Rasper, J., and Kauzmann, W. (1962), *J. Amer. Chem. Soc. 84*, 1771.
Schechter, A. N., and Epstein, C. J. (1968), *J. Mol. Biol. 35*, 569.
Shen, L. L., and Hermans, J., Jr. (1972), *Biochemistry 11*, 1836.
Smith, D. W., and Williams, R. J. P. (1970), *Struct. Bonding (Berlin) 7*, 1.
Suzuki, C., Kitamura, K., Suzuki, K., and Osugi, J. (1963a), *Rev. Phys. Chem. Jap. 32*, 30.
Suzuki, K., Miyosawa, Y., and Suzuki, C. (1963b), *Arch. Biochem. Biophys. 101*, 225.
Suzuki, K., Tsuchiya, M., and Kadono, H. (1970), *Bull. Chem. Soc. Jap. 43*, 3083.
Theorell, H., and Ehrenberg, A. (1951), *Acta Chem. Scand. 5*, 823.
Vedam, R., and Holton, G. (1968), *J. Acoust. Soc. Amer. 43*, 108.
Weber, H. H. (1930), *Biochem. Z. 218*, 1.
Zipp, A. (1973), Ph.D. Thesis, Princeton University.
Zipp, A., Ogunmola, G., Neuman, R. C., Jr., and Kauzmann, W. (1972), *J. Amer. Chem. Soc. 94*, 2541.

Physicochemical and Biological Studies on the Metal-Induced Conformational Change in Troponin A. Implication of Carboxyl Groups in the Binding of Calcium Ion†

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ABSTRACT: The Ca^{2+}-induced conformational change in which troponin A assumes a more helical conformation has been explored in greater detail, using circular dichroism, molecular weight, fluorescence, and biological techniques. It has been demonstrated that other bivalent ions, such as Mg^{2+}, Ba^{2+}, and Cd^{2+}, are capable of inducing a similar type of change in the protein molecule, the effect, however, being only about 50–70% the magnitude of that produced by Ca^{2+}. A possible explanation for this finding is the much lower (~1/500) binding constants of these metals for troponin A, compared with Ca^{2+}. Circular dichroism studies extended to higher total ionic strength than before demonstrated that the Ca^{2+}-induced change was not an artifact of the initial relatively low ionic strength buffer system employed. Several derivatives of troponin A have been prepared in which a varying number of carboxyl groups have been replaced by uncharged glycinate residues. Circular dichroism and fluorescence studies indicated that these modified samples were affected only slightly by Ca^{2+} ions. Biological studies on these derivatives employing desensitized actomyosin, the Mg^{2+}-activated ATPase of which was partially inactivated by "inhibitory protein," indicated a great loss in their ability to overcome or neutralize the effect of inhibitory protein. At the same time, sedimentation equilibrium studies revealed no significant alteration in molecular weight or molecular weight behavior (*e.g.*, association–dissociation phenomena), compared to native troponin A. It is concluded that the main sites on the troponin A molecule available for interaction with Ca^{2+} are certain carboxyl groups of aspartic and glutamic acid residues. Removal of these regions of negativity allows the molecule to adopt a slightly different conformation, comparable to that assumed by the molecule when Ca^{2+} is added, and to become essentially insensitive to the presence or absence of Ca^{2+} ions.

The troponin complex of skeletal muscle is composed of three distinct proteins (Drabikowski *et al.*, 1971; Ebashi *et al.*, 1971; Greaser and Gergely, 1971; Wilkinson *et al.*, 1971; Murray and Kay, 1971). These are the 37,000-dalton component or TN-T,[1] the function of which is not understood, but which is believed to interact with tropomyosin; the inhibitor protein, TN-I, which inhibits the Mg^{2+}-activated ATPase activity of desensitized actomyosin; and finally, the calcium binding protein, TN-C or troponin A, which in the presence of Ca^{2+} ions neutralizes the effect of inhibitory protein.

It has been demonstrated that troponin A binds Ca^{2+} very strongly (Fuchs, 1971), with a binding constant of about 10^6 M^{-1} (Ebashi *et al.*, 1968; Hartshorne and Pyun, 1971). Upon binding Ca^{2+}, troponin A undergoes a remarkable conformational change, with no alteration in molecular weight (Murray and Kay, 1972; Van Eerd and Kawasaki, 1972). As this conformational change occurs over a physiological range of concentration of Ca^{2+} ions, it may well be involved in the ability of troponin A to neutralize the effects of inhibitory protein.

This study was initiated with a view to defining more precisely the molecular basis of the Ca^{2+}-induced conformational change in troponin A. The conformational studies cited above involved working at rather low ionic strength (~50 mM) and the possibility could not be excluded that the conformational change noted may be a partial artifact as a result of the Donnan effect. To eliminate this possibility, in the present study 0.15 M KCl was included in the solvent system, and circular dichroism and molecular weight measurements were employed to monitor any changes noted in troponin A upon

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[1] Abbreviations used are: TN-T, the 37,000-dalton component of the troponin complex, or tropomyosin binding factor; TN-I, inhibitory protein; EGTA, ethylene glycol bis(β-aminoethyl ether) *N,N'*-tetraacetic acid; CD, circular dichroism.

Ca^{2+} addition. The ability of other bivalent metals such as Mg^{2+} to replace Ca^{2+} was also studied by similar techniques, because in living muscle the intracellular concentration of Mg^{2+} exceeds that of Ca^{2+} (Nanninga, 1961).

Nockolds *et al.* (1972) have recently shown that carboxyl groups are implicated in the binding of Ca^{2+} to a carp myogen, which is believed to be analogous to troponin A of mammalian muscle. In order to ascertain whether these groups are in fact involved in such binding in troponin A, they were chemically modified by the procedure of Hoare and Koshland (1967). Sedimentation equilibrium, circular dichroism, and fluorescence studies were carried out on these derivatives and the results compared with native troponin A. Parallel biological studies involving desensitized actomyosin and inhibitory protein were also performed on these modified samples. The results indicate that certain key carboxyl groups in the troponin A molecule are indeed involved in the binding of Ca^{2+}.

Materials and Methods

Isolation and Purification of Troponin A. Crude troponin A was prepared as described previously (Murray and Kay, 1971) using the method of Hartshorne and Mueller (1968) in which the pH 1 precipitation step was carried out either once or twice. The resulting protein (70–80% pure) was then subjected to chromatography on DEAE-Sephadex A-25 at 4° in a solvent system of 50 mM Tris-HCl (pH 7.6)–6 M urea–0.5 mM dithiothreitol. The protein was eluted with the same buffer containing a linear gradient to 0.6 M KCl (Murray and Kay, 1972).

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. These experiments were performed on 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate as outlined by Murray and Kay (1972). Samples were prepared in 1% sodium dodecyl sulfate solution containing 1 mM dithiothreitol by heating in a boiling water bath for 5–10 min.

Amino Acid Analysis. Amino acid analysis was carried out on a Beckman 120B amino acid analyzer. Protein samples were hydrolyzed *in vacuo* with 6 N HCl for 24 hr.

Protein Concentration. For native troponin A, protein concentrations were routinely measured by ultraviolet absorption employing an $E^{1\%}_{1\text{ cm},\ 277.5\text{ nm}}$ of 2.3 (Murray and Kay, 1972). For chemically modified derivatives, concentrations were determined in the ultracentrifuge employing the Rayleigh interference optical system, assuming 41 fringes equivalent to 10 mg/ml (Babul and Stellwagen, 1969).

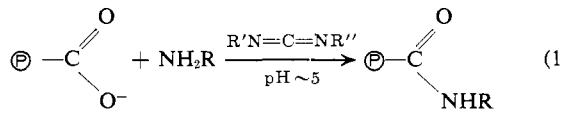
Ultracentrifugation. Molecular weight studies were carried out at 20° in a Beckman Spinco Model E ultracentrifuge equipped with a photoelectric scanner, multiplex accessory, and high intensity light source. The Rayleigh interference optical system was also used on occasion. Double sector charcoal filled epon cells with wide aperture window holders were used. Conventional sedimentation equilibrium experiments were carried out according to the methodology of Chervenka (1969). Meniscus depletion runs employed the procedures of Yphantis (1964). The conventional runs were executed at 16,000–18,000 rpm and the meniscus depletion ones were performed at 40,000–42,000 rpm. A value of 0.73 ml/g for the partial specific volume of troponin A was employed, as calculated by Murray and Kay (1972).

Circular Dichroism. The circular dichroism measurements were made on a Cary Model 6001 circular dichroism attachment to a Cary 60 recording spectropolarimeter in accordance with previously described methodology (Oikawa *et al.*, 1968).

Fluorescence. A Turner Model 210 recording fluorescence spectrophotometer was used for fluorescence measurements. The sample compartment was water jacketed and connected with a Lauda thermoregulator and measurements were conducted at 20°. The tyrosine fluorescence was observed at 306 nm, after excitation at 276 nm. The $OD_{278\text{ nm}}$ of the sample was 0.10 or less.

Bivalent Metal Concentration. Ca^{2+} concentrations were adjusted by means of a Ca buffer, consisting of Ca^{2+} and 1 mM EGTA in 50 mM Tris-HCl buffer at pH 7.6. In the calculation of free or available Ca^{2+} ion concentration, a binding constant for EGTA of $2.5 \times 10^5\ M^{-1}$ was assumed (Ebashi and Endo, 1962). We neglected the binding between Mg^{2+} and EGTA because the binding constant for Mg^{2+} is only 40 (Kawasaki and van Eerd, 1972). A similar assumption was made for Ba^{2+} and Cd^{2+}.

Chemical Modification of Carboxyl Groups. Carboxyl groups of proteins can be converted into amides by a two-step reaction with a water soluble carbodiimide and an amine according to eq 1 (Hoare and Koshland, 1967). Partially



(1)

modified derivatives of troponin A were prepared in the presence and absence of 1 mM EGTA. Troponin A (10 mg/ml) was dissolved in 1 M glycinamide · HCl and the pH adjusted to 4.75. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide dissolved in water (0.4 M) solution was added in aliquots over a period of 15 min until the final concentration of the carbodiimide was 0.1 M. The pH was maintained at 4.75 by the automatic addition of 0.1 M HCl from a Radiometer titrimeter. Aliquots were removed from the stirred solution at periods up to 22 hr. These aliquots were chromatographed on a column of Bio-Gel P2 equilibrated with 0.15 M KCl–50 mM Tris-HCl (pH 7.6)–1 mM EGTA. Fractions corresponding to the modified proteins were collected individually and dialyzed extensively against water and lyophilized. The number of carboxyl groups modified was determined by amino acid analysis after acid hydrolysis to detect the increase in the amount of glycine.

Biological Activity Studies. ATPase activities were determined by electrometric titration, following proton liberation in the pH-Stat, Radiometer TTT1, equipped with a titrator and titrigraph. The pH-Stat reaction vessel was stirred by passage of water from a large constant temperature bath, through the jacket surrounding the vessel. The reaction mixture consisted of 500–750 μg of desensitized actomyosin in a solution containing 1 mM Tris-HCl (pH 7.6), 2.5 mM ATP, 2.5 mM $MgCl_2$, 50 μg of inhibitory protein, and 50 μg of tropomyosin in a total volume of 10 ml. The reaction was carried out at 20°. Desensitized actomyosin was prepared from natural actomyosin by a modification (Ebashi *et al.*, 1968) of the original procedure, wherein a suspension of natural actomyosin is washed up to six times with a large excess of 2 mM $NaHCO_3$. The ability of troponin A or the carboxyl-modified derivatives to neutralize the effect of inhibitory protein was correlated with the alteration in ATPase activity upon addition of these samples to the partially inhibited desensitized actomyosin in the basic reaction mixture.

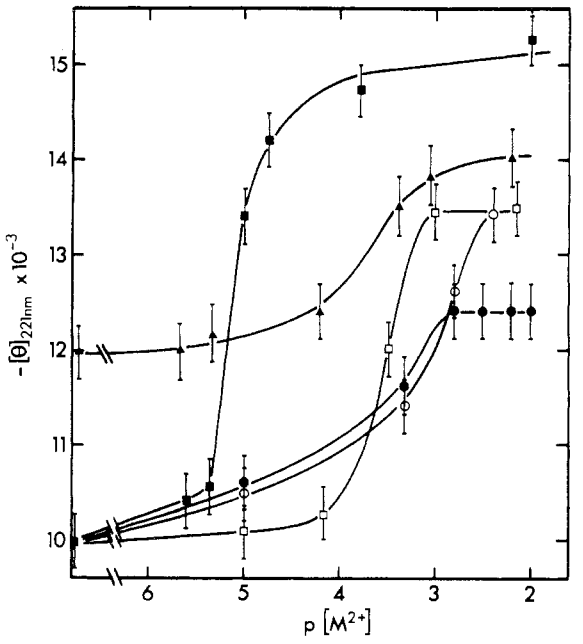


FIGURE 1: The change in ellipticity at 221 nm as a function of the free concentration of various bivalent metal ions: (■) Ca^{2+}; (▲) Ca^{2+} in the presence of 1 mM Mg^{2+}; (●) Ba^{2+}; (□) Cd^{2+}; (○) Mg^{2+}. Vertical bars represent estimate of error.

Results and Discussion

Circular Dichroism and Molecular Weight Studies on Native Troponin A

Effect of Bivalent Ions Other than Ca^{2+}. In order to establish the metal specificity of the conformational change induced in troponin A, CD studies were carried out on this protein, in the presence of Mg^{2+}, Ba^{2+}, and Cd^{2+}, individually. The results with Mg^{2+} were felt to be particularly relevant, since the concentration of Mg^{2+} in living muscle is higher than Ca^{2+} (Nanninga, 1961), and consequently may produce an effect on troponin A. The measurements were made in 0.15 M KCl–50 mM Tris-HCl (pH 7.6)–1 mM EGTA, hereinafter referred to as the "no Ca^{2+} reference solvent." Aliquots of the various metals were added and $[\theta]_{221\ nm}$ monitored as a function of metal concentration. It can be seen from Figure 1 that the change in $[\theta]_{221\ nm}$ produced by these three bivalent ions varies from approximately 50 to 70% of that induced by Ca^{2+} ions under the same conditions. The binding constants for Mg^{2+}, Cd^{2+}, and Ba^{2+}, as evaluated from the midpoints of these titration curves, were 8.9×10^3, 3.98×10^3, and 1.58×10^3 M^{-1}, respectively. These values are two orders of magnitude less than the value obtained here for Ca^{2+} (1.3×10^5 M^{-1}) and may well constitute the underlying reason as to why these metals are not as effective as Ca^{2+} in inducing a conformational change in troponin A. Kawasaki and van Eerd (1972) have recently obtained a comparable result in a CD and fluorescence study of the effect of Mg^{2+} on troponin A, where they noted with Mg^{2+} 36% of the change in fluorescence intensity and 80% of the change in ellipticity induced by Ca^{2+}. Also in agreement with these authors, the data presented in this study (see Figure 1) indicate that in the presence of 1 mM Mg^{2+}, the Ca^{2+}-induced conformational change is appreciably smaller, presumably due to a weaker binding of Ca^{2+} by troponin A in the presence of Mg^{2+}. Clearly, cations which are bound more tightly by troponin A induce larger conformational changes in the molecule.

Alteration in Ionic Strength. In the absence of Ca^{2+}, $[\theta]_{221\ nm}$ is $-10{,}000 \pm 300$ (deg cm²)/dmol and upon raising the available Ca^{2+} level to 1 mM, $[\theta]_{221\ nm}$ is $-15{,}500 \pm 300$ (deg cm²)/dmol. In the present study, the highest ionic strength solvent examined was 0.6 M KCl–50 mM Tris-HCl at pH 7.6. Here also $[\theta]_{221\ nm}$ showed the same approximately 60% increase upon addition of 1 mM Ca^{2+}, suggesting that whatever the mode of action of Ca^{2+} may be, ionic strength of the medium is relatively unimportant.

Molecular Weight Measurements. Meniscus depletion sedimentation equilibrium studies on troponin A in the presence of EGTA indicated that the mol wt of the protein was 22,000 daltons (Murray and Kay, 1972). Measurements were extended in this study to the higher ionic strength solvent, 0.15 M KCl–50 mM Tris-HCl (pH 7.6)–1 mM EGTA. The majority of runs were conventional sedimentation equilibrium ones employing the ultraviolet absorption optics of the photoelectric scanner. Plots of the natural logarithm of the concentration (optical density, OD) *vs.* the square of the radial distance were essentially linear except at high concentration, where slight upward curvature indicative of aggregation was observed. Meniscus depletion experiments carried out at lower concentrations gave no sign of aggregation. Values of the molecular weight of the protein estimated from both approaches did not significantly differ from the figure of 22,000 daltons cited above.

It had been shown earlier that at the free Ca^{2+} concentration just needed to complete the conformational change in troponin A ($\sim 10^{-4}$ M) the molecular weight *vs.* concentration plots were similar and showed little evidence for aggregation; however, addition of 10^{-3} M $CaCl_2$ resulted in much increased weight average molecular weights, of the order of 40,000 daltons (Murray and Kay, 1972). It seemed opportune to examine those samples of troponin A which had been exposed to the bivalent metal ions Mg^{2+}, Ba^{2+}, and Cd^{2+}, at a high enough concentration to complete the conformational change. In all cases it was found that the molecular weight behavior of troponin A in the presence of Mg^{2+}, Ba^{2+}, or Cd^{2+} was not significantly different from the protein in the presence of EGTA.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. These runs demonstrated that troponin A was essentially homogeneous (>99% as judged by densitometric scans) with an estimated mol wt of 19,000 daltons. It is of interest to note that this value is somewhat below the figure obtained by ultracentrifugal analysis. However it is extremely reproducible, the best value being 18,500 ± 500 daltons. It is possible that the very acidic nature of this protein affects its ability to interact with sodium dodecyl sulfate, so that the complex may migrate faster on the gel than its actual molecular weight would dictate. On some occasions the technique of conventional sedimentation equilibrium gave molecular weights of the order of 19,000 daltons. No satisfactory explanation for this discrepancy can be offered.

Circular Dichroism, Molecular Weight, Fluorescence, and Biological Activity Studies on Carboxyl-Modified Troponin A

Extent of Hoare–Koshland Reaction with Troponin A. Amino acid analyses were carried out to determine how many glycinate residues had been introduced into troponin A by carrying out the Hoare–Koshland reaction (1967) under two different conditions, *viz.*, the absence and presence of EGTA. Table I documents the data that were obtained. It should be noted that there is very little difference in the glycine content of all derivatives. This finding suggests that the reaction proceeds quite quickly with some 40 or so carboxyl residues out

TABLE I: Moles of Glycine Incorporated per Mole of Troponin A, in Accordance with the Hoare–Koshland Reaction.

Reaction Conditions		Time of Reaction (hr)	mol of Glycine Introduced/ 22,000 g of Troponin A
Presence of EGTA	Absence of EGTA		
	+	4	38
+		1	37
+		4	41
+		22	42

of a potential reactive group of 62 in the native protein, the remainder being probably inaccessible to reagent.

CD Studies. Initially the sample reacted for some 4 hr in the absence of EGTA was subjected to CD analysis. In the usual "no Ca^{2+} reference solvent" $[\theta]_{221\ nm}$ was found to be $-12{,}900$ (deg cm²)/dmol, while upon raising the available Ca^{2+} concentration to 4×10^{-2} M, $[\theta]_{221\ nm}$ becomes $-13{,}800$ (deg cm²)/dmol. The percentage change in $[\theta]_{221\ nm}$ upon adding a large excess of Ca^{2+} is thus approximately 10% compared with 60% for the native molecule. It should also be noted that the value of $[\theta]_{221\ nm}$ in the presence of EGTA, *viz.*, $-12{,}900$ (deg cm²)/dmol, is considerably higher than the value for native troponin A in EGTA [$-10{,}000$ (deg cm²)/dmol]. In other words, it would appear that introduction of the 38 glycinate residues, with the resulting removal of areas of negative charge from the molecule, has partially induced a similar sort of conformational change as did Ca^{2+}. Also, the derivatized molecule is much less sensitive to the presence of Ca^{2+} ions. Samples allowed to react in the presence of 1 mM EGTA for different periods of time were also studied. Table II shows the changes obtained in $[\theta]_{221\ nm}$ upon addition of Ca^{2+} to each of the derivatives. Again, in each case, the minimum value of $[\theta]_{221\ nm}$ is higher than for the native molecule, and the Ca^{2+}-induced change is about 7–10%.

It would thus appear to matter little whether or not the carboxyl modification is carried out in the absence or presence of EGTA. The resulting derivatives seem to be very similar in terms of the number of residues modified, as well as their relative insensitivity to a Ca^{2+}-induced conformational change.

Tyrosine Fluorescence Intensity Studies. Since troponin A contains no tryptophan, the fluorescence spectrum is essentially that of tyrosine. Upon excitation at 276 nm, the emission maximum is observed at 306 nm. The tyrosine fluorescence intensity of troponin A as a function of the Ca^{2+} concentration is depicted in Figure 2. There is a pronounced increase in the fluorescence intensity of the tyrosine residues as Ca^{2+} is added, paralleling completely the CD observations. This is in agreement with the work of van Eerd and Kawasaki (1972). From the studies of Cowgill (1968) the observed fluorescence intensity enhancement may be directly correlated with the considerable increase in helix content in the protein molecule (from 28 to 40%), upon adding Ca^{2+}. The increase in fluorescence intensity is not caused by aggregation of troponin A molecules, since previous direct molecular weight measurements (Murray and Kay, 1972, and this study (see below)) preclude this possibility. Figure 2 also includes representative data showing the fluorescence intensities as a function of Ca^{2+} concentration for the carboxyl-modified derivatives of troponin A. The changes in intensity produced by adding Ca^{2+} are in all cases quite small (~5%), which again parallels the CD observations.

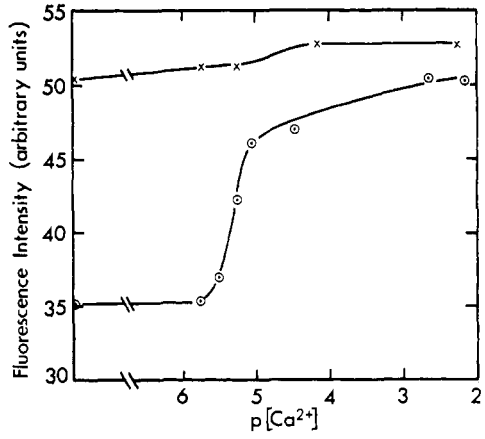


FIGURE 2: The change in the fluorescence intensity of the tyrosine residues of troponin A (⊙) and the carboxyl-modified derivatives (×), as a function of the free Ca^{2+} concentration (p[Ca^{2+}]). The protein concentration is 0.25 mg/ml and the temperature is 20°. The intensity of fluorescence is uncorrected and given in arbitrary units.

Biological Activity Studies. Inhibitory protein acting in conjunction with tropomyosin inhibits the Mg^{2+}-activated ATPase of desensitized actomyosin in the presence or absence of Ca^{2+}. If troponin A is added to the system, little effect is observed in the absence of Ca^{2+}, but in the presence of Ca^{2+} this protein overcomes the effect of inhibitory protein, *i.e.*, there is a restoration in the Mg^{2+}-activated ATPase of desensitized actomyosin. It seemed opportune to compare the biological activity of the carboxyl-modified derivatives of troponin A with the native molecule. Figure 3 summarizes data that were obtained in this assay for the native protein and its derivatives. Here, 750 μg of desensitized actomyosin was inactivated to an extent of about 50% by a mixture of 50 μg of inhibitory protein and 50 μg of tropomyosin. Some 90% of the initial ATPase activity was regained by addition of 100 μg of troponin A. The carboxyl-modified derivatives, on the other hand, showed no restorative effect; in fact a further slight inactivation was noted at high concentration, ~150 μg or so, on some occasions.

Earlier, Murray and Kay (1972) had suggested that the Ca^{2+}-induced conformational change in troponin A is involved in releasing the restraints imposed upon the actomyosin system by the inhibitory protein. This view seems to be substantiated by the present CD and fluorescence studies which indicate that the modified derivatives are relatively insensitive to conformational change and hence, using the above argument, will not be effective in neutralizing inhibitory protein. Another point of view not necessarily related to this one would be simply that the decreased neutralizing ability

TABLE II: Ellipticity Values at 221 nm for Carboxyl-Modified Derivatives of Troponin A, Prepared in Accordance with the Hoare–Koshland Reaction.

Reaction Time of Sample (hr)	$[\theta]_{221\ nm}$ (deg)		% Change in $[\theta]_{221\ nm}$
	No Ca^{2+}	+ Ca^{2+}	
1	$-11{,}500$	$-12{,}750$	10
4	$-12{,}200$	$-13{,}100$	7
22	$-12{,}900$	$-14{,}000$	8

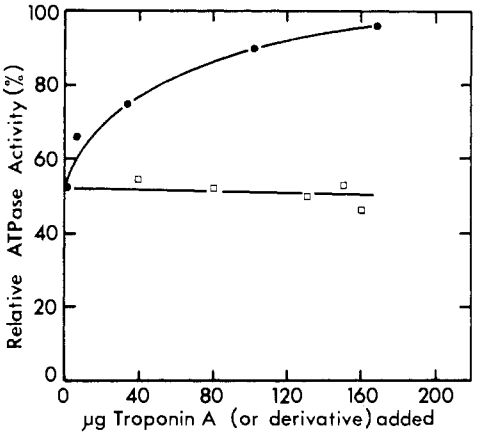


FIGURE 3: The effect of addition of troponin A (●) or the carboxyl-modified derivatives (□) on the Mg^{2+}-activated ATPase of desensitized actomyosin, which has been partially inhibited by inhibitory protein and tropomyosin. The reaction was carried out at 20°. Other conditions are described in the text.

is due to the reduction in the charged nature of the modified troponin A molecule.

Molecular Weight Studies. The molecular weight of the carboxyl-modified derivatives of troponin A was determined by meniscus depletion experiments employing the interference optical system both in the usual "no Ca^{2+} solvent" and in the presence of 5×10^{-4} M available Ca^{2+}. Plots of the natural logarithm of the concentration *vs.* the square of the radial distance obtained in either solvent system were linear and the molecular weight obtained, as the average of several runs, was 22,200 ± 500 daltons. If higher concentrations of Ca^{2+} were employed, 10^{-2} M for example, the plots showed distinct upward curvature, and aggregation to weight average molecular weights of the order of 50,000–60,000 daltons was obvious.

In summary, the results presented in this portion of the investigation support the idea that the Ca^{2+} binding sites on troponin A include certain key carboxylate groups of aspartic and/or glutamic acid residues. If these are replaced by neutral glycinate moieties the modified troponin A loses most of its responsiveness to Ca^{2+}, in terms of undergoing a much smaller conformational change than the native molecule. The loss of areas of negative charge is reflected by the inability of the derivative to neutralize the effect of inhibitory protein on the Mg^{2+}-activated ATPase activity of desensitized actomyosin.

Acknowledgments

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References

Babul, G., and Stellwagen, E. (1969), *Anal. Biochem. 28*, 216.
Chervenka, C. H. (1969), A Manual of Methods for the Analytical Ultracentrifuge, Spinco Division of Beckman Instruments, Inc., Palo Alto, Calif.
Cowgill, R. W. (1968), *Biochim. Biophys. Acta 168*, 417.
Drabikowski, W., Dabrowska, R., and Barylko, B. (1971), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 12*, 148.
Ebashi, S., and Endo, M. (1962), *in* Biochemistry of Muscle Contraction, Gergely, J., Ed., Little Brown and Co., Boston, Mass., p 199.
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.
Greaser, M. L., and Gergely, J. (1971), *J. Biol. Chem. 246*, 4226.
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.
Hoare, D. G., and Koshland, D. E. (1967), *J. Biol. Chem. 242*, 2447.
Kawasaki, Y., and van Eerd, J. P. (1972), *Biochem. Biophys. Res. Commun. 49*, 898.
Murray, A. C., and Kay, C. M. (1971), *Biochem. Biophys. Res. Commun. 44*, 237.
Murray, A. C., and Kay, C. M. (1972), *Biochemistry 11*, 2622.
Nanninga, L. B. (1961), *Biochim. Biophys. Acta 54*, 338.
Nockolds, C. E., Kretsinger, R. H., Coffee, C. J., and Bradshaw, B. A. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 581.
Oikawa, K., Kay, C. M., and McCubbin, W. D. (1968), *Biochim. Biophys. Acta 168*, 164.
Van Eerd, J., and Kawasaki, Y. (1972), *Biochem. Biophys. Res. Commun. 47*, 859.
Wilkinson, J. M., Perry, S. V., Cole, H. A., and Trayer, I. P. (1971), *Biochem. J. 124*, 44.
Yphantis, D. A. (1964), *Biochemistry 3*, 297.