Caldwell, P. C. (1958), *J. Physiol. (London) 142,* 22.
Cohen, C., and Tooney, N. M. (1974), *Nature (London) 251,* 659.
Colwell, R. R., and Liston, J. (1960), *J. Appl. Environ. Microbiol. 8,* 104.
Delaney, D., and Krause, S. (1976), *Macromolecules 9,* 455.
Ebashi, S., and Kodama, A. (1965), *J. Biochem. (Tokyo) 58,* 107.
Hodge, A. J. (1952), *Proc. Natl. Acad. Sci. U.S.A. 38,* 850.
Johnson, W. H. (1962), *Physiol. Rev. 42, Suppl. 5,* 113.
Johnson, W. H., Kahn, J., and Szent Györgyi, A. G. (1959), *Science 130,* 169.
Kominz, D. R., Saad, F., and Laki, K. (1957), *Med. Sci. (Tokyo) 9,* 118.
Ruegg, J. C. (1961), *Proc. R. Soc. London, Ser. B 160,* 224.
Ruegg, J. C. and Weber, H. H. (1963), in Perspectives in Biology, Cori, C. F., et al., Ed., London, Elsevier.
Shapiro, A. L., Vinuela, E., and Maizel, J. V. (1967), *Biochem. Biophys. Res. Commun. 28,* 815.
Stafford, W. F., and Yphantis, D. (1972), *Biochem. Biophys. Res. Commun. 49,* 848.

Solubility Properties of α-Reduced Paramyosin†

Jacqueline P. Merrick and William H. Johnson*

ABSTRACT: It is now believed that the reduced form of α-paramyosin is that found in the living adductor muscles of molluscs. We have studied the solubility of a preparation of α-paramyosin obtained under reducing conditions. In contrast to the solubility profile of β-paramyosin, the α-preparation showed a rapid, almost linear decrease in solubility over the ionic strength range 0.35–0.25 at neutral pH. Solubility in this range was further decreased by the presence of physiologically small amounts of calcium ion. Lactate ion, which can accumulate during anaerobic glycolysis in molluscan muscles, also decreases the solubility at a level of 50 mM. In addition, the type of paracrystal formed by α-paramyosin differs greatly from those of β-paramyosin and paracrystal formed in the presence of lactate differs from those formed in buffer solutions. Reduced α-paramyosin is more sensitive to the above parameters than the preparations made without reducing agents. Moreover, the pH and ionic strength ranges in which greatest change in solubility behavior occurs are physiologic, as are the calcium and lactate ion levels effective in increasing intermolecular interactions. A model is proposed for α-paramyosin in which the extra 5% presumably removed in β preparations is a "sticky head" which protrudes from one end of the molecule and confers on it an increased tendency for interaction, particularly at physiological ionic strengths. Such molecules would be capable of promoting interactions between thick filaments which contain them, providing a means of accounting for the pH dependent stiffness observed in glycerinated preparations of molluscan catch muscles.

Paramyosin is a fibrous, α-helical protein found in many invertebrates which, in addition to purely structural functions, may also play a role in tonic (catch) contractions of such muscles as anterior byssus retractor muscle (ABRM) of *Mytilus edulis* and the white adductor of *Mercenaria mercenaria* (Johnson et al., 1959; Ruegg, 1961). In the past, this protein has been extracted with a high ionic strength buffer, followed by ethanol denaturation of actomyosin (Bailey, 1956). Another method of extraction is that of Hodge (1952) in which organic acids are used first to extract and then to denature proteins other than paramyosin. These methods yield preparations with different solubility, viscosity, and electrophoretic behavior. Until recently the reason for these differences was not known.

Stafford and Yphantis (1972) showed that paramyosin, extracted by the ethanol method, appears to be attacked by at least two proteolytic enzymes, active at high ionic strength and neutral pH. When EDTA[1] is added to the extracting solution in short (<1 hr) extractions, a new molecular species is isolated, with an apparent subunit molecular weight 5000 greater than that of protein extracted by the earlier ethanol procedure. We have shown in the preceding paper (Edwards et al., 1977) that this new protein, given the name α-paramyosin by Stafford and Yphantis (1972), has solubility characteristics very similar to the acid preparation of Hodge and also has the same apparent molecular weight.

It has been further shown that the four sulfhydryl groups of paramyosin exist in the reduced state in vivo (Stafford, 1973; Cowgill, 1974) and, for at least the adductor muscle of *Mercenaria mercenaria,* the reduced form of α-paramyosin seems to be the functional molecular species.

A relationship has been found between the solubility behavior of paramyosin and the mechanical behavior of catch muscles. The elastic modulus of the anterior byssus retractor muscle (ABRM) of *Mytilus edulis* is reduced drastically in pH ranges where α-paramyosin is soluble (Ruegg and Weber, 1963); however, the zone of pH in which the elastic modulus changes was not found to be nearly as sharp as the solubility changes of β-paramyosin (Johnson, 1962), and, in subsequent work, we found that there was considerable hysteresis in the pH dependence of the elastic modulus of the ABRM. In the search for better agreement between mechanical and molecular properties, solubility experiments previously done on acid, α-, and β-paramyosin were done on α-paramyosin prepared under

† From the Department of Biology, Rensselaer Polytechnic Institute, Troy, New York 12181. *Received November 7, 1975.* This research was supported in part by the United States Public Health Service through NIDR Grant 5-T1-DE-117-11. This work was used by J. P. Merrick in partial fulfillment of the requirements for the Ph.D. degree at Rensselaer.

[1] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; ABRM, anterior byssus retractor muscle.

reducing conditions (αr-paramyosin). We found that the solubility transition zones along the pH scale are like acid and previous α preparations not sharp at lower ionic strengths and are shifted somewhat with respect to previous preparations. β-Paramyosin has been shown to form polymorphic paracrystals (Cohen et al., 1973); however, αr-paramyosin forms bundles of paracrystals with a single 14.5-nm periodicity.

Materials and Methods

Extraction Procedures for αr-Paramyosin. The white portion of adductor muscles of *Mercenaria mercenaria* was excised, washed, and blended with 0.1 M KCl and then extracted at 0 °C for 1 h in a solution containing 0.6 M KCl, 0.04 M Tris-HCl, 0.01 M EDTA, 0.5 mM dithiothreitol, and 3 mM azide. The extract was centrifuged for 20 min at 18 000 rpm. Three volumes of 95% ethanol was then added to the supernatant. This was allowed to stand for 2 to 4 h and centrifuged and the pellet resuspended in a buffer of 0.6 M high ionic strength. All solutions for extraction and purification contained 0.5–1.0 mM dithiothreitol. Three recrystallizations from the high ionic strength buffer were generally sufficient to purify the α-paramyosin prepared under these reducing conditions.

β-Paramyosin was prepared by the usual Bailey ethanol method, as described by Johnson et al. (1959).

Sodium Dodecyl Sulfate Gel Electrophoresis. Whole and split polyacrylamide gels were run using the technique of Shapiro et al. (1967), as modified by Stafford and Yphantis (1972) with the exception that a 5% gel was used in place of the 4% gel employed by these workers. Purity of various preparations was continuously checked by this method.

Microscopy. Paramyosin tactoids, formed by dialyzing purified αr-paramyosin against 0.1 M phosphate buffer at pH 6, were examined by phase contrast using a Wild M-2 microscope. For electron microscopy, a dilute suspension of such paracrystals was placed on formvar coated, carbon stabilized grids and negatively stained with 1% uranyl acetate. These were examined on a Philips 200 microscope.

Aggregation Studies. The solubility behavior of αr-paramyosin as a function of ionic strength was studied by an equilibrium dialysis technique. Samples, at an initial concentration of 1 mg/mL, were dialyzed to equilibrium at 20 °C against a buffer containing 10 mM phosphate, 0.5 mM dithiothreitol, and 3 mM azide, and adjusted to the appropriate ionic strength with added KCl. The pH was held constant at 7.0 ± 0.03. Dialysates were then centrifuged at 9000 rpm for 45 min and the optical density of the supernatant was measured at 277 and 320 nm. The difference between the two optical densities was used as a measure of the amount of protein remaining in the supernatant. Ionic strengths were calculated from known constituents of the solutions at each pH.

Results

Paramyosin isolated from the adductor muscles of *Mercenaria mercenaria* and extracted in the presence of EDTA and dithiothreitol is considered to be identical with the α-reduced species isolated by Stafford (1972). Our preparation ran with the same mobility when compared on split gels with paramyosin kindly supplied by Stafford's laboratory or prepared by their methods. In densitometer traces of these gels, the peak for α-paramyosin can clearly be distinguished from that of β-paramyosin, as previously shown by Stafford and Yphantis.

αr-Paramyosin forms very long (on the average about 30 nm), wide birefringent paracrystals, readily observable in phase contrast microscopy (Figure 1A). These may be compared with paracrystals prepared by the same method from β-paramyosin (Figure 1B). When the paracrystals of αr-paramyosin are viewed in the electron microscope, bundles of paracrystals with a 14.5-nm banding pattern are observed (Figure 1C,D). β-Paramyosin paracrystals, in contrast, form single structures of thickness comparable to that of the individual units of the α-paramyosin paracrystal. We have observed only the 14.5-nm periodicity in our preparations of αr-paramyosin, and those are in register across the paracrystalline bundle.

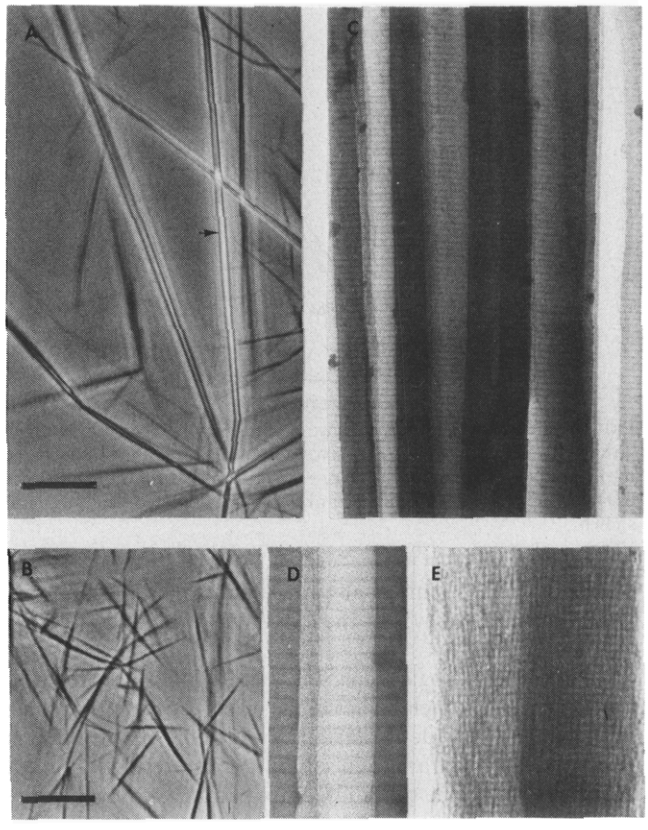

FIGURE 1: Photomicrographs of paracrystals of paramyosin. (A) Light micrograph of paracrystals of αr-paramyosin prepared as given in text; (B) light micrograph of paracrystals of β-paramyosin prepared by the ethanol method; (C) electron micrograph of one paracrystal bundle equivalent to the bundle shown by arrow in light micrograph A; (D) higher magnification of portion of C; (E) electron micrograph of paracrystals of αr-paramyosin prepared in the presence of 50 mM lactate ion. Bars in A and B, 5 μ. Periodicity in C, D, and E is 14.5 nm.

Aggregation studies on αr-paramyosin reveal that the protein prepared under these conditions is slightly more soluble at neutral pH than preparations made in the absence of a reducing agent. When protein remaining in solution is plotted against ionic strength, as in Figure 2, the curve is steeper for the reduced sample, particularly in the range 0.25–0.35. Differences at ionic strengths below 0.25 were not significant.

When calcium ion is included in the buffer at levels of 10^{-7}, 10^{-5}, and 10^{-3} M, a marked decrease in solubility of αr-paramyosin is seen at the higher ionic strengths (Figure 3). The lowest calcium ion concentration, 10^{-7} M, seems to be just as effective as the higher concentrations in modifying the solubility; thus very small amounts of Ca^{2+} are fully effective in bringing about a tendency toward aggregation in the physiological range. Increases in Ca^{2+} in the range of activation of the contractile system in most muscles, i.e., 10^{-7} to 10^{-5} M, do not enhance aggregation. The greatest change in solubility again occurs in the 0.35–0.25 M range, with a maximum

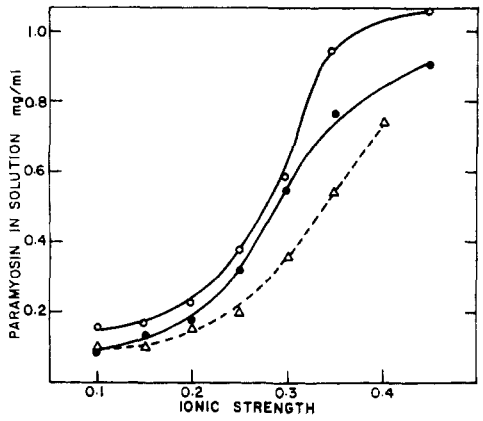

FIGURE 2: Ionic strength dependence of paramyosin prepared by methods given in the text. (O—O) αr-paramyosin; (●—●) α-paramyosin prepared in the absence of dithiothreitol; (Δ---Δ) αr-paramyosin in the presence of 50 mM lactate ion. All curves at pH 7.0 and calculated ionic strengths shown.

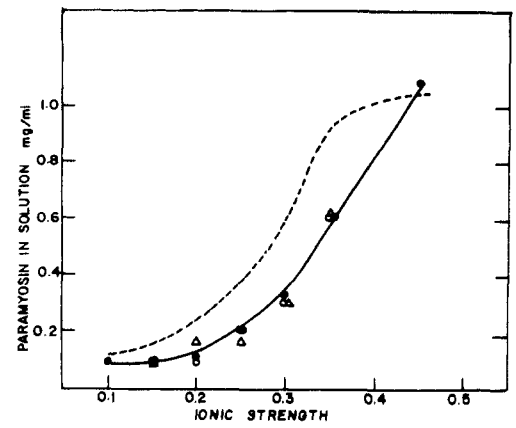

FIGURE 3: Effect of Ca^{2+} on the solubility of αr-paramyosin. (---) Ca^{2+} reduced to low level by presence of only 2 mM EDTA; (Δ) 10^{-7} M Ca^{2+}; (●) 10^{-5} M Ca^{2+}; (O) 10^{-3} M Ca^{2+}. All Ca^{2+} concentrations calculated by the method of Jewell and Ruegg (1966) using mixtures of 2 mM EDTA and 2 mM CaEDTA.

change at 0.25 M ionic strength. Calcium also affects the α-paramyosin solubility, but the maximum change in this case occurs at $\mu = 0.30$.

Another physiological ion, the lactate ion, the *d* form of which is one of the metabolites of the glycolytic scheme of some molluscan muscles (de Zwaan and Zandee, 1972), also causes a decrease in αr-paramyosin solubility. At a level of 50 mM, lactate depresses the solubility of αr-paramyosin by 35–50% in the ionic strength range 0.35–0.25 M (Figure 2). The presence of lactate ion in the buffer also changes the paracrystal appearance. The bundles were found to have a looser structure than those shown in Figure 1D with the boundaries between individual paracrystals in the bundle becoming less distinct, as though they had become fused together. Two adjacent subunits of paracrystals from the lactate preparation are shown in Figure 1E.

Discussion

The role of paramyosin in the catch contraction of certain molluscan muscles has long been debated. There are those who believe that paramyosin is not involved in catch, but that actomyosin linkages turn over very slowly and thus maintain the catch tension without energy expenditure (Lowy and Millman, 1959, 1963; Lowy et al., 1964). Others theorize that paramyosin, located in the core of the thick filament, modifies the myosin–actin interaction because of its ability to change phase in response to very small environmental changes (Szent-Gyorgyi et al., 1971). A third position (Ruegg, 1961; Johnson et al., 1959; Johnson, 1962) holds that paramyosin is directly involved in thick filament interaction and it is this interaction that maintains the catch state.

Mechanical studies on glycerinated ABRM (Ruegg and Weber, 1963; Johnson, et al., 1959; Johnson, 1962) have shown that the catch-like elastic behavior exhibited by glycerinated preparations of the ABRM is a function of pH, ionic strength, and temperature. However, the detailed behavior of the stretch resistance of this preparation in the range of pH between 6.0 and 8.0 differs considerably from that of the solubility of β-paramyosin. The latter changes sharply with pH whereas the former changes slowly. The former also shows considerable hysteresis in that the same pH dependence is not seen in traversing the pH scale in opposite directions. Thus the solubility behavior of β-paramyosin could not account for the details of mechanical behavior of catch muscles, in particular the ABRM, and thus may be suspected as having a secondary role in the catch process, perhaps regulatory.

In the preceding paper (Edwards et al., 1977), the α preparation of Stafford and Yphantis (1972) exhibits hysteresis in its pH dependent solubility behavior. Also, solubility does not change sharply with pH below ionic strengths which are probably in the physiological range, in fact, solubility depends more on the ionic strength than pH in this range. This is very similar to pH dependent stiffness of the glycerinated ABRM and considerably improves the correlation between the two phenomena.

When α-paramyosin is prepared under reducing conditions, the general solubility dependences on pH and ionic strength are not modified greatly, but the dependence on ionic strength is sharpened somewhat in the physiological range. Although we are not certain that paramyosin prepared in the absence of reducing agents is fully oxidized, we may conclude that it is not the redox state of the protein which accounts for the great difference between the α and β forms of paramyosin, rather some structural alteration such as removal of a small piece of the molecule in the beta preparation, as suggested by Stafford and Yphantis (1972).

Conditions within the cell are probably reducing; thus the protein prepared in the presence of dithiothreitol is probably closer to the native form. Experiments are now in progress in which detailed differences between preparations known to be fully oxidized and reduced (both α- and β-paramyosin) are examined both in solubility behavior, and also in size and shape using the techniques of electric birefringence. Delaney and Krause (1976) have found that the latter technique can detect very small changes in the radius of gyration of the molecule when used at very low ionic strengths where paramyosin is soluble (see previous paper).

Ca^{2+} may be associated with the control of the catch mechanism as well as the active contractile system, as suggested by Twarog and Mineoka (1972). This ion has little effect on the solubility of αr-paramyosin in the range between 10^{-7} and 10^{-3} M Ca^{2+}, but reduction to levels of the Ca^{2+} below 10^{-7} M by use of EDTA increases the solubility of this preparation. Apparently some Ca^{2+} will enhance the aggregation of the protein, but further increases within what might be called the physiological range of control have little effect. Perhaps the effects at low Ca^{2+} levels are pure charge effects. Divalent cations are used in some cases to enhance the aggre-

gation of paracrystal of paramyosin having a highly ordered morphology (Cohen et al., 1973).

Of particular interest is the effect of lactate ion on the solubility of αr-paramyosin. This ion enhances the aggregation of paramyosin at concentrations below 50 mM. Experiments with glycerinated preparations of the ABRM indicate that *d*-lactate quite specifically increases the stretch resistance. Pyruvate and acetate have small effects, but lactate seems to be most effective (Hartt and Johnson, in preparation). de Zwaan and Zandee (1972) have shown that the *d* form of lactate accumulates in the metabolism of bivalves, especially *Mytilus edulis*. Thus this ion would tend to add to any aggregation tendency brought about by decrease in ionic strength and decrease in pH. We have found that both *d*- and *l*-lactate have similar effects on solubility.

Our experiments indicate that this is an effect of the lactate ion and not an effect due to changes in ionic strength which might be brought about by addition of potassium lactate. If the latter were the case, the solubility should increase rather than decrease with increasing amounts of lactate. Certainly, if lactate ion enhanced the action of the protease thought to be responsible for the production of β-paramyosin, the solubility would also increase.

Striking differences appear in the aggregation of α- and β-paramyosin under conditions where paracrystals are formed. The β form as isolated by us produces paracrystals similar to those observed by Cohen et al. (1973), whereas the α form yields bundles of paracrystals which are much larger and are readily seen in the light microscope. These are shown in electron micrographs to be true bundles instead of larger individual paracrystals (Figure 1C). The effect of lactate ion is not so much to open up the structure of the individual paracrystal but to obscure the junction between adjacent paracrystals, which is in keeping with the tendency of this ion to enhance aggregation. It is also significant that the 14.5-nm band remains in register across the bundles in both cases. This periodicity is the only longitudinal period which we have seen in our work. In contrast, β-paramyosin is highly polymorphic (Cohen et al., 1973).

Thus, if the individual paracrystal can be taken as an analogue of the core of the large filaments of catch muscles, and much evidence suggests that this is the case (Szent-Gyorgyi et al., 1971; Kahn and Johnson, 1960), the formation of bundles of paracrystals suggests that any αr-paramyosin available on the surface of the filament would have a strong tendency to interact with paramyosin on adjacent filaments. Large filament interaction has been suggested as a basis for catch by a number of authors (Heumann and Ruegg, 1968; Rosenbluth, 1967; and more recently on the basis of filament positions in molluscan catch muscles, by Morrison and Odense, 1974). Thus the formation of bundles from αr-paramyosin solutions might be a model for large filament interaction under conditions where αr-paramyosin would strongly aggregate. Small amounts of Ca^{2+} may stabilize intrafilament structure, whereas surface interactions may be a function of other ambient conditions, such as pH, ionic strength, and lactate ion concentrations.

Evidence recently published by Halsey and Harrington (1973) and Cowgill (1972), taken together with our data and that of Stafford and Yphantis (1972), would suggest a model for the paramyosin molecule and its interactions. These workers have shown independently that approximately two-thirds of the β-paramyosin molecule forms a protease resistant core while one-third of the molecule is more vulnerable to protease activity, which would suggest that the latter region possesses a looser structure. Our data, and that of Stafford and Yphantis (1972), would suggest that a small portion of the molecule is removed in going from α to β. The present work indicates that, if this were true, this small piece confers drastically different solubility properties on the αr-paramyosin molecule. If this piece were located at the end of the protease sensitive portion, it could act as a "sticky head", protruding out of the paracrystal and giving rise to strong interactions between adjacent paracrystals. The increased tendency for interaction would be present at physiological ionic strength (0.20 to 0.35) and would be sensitive to pH, ionic strength, and also presumably to physiological ions involved in metabolism, such as lactate.

The sensitivity of the native paramyosin molecule to such physiological parameters as pH, ionic strength, calcium ion and metabolite accumulation, in vitro at least, suggests a poised system. Paramyosin molecules could easily interact to cause interactions between and possible aggregation of the thick filaments, and thus maintenance of the catch state. If the net ionic strength of the cell is in the range between 0.25 and 0.35, the system would require relatively small changes in surface properties of large filaments, perhaps small changes in charge, to bring about filament aggregation.

Acknowledgments

The authors gratefully acknowledge the cooperation of Dr. R. V. Rice in the use of Philips 200 Electron Microscope and also the technical assistance of Mr. Martin Haller, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213.

References

Bailey, K. (1956), *Pubbl. Stn. Zool. Napoli 29,* 96.
Cohen, C., Kendrick-Jones, J., and Szent-Gyorgyi, A. G. (1973), *J. Mol. Biol. 45,* 223.
Cowgill, R. W. (1972), *Biochemistry 11,* 4532.
Cowgill, R. W. (1974), *Biochemistry 13,* 2467.
Delaney, D., and Krause, S. (1976), *Biopolymers 9,* 455.
de Zwaan, A., and Zandee, D. I. (1972), *Comp. Biochem. Physiol. B 43,* 47.
Edwards, H. H., Merrick, J. P., and Johnson, W. H. (1977), *Biochemistry 16* (preceding paper in this issue).
Halsey, J. P., and Harrington, W. F. (1973), *Biochemistry 12,* 693.
Heumann, H. G., and Ruegg, J. C. (1968), *Pflugers Arch. 300,* 87.
Hodge, A. (1952), *Proc. Natl. Acad. Sci. U.S.A. 38,* 850.
Jewell, B. R., and Ruegg, J. C. (1966), *Proc. R. Soc. London, Ser. B 164,* 428.
Johnson, W. H. (1962), *Physiol. Rev. 42, Suppl. 5,* 113.
Johnson, W. H., Kahn, J., and Szent-Gyorgyi, A. G. (1959), *Science 130,* 1190.
Kahn, J., and Johnson, W. H. (1960), *Arch. Biochem. Biophys. 86,* 138.
Lowy, J., and Millman, B. R. (1959), *Nature (London) 183,* 1730.
Lowy, J., Millman, B. R., and Hanson, J. (1964), *Proc. R. Soc. London 160,* 525.
Morrison, C. M., and Odense, P. H. (1974), *J. Ultrastruct. Res. 49,* 228.
Rosenbluth, J. (1967), *J. Cell Biol. 34,* 15.
Ruegg, J. C. (1961), *Proc. R. Soc. London, Ser. B 154,* 209, 224.
Ruegg, J. C., and Weber, H. H. (1963), in Perspectives in Biology, Corl, C. F., et al., Ed., Amsterdam, Elsevier.

Shapiro, A. L., Vinuela, E., and Maizel, J. V. (1967), *Biochem. Biophys, Res. Commun. 28,* 815.
Stafford, W. (1973), Ph.D. Thesis, University of Connecticut.
Stafford, W., and Yphantis, D. A. (1972), *Biochem. Biophys. Res. Commun. 49,* 848.
Szent-Gyorgyi, A. G., Cohen, C., and Kendrick-Jones, J. (1971), *J. Mol. Biol. 56,* 239.
Twarog, B., and Mineoka, Y. (1972), *Cold Spring Harbor Symp. Quant. Biol. 37,* 489.

Polymerizability of Rabbit Skeletal Tropomyosin: Effects of Enzymic and Chemical Modifications†

Peter Johnson‡ and Lawrence B. Smillie*

ABSTRACT: Polymerizability of tropomyosin was unaffected by the removal of the three terminal residues 282, 283, and 284 using carboxypeptidase A. However, when residue 281 was removed, polymerizability was abolished. These results are consistent with a 9-residue molecular head-to-tail overlap in polymerized tropomyosin, in which residue 281 plays a space-filling role at the center of the overlap core. In acetylation studies, loss of polymerizability closely paralleled the extent of acetylation of lysine-7, and this residue was more susceptible to acetylation than any other. The effect of acetylation on polymerizability was probably caused not only by cleavage of salt-bridge between lysine-7 ϵ-NH_2 and residue 284 α-COOH but also by distortion of the overlap core by the *N*-acetyl group. Specific modification of methionine in tropomyosin indicated that, in addition to residue 281, methionine-8 is also involved in formation of the overlap core. Modified nonpolymerizable tropomyosins could still bind to F-actin, indicating that the head-to-tail polymerization of tropomyosin is not a prerequisite for actin binding, although the regularity of tropomyosin molecules along the actin helix is presumably disrupted.

The tropomyosin molecule is a highly helical asymmetric protein (Cohen and Szent-Gyorgyi, 1957) of molecular weight 66 000 (Woods, 1967) in which the two identical or near-identical polypeptide chains (Hodges and Smillie, 1970; Cummins and Perry, 1974) are aligned in parallel (Caspar et al., 1969) and in register (Johnson and Smillie, 1975; Lehrer, 1975; McLachlan and Stewart, 1975; Stewart, 1975). The complete amino acid sequence of one of the two major forms of rabbit skeletal tropomyosin (α-tropomyosin) has been established (Stone et al., 1975), and, on the basis of the regularities in the distributions of hydrophobic, acidic, and basic residues, it has been proposed that the molecule is a coiled-coil α-helical structure stabilized by hydrophobic and electrostatic interactions between the two polypeptide chains (Stone et al., 1975; Sodek et al., 1972; McLachlan and Stewart, 1975).

In solutions of low ionic strength, the tropomyosin coiled coils interact with each other to form head-to-tail linear aggregates (Kay and Bailey, 1960), and, from x-ray measurements (Caspar et al., 1969) and the fact that the polypeptide chains are in register, the extent of the head-to-tail molecular overlap has been calculated to be 13 Å or approximately 8–9 residues (Johnson and Smillie, 1975; Stewart, 1975). This head-to-tail arrangement of tropomyosin molecules also exists in vivo where the molecules are located along the grooves of the I filament such that each tropomyosin spans 7 actin monomers in one strand (or 14 in both strands) and specifically binds 1 unit of the troponin complex (Ebashi et al., 1969; Potter 1974; Potter and Gergely, 1974). In this assembly, the tropomyosin molecules govern the relative positioning of the troponin complexes to the actin strands (Potter and Gergely, 1974), and the arrangement of the tropomyosin molecules themselves along the I filament must be an important factor in the mechanism by which the troponin complex can regulate the actin–myosin interaction.

Because of the importance of the head-to-tail overlap between tropomyosin molecules in dictating the periodicity of the troponin complex along the I filament, we decided to investigate the head-to-tail polymerization process in detail. As the complete amino acid sequence of α-tropomyosin and the precise arrangement of the two polypeptide chains are known, we have been able to study the involvement of specific amino acid residues in the head-to-tail overlap by chemical and enzymic modifications of tropomyosin and by model building studies. These results are consistent with the molecular model proposed by McLachlan and Stewart (1975) for the 8–9 residue overlap and demonstrate the importance of both electrostatic and hydrophobic interactions between the NH_2-terminal and COOH-terminal regions of adjacent molecules.

Materials and Methods

Protein Preparations. Tropomyosin was prepared from rabbit skeletal muscle and resolved into α and β components by the method of Cummins and Perry (1973). Tropomyosin-free actin was prepared from rabbit skeletal muscle by the method of Spudich and Watt (1971).

Helix Determinations. The method of Chen et al. (1974) was used to calculate helix contents from circular dichroism spectra at 10 °C of tropomyosin preparations at concentrations

† From the Medical Research Council of Canada Group on Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7. *Received September 16, 1976.* This work was supported by the Medical Research Council of Canada.

‡ Present address: Department of Chemistry, Ohio University, Athens, Ohio 45701.