher glutamic acid content of troponin A prepared by Schaub *et al.* (1972).

It should be noted that troponin A, prepared by either of the two preparative procedures outlined above, was effective in neutralizing the effect of the inhibitory factor on the ATPase activities of synthetic actomyosin systems in the presence of Ca^{2+} (Murray and Kay, 1971).

Sedimentation Equilibrium Studies. High-speed sedimentation equilibrium experiments on troponin A resulted in linear plots of ln y *vs.* r^2. Troponin A prepared by the Hartshorne and Mueller approach was shown, from molecular weight analysis of six runs, to have a minimum molecular weight of 22,200 ± 300 in 50 mM Tris buffer at pH 7.6. Troponin A prepared by ion exchange chromatography in urea was found from the analysis of seven high-speed experiments to have a minimum molecular weight of 22,200 ± 400. In this case the solvent was 50 mM Tris buffer at pH 7.6 with $CaCl_2$ concentrations ranging from 10^{-5} M to 5×10^{-4} M. In the presence of 10^{-4} M EGTA[1] three distinct experiments gave a minimum molecular weight for troponin A of 21,800 ± 400. These minimum molecular weights agree very well with that calculated from the amino acid analysis of our material, which was established as 21,700.

The relationship between the molecular weight and protein concentration is illustrated in Figure 4. At free Ca^{2+} concentrations of 10^{-5}, 5×10^{-5}, and 10^{-4} M, the molecular weight *vs.* concentration plots are similar and show little evidence of aggregation. However, addition of 10^{-3} M $CaCl_2$ resulted in increased weight average molecular weights. Plots of molec-

[1] Abbreviation used is: EGTA, ethylene glycol bis(β-aminoethyl ether) *N,N'*-tetraacetic acid.

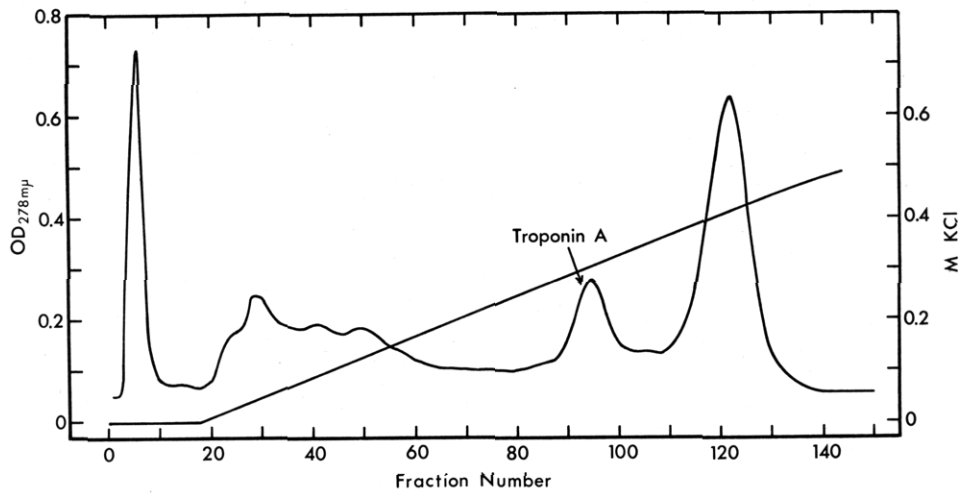

FIGURE 2: DEAE-Sephadex A-25 chromatography of crude troponin A. Protein (180 mg) in 10 ml of 50 mM Tris (pH 8.0), 6 M urea, and 0.5 mM dithiothreitol was applied to a gel bed (2.1 × 16 cm) equilibrated against the same buffer and eluted at 21 ml/hr with a gradient of KCl to 0.6 M. Each fraction contained 6.9 ml.

ular weight *vs.* concentration from sedimentation equilibrium experiments at different initial loading concentrations did not overlap. Aggregation to weight average molecular weights in the order of 40,000 is obvious.

In the case of troponin A prepared by the Hartshorne and Mueller approach, the $CaCl_2$ level is not controlled but is probably less than 10^{-5} M. Weight-average molecular weights of this material were as high as 60,000. This is probably due to the presence of high molecular weight contaminants in the preparation, or to complexing of troponin A with low molecular weight contaminants such as inhibitory factor.

These studies suggest that troponin A, prepared by either of the two preparative procedures outlined, has a minimum molecular of 22,200. In 50 mM Tris buffer (pH 7.6) the weight-average molecular weight increases to 25,000–26,000 in Ca^{2+} concentrations up to 10^{-4} M. Above this Ca^{2+} level, more extensive aggregation of the protein occurs.

Circular Dichroism Studies. Circular dichroism studies on troponin A prepared by ion-exchange chromatography in 6 M urea revealed the fact that the conformation of the protein

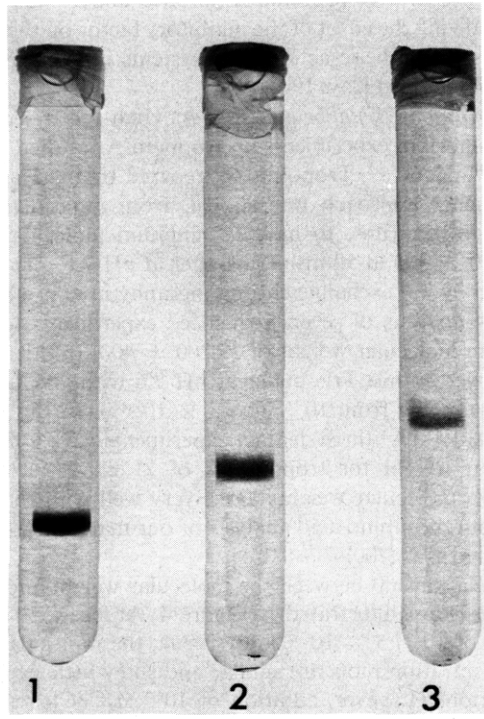

FIGURE 3: 10% sodium dodecyl sulfate–polyacrylamide gels showing (1) 10 μg of lysozyme, (2) 25 μg of troponin A prepared by ion-exchange chromatography in 6 M urea, and (3) 10 μg of chymotrypsinogen A.

TABLE I: Amino Acid Content of Troponin A Prepared by the Hartshorne and Mueller Approach Compared to Preparations Made by Other Workers.

	Residues/100,000 g of Protein		
Amino Acid	This Study	Hartshorne and Pyun (1971)	Schaub *et al.* (1972)
Lys	58.2	51.2	56.1
His	8.2	5.8	10.0
Arg	36.8	36.1	42.2
Asp	114.1	113.9	111.1
Thr	32.7	36.0	29.4
Ser	38.1	41.6	37.2
Glu	149.1	142.3	193.9
Pro	18.5	7.3	19.4
Gly	67.7	71.7	62.2
Ala	71.8	71.4	78.9
Val	44.1	38.3	43.9
Met	45.9	61.1	40.6
Ile	48.6	50.8	44.4
Leu	58.6	52.4	56.1
Tyr	13.4	16.7	9.4
Phe	49.8	57.4	42.2
Trp			*Nil*
Cys			8.9

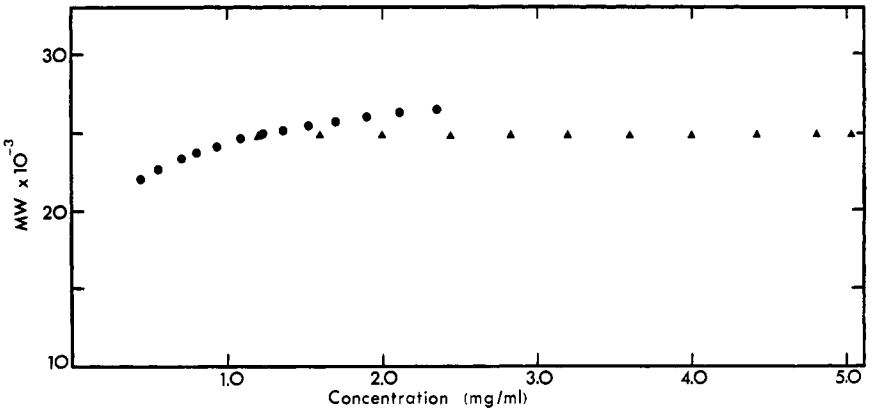

FIGURE 4: Molecular weight of troponin A prepared by ion-exchange chromatography in urea as a function of protein concentration. The results are from two low speed experiments with initial loading concentrations of 1.11 (●) and 2.39 mg per ml (▲). The rotor speed was 16,000 rpm in each case. The solvent was 50 mM. Tris buffer at pH 7.6 containing 10^{-5} M $CaCl_2$.

depends to a great extent on the concentration of Ca^{2+} present. Figure 5 represents typical far-ultraviolet circular dichroism spectra of troponin A in the absence and in the presence of Ca^{2+}. In the absence of Ca^{2+} or in the presence of EGTA the $[\theta]_{221\,m\mu}$ is $-10{,}000 \pm 300$ (deg cm^2)/dmole, while the addition of 5×10^{-4} M $CaCl_2$ causes a 60% increase to $-16{,}000 \pm 400$ (deg cm^2)/dmole. The apparent per cent α helix can be estimated using polyglutamic acid at pH 3.8 as a prototype for 100% α helix and the same polymer at pH 9.3 as a model for a random coil (Cassim and Yang, 1967). The change due to addition of 5×10^{-4} M $CaCl_2$ corresponds to an increase in apparent α helix from 28 to 40%. The ellipticity band at about 207 mμ undergoes a corresponding change, but of smaller magnitude. In the absence of Ca^{2+} the $[\theta]_{207\,m\mu}$ is $-13{,}000$ (deg cm^2)/dmole corresponding to 21% α helix. Addition of 5×10^{-4} M $CaCl_2$ causes an increase in $[\theta]_{207\,m\mu}$ to $-16{,}500$ (deg cm^2)/dmole corresponding to 43% α helix. These results indicate that the apparent helical content of troponin A increases significantly by about 50% upon the addition of 5×10^{-4} M Ca^{2+}. Ahmed *et al.* (1970) observed similar coil to helix transition in tropocalcin with addition of similar quantities of Ca^{2+}. Tropocalcin, however, appears to be a different protein than troponin A, since neither magnitudes nor positions of far-ultraviolet ellipticity bands coincide with our preparations of troponin A.

The near-ultraviolet circular dichroism spectra of troponin A in the presence and absence of Ca^{2+} are shown in Figure 6. The contribution appears to be due largely to Phe with negative dichroic bands at 259 and 265 mμ, since their sign and position are in complete agreement with the CD spectrum of acetyl-L-phenylalanine methyl ester (Goodman and Toniolo, 1968). Ca^{2+} appears to cause only a slight sharpening of the ellipticity bands, with no alteration in their wavelength position. It would thus appear that the conformational change results primarily in an alteration of the secondary structure of the protein.

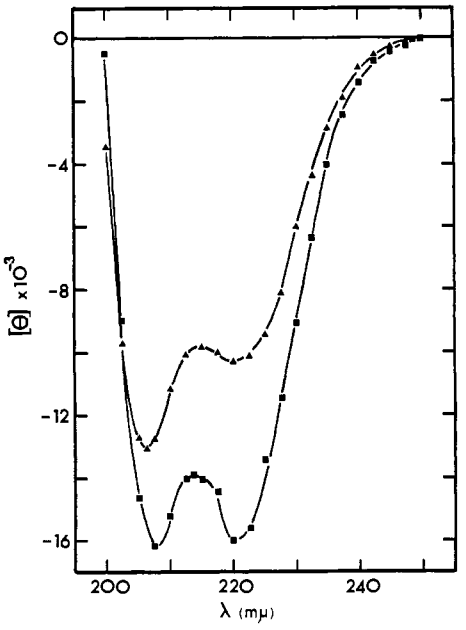

FIGURE 5: Far-ultraviolet circular dichroism spectra of troponin A, prepared by ion-exchange chromatography in urea, in 50 mM Tris (pH 7.6)–1 mM EGTA (▲) and in 50 mM Tris (pH 7.6)–5×10^{-4} M $CaCl_2$ (■).

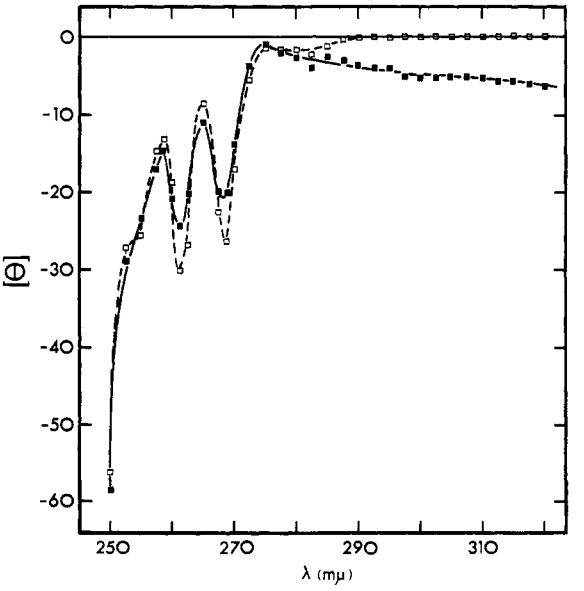

FIGURE 6: Near-ultraviolet circular dichroism spectra of troponin A, prepared by ion-exchange chromatography in urea, in 50 mM Tris (pH 7.6)–1 mM EGTA (■) and in 50 mM Tris (pH 7.6)–5×10^{-4} M $CaCl_2$ (□).

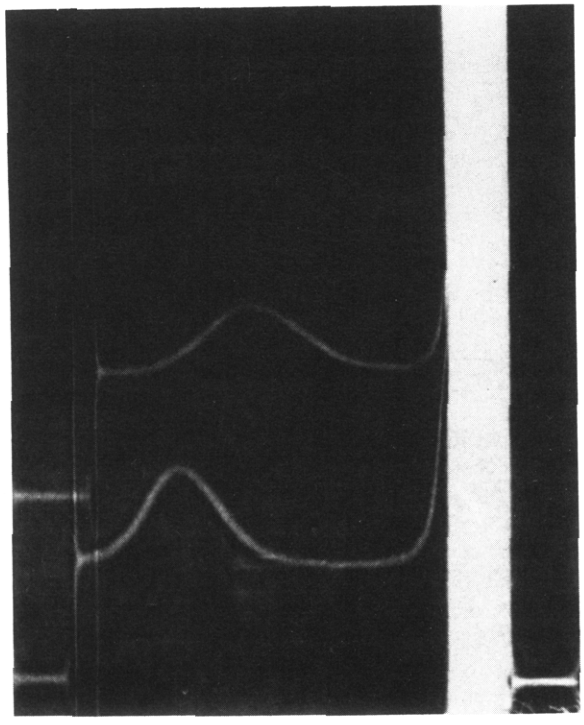

FIGURE 7: Sedimentation profiles of troponin A in 50 mM Tris (pH 7.6)–5 × 10^{-5} M $CaCl_2$ (upper curve) and in 50 mM Tris (pH 7.6)–10^{-3} M EGTA (lower curve). The protein concentration was 6 mg/ml in both cases. This photograph was taken after 156 min at speed 60,000 rpm.

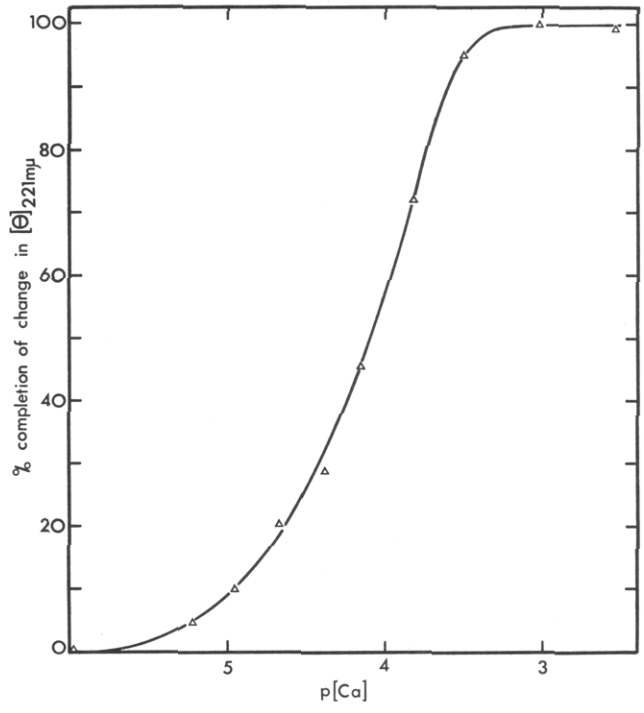

FIGURE 8: Percent completion of change in ellipticity at 221 mμ as a function of total Ca^{2+} added. The change is essentially 0% complete at 10^{-6} M $CaCl_2$ when $[\theta]_{221\,m\mu}$ is −10,300 (deg cm²)/dmole and 100% completion at above 10^{-3} M $CaCl_2$ when $[\theta]_{221\,m\mu}$ is −16,100 (deg cm²)/dmole.

The induction of this large conformational change upon the addition of Ca^{2+} was corroborated by sedimentation velocity experiments on this system. Samples of the same concentration (6 mg/ml) of troponin A were run in two Kel f cells, one equipped with a wedge window (Figure 7). One sample contained 5 × 10^{-5} M $CaCl_2$ (top) while the other contained 10^{-4} M EDTA (bottom). It is obvious that Ca^{2+} causes an increase in the rate of sedimentation and an increase in the diffusion rates. The apparent sedimentation coefficients were 1.92 and 1.36 S in the presence of 5 × 10^{-5} M $CaCl_2$ and 10^{-4} M EGTA, respectively. The increase in sedimentation rate with no alteration in molecular weight (see above) upon addition of 5 × 10^{-5} M Ca^{2+} implies a compacting of the molecule, and an overall increase in symmetry.

The interaction of Ca^{2+} with troponin A was studied by circular dichroism in more detail. Troponin A, isolated with no precautions taken to reduce Ca^{2+} levels, usually has some bound Ca^{2+}. This Ca^{2+} was removed by dialysis against EGTA, followed by extensive dialysis against Ca^{2+}-free buffer to remove the EGTA. The Ca^{2+} level was then increased and the ellipticity band at 221 mμ monitored. Assuming the change in $[\theta]_{221\,m\mu}$ to be complete by the addition of 5 × 10^{-3} M $CaCl_2$, the results were expressed as % completion in the change in $[\theta]_{221\,m\mu}$ *vs.* Ca^{2+} concentration (Figure 8). The troponin A concentration for the above experiments was about 5.5 × 10^{-5} M (1.2 mg/ml). The ellipticity change is almost complete after adding 3 moles of Ca^{2+}/mole of troponin. Most likely 2 moles of Ca^{2+} is bound per 22,200 g of troponin A (Hartshorne and Pyun, 1971). The discrepancy may be due to insufficient removal of EGTA from troponin A before the addition of Ca^{2+} or to a decrease in the Ca^{2+} binding constant of troponin A because of the harsh treatment with 6 M urea.

Rabbit white muscle contains about 0.07 μmole of troponin/g (Ebashi *et al.*, 1969). This corresponds to a concentration of troponin A of 1.5 mg/g of muscle, which is comparable to the concentrations used in our circular dichroism experiments. The change we observe occurs between total Ca^{2+} concentrations of 10^{-6} and 5 × 10^{-4} M. However, if the Ca^{2+} binding constant of troponin A is 10^6 M^{-1} (Ebashi *et al.*, 1968; Hartshorne and Pyun, 1971), the change occurs between free Ca^{2+} levels of 10^{-8} and 10^{-5} M. During the excitation leading to contraction, the free Ca^{2+} level increases from about 10^{-8} to 10^{-5} M. This level is quite adequate to induce a complete conformational change in troponin A. We suggest that this conformational change is involved in releasing the restraints imposed upon the actomyosin system by the inhibitory factor.

Acknowledgments

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References

Ahmed, A. K., Han, M. H., Benson, E. S., and Wetlaufer, D. B. (1970), *Biophys. Soc. Abstr.*, 89a.

Cassim, J. Y., and Yang, J. T. (1967), *Biochem. Biophys. Res. Commun. 26*, 58.

Drabikowski, W., Dabrowska, R., and Barylko, B. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 12*, 148.

Ebashi, S., Endo, M., and Ohtsuki, I. (1969), *Quart. Rev. Biophys. 2*, 351.

Ebashi, S., Kodama, A., and Ebashi, F. (1968), *J. Biochem. (Tokyo) 64*, 465.

Ebashi, S., Wakabayashi, T., and Ebashi, F. (1971), *J. Biochem. (Tokyo) 69*, 441.

Fuchs, F. (1971), *Biochim. Biophys. Acta 245*, 221.
Goodman, M., and Toniolo, C. (1968), *Biopolymers 6*, 1673.
Greaser, M. L., and Gergely, J. (1971), *J. Biol. Chem. 246*, 4226.
Han, M. H., and Benson, E. S. (1970), *Biochem. Biophys. Res. Commun. 38*, 378.
Hartshorne, D. J., and Mueller, H. (1968), *Biochem. Biophys. Res. Commun. 31*, 647.
Hartshorne, D. J., and Pyun, H. Y. (1971), *Biochim. Biophys. Acta 229*, 698.
Murray, A. C., and Kay, C. M. (1971), *Biochem. Biophys. Res. Commun. 44*, 237.
Richards, E. G., Teller, D. C., and Schachman, H. K. (1968), *Biochemistry 7*, 1054.
Schaub, M. C., Perry, S. V., and Hacker, W. (1972), *Biochem. J. 126*, 237.
Shapiro, A. L., Vinuela, E., and Maizel, J. V. (1967), *Biochem. Biophys. Res. Commun. 28*, 815.
Yphantis, D. A. (1964), *Biochemistry 3*, 297.

Metabolic Profiles of Three Fiber Types of Skeletal Muscle in Guinea Pigs and Rabbits†

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ABSTRACT: Certain guinea pig and rabbit skeletal muscles, which are composed solely or predominantly of a single type of fiber as defined histochemically, were analyzed for various enzymatic and substrate characteristics. The results show that fiber types in the adult guinea pig and rabbit limb muscles can be conveniently classified into three categories on the basis of three criteria, (1) the contraction time of the fiber relative to others within the same animal, (2) glycolytic capacity, and (3) oxidative capacity. The rabbit semimembranosus accessorius and the white portion of the guinea pig vastus lateralis are fast twitch and have a very high anaerobic capacity, as indicated by glycogen concentration and phosphorylase, lactate dehydrogenase and mitochondrial α-glycerophosphate dehydrogenase activities. Cytochrome concentration and succinate dehydrogenase activity are low, indicating a low aerobic capacity. Consequently these fibers, formerly termed fast-twitch white, are more explicitly called fast-twitch-glycolytic fibers. The red portion of the guinea pig vastus lateralis has a high glycogen concentration, moderate lactate dehydrogenase, and high phosphorylase and α-glycerophosphate dehydrogenase activities. The red vastus lateralis has the highest cytochrome concentration and succinate dehydrogenase activity, indicating a high aerobic capacity in addition to moderate to high glycolytic capacity. Consequently these fibers, previously labeled fast-twitch red, can more explicitly be called fast-twitch-oxidative-glycolytic fibers. Although this fiber type is found in the rabbit, no muscle consisting predominantly of fibers that are fast-twitch-oxidative-glycolytic was found. The soleus muscle is slow twitch and chemically is characterized by low glycogen concentration and low phosphorylase, lactate dehydrogenase, and mitochondrial α-glycerophosphate dehydrogenase activities together with a moderate cytochrome concentration and succinate dehydrogenase activity. These features indicate a moderate to high aerobic and a relatively low glycolytic capacity. Formerly labeled the slow-twitch intermediate fiber they are more appropriately called slow-twitch-oxidative fibers. These data provide quantitative information on the metabolic characteristics of the three distinct muscle fiber types previously described by histochemical techniques. Furthermore, since muscles composed of fast-twitch-oxidative-glycolytic or slow-twitch-oxidative fibers are red in appearance the classification of skeletal muscle solely as "red" and "white" is imprecise and incomplete. Failure to recognize this can complicate the interpretation of studies with skeletal muscle.

In 1873 Ranvier reported that muscles which were slow contracting appeared red whereas fast contracting muscles appeared white. Exceptions to this general relationship between "redness" of a muscle and speed of contraction were later reported by Denny-Brown (1929). More recent investigations using a variety of histochemical techniques have demonstrated that most skeletal muscles are composed of different types of fibers and that muscles cannot be simply categorized as "red" or "white" (Barnard *et al.*, 1971; Edgerton and Simpson, 1969; Guth and Samaha, 1969; *cf.* Peter, 1972).

Histochemical, biochemical, and physiological studies of hind-limb skeletal muscle of guinea pigs have demonstrated the presence of three fiber types, each of which possesses a distinctive combination of histochemical and mechanical characteristics (Barnard *et al.*, 1971). These three fiber types were classified as fast-twitch red, fast-twitch white, and slow-twitch intermediate based on (1) their twitch characteristics which correlate with the specific activity of myosin ATPase and (2) histochemical assessment of their oxidative and glycolytic capacities (Edgerton and Simpson, 1969; Gillespie *et al.*, 1970; Barnard *et al.*, 1971; Peter, 1972).

The purpose of this paper is to provide quantitative data on the enzyme activities and glycogen concentration in muscles

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