In-Register Homodimers of Smooth Muscle Tropomyosin†

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ABSTRACT: Gizzard smooth muscle tropomyosin dimer molecules were dissociated by guanidinium chloride and reassociated by dialysis against 1 M NaCl. Several properties of the protein were changed by this treatment. There was a large decrease in tropomyosin's low-salt viscosity, owing to reduced end-to-end polymerization, the helix unfolding profile changed from a one-step to a two-step process, and the ability to form intramolecular, interchain, disulfide-cross-linked homodimers increased dramatically. Thus, the native molecule, thought to exist predominantly as the $\beta\gamma$ heterodimer which cannot form disulfide cross-links [Sanders, C., Burtnick, L. D., & Smillie, L. B. (1986) *J. Biol. Chem. 261*, 12774–12778], reassembles, after dissociation, to form predominantly parallel, in-register $\beta\beta$ and $\gamma\gamma$ homodimers able to form disulfide cross-links. This suggests that the physical properties, including the end-to-end interaction, of gizzard tropomyosin homodimers differ considerably from those of the heterodimer. This is a first step toward a molecular understanding of the end-to-end interaction of smooth muscle tropomyosin.

Muscle contraction involves the interaction of the myosin-containing thick filaments with the actin-containing thin filaments, and the hydrolysis of ATP by this actomyosin complex provides energy for the contractile process. Rod-shaped tropomyosin binds end-to-end along the actin thin filament and together with troponin in striated skeletal muscle [see Leavis and Gergely (1984) for a review] or with caldesmon in smooth gizzard muscle [see Marston and Smith (1985) for a review] takes part in the Ca^{2+} regulation of contraction. Tropomyosin's end-to-end interaction is thought to play a crucial role in the cooperative features of this regulation in skeletal muscle [see Leavis and Gergely (1984) for a review]. It has been observed that gizzard tropomyosin regulates with a greater cooperativity than does skeletal muscle tropomyosin (Lehrer & Morris, 1984). The fact that gizzard tropomyosin has a greater end-to-end interaction than skeletal tropomyosin (Tsao et al., 1956; Dabrowska et al., 1980; Sanders & Smillie, 1984; Nowak & Dabrowska, 1985) may, in part, account for this observation.

The end-to-end polymerization of tropomyosin increases with decreasing salt concentration as inferred from the increase in viscosity with decreasing salt concentration for both skeletal (Bailey, 1948; Kay & Bailey, 1960; Ooi et al., 1962) and gizzard (Tsao et al., 1956; Dabrowska et al., 1980; Sanders & Smillie, 1984; Nowak & Dabrowska, 1985) tropomyosins. The end-to-end interaction of skeletal tropomyosin involves the overlap of eight to nine terminal residues (Johnson & Smillie, 1975, 1977; McLachlan & Stewart, 1975; Phillips et al., 1979). However, the molecular basis of gizzard tropomyosin's greater end-to-end interaction is unknown.

Gizzard tropomyosin, like skeletal tropomyosin, is composed of two coiled-coil, α-helical polypeptide chains with an α-helical content close to 100%, giving rise to a high ellipticity at 222 nm in the circular dichroism spectrum (Woods, 1969a, 1976). Skeletal tropomyosin contains 2 types of chains (Cummins & Perry, 1973), α and β, both containing 284 residues with a cysteine residue at position 190 (Mak et al., 1980). Gizzard tropomyosin also contains 2 types of chains, β and γ [also referred to as α and β, respectively; see Lau et al. (1985)], of roughly equal concentration (Cummins & Perry, 1974) and each containing 284 residues (Helfman et al., 1984; Sanders & Smillie, 1985; Lau et al., 1985). The β chain contains one cysteine residue at position 36 (Helfman et al., 1984; Sanders & Smillie, 1985), and the single cysteine of the γ chain is located at position 190 (Lau et al., 1985). The two chains of a skeletal tropomyosin molecule are in-register and parallel since they can easily and quantitatively form an intramolecular, interchain disulfide bond at cysteine-190 by oxidation (Johnson & Smillie, 1975; Stewart, 1975) or disulfide exchange (Lehrer, 1975). In contrast to skeletal tropomyosin, gizzard tropomyosin forms only a fraction of disulfide cross-links with difficulty by oxidation (Strasburg & Greaser, 1976; Lehrer et al., 1984), and none are formed by disulfide exchange (Lehrer et al., 1984). Cross-linking of gizzard tropomyosin with succinimide cross-linkers results in small amounts of cross-linked dimers, predominantly heterodimers, suggesting that gizzard tropomyosin is composed predominantly of heterodimers, which cannot form disulfide cross-links since the cysteines on the two chains are widely separated (Sanders et al., 1986).

As part of a study to understand the role of tropomyosin in smooth muscle regulation, we observed that there was a large drop in the low-salt viscosity of gizzard tropomyosin after the protein was dissociated with guanidinium chloride and reassociated by dialysis. Concomitant with this change there was a change in gizzard tropomyosin's helix unfolding profile and a dramatic increase in the ability to form disulfide-cross-linked $\beta\beta$ and $\gamma\gamma$ homodimers. These results, together with the view that native gizzard tropomyosin is composed primarily of heterodimers (Sanders et al., 1986), suggest that the physical properties, including the end-to-end interaction, of the heterodimer and homodimers of gizzard tropomyosin differ considerably.

Experimental Procedures

Chicken gizzard tropomyosin was prepared at 4 °C as described previously (Graceffa, 1987) and will be referred to as native gizzard tropomyosin (N-TM).[1] In some cases, N-TM

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[1] Abbreviations: Mops, 3-(*N*-morpholino)propanesulfonic acid; N-TM, native gizzard tropomyosin; DR-TM, N-TM chains dissociated and then reassociated; DRDR-TM, DR-TM chains dissociated and then reassociated.

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was further purified by chromatography at room temperature. The protein was first chromatographed on hydroxylapatite as carried out previously on skeletal tropomyosin (Eisenberg & Kielley, 1974). All of the tubes of the main peak were combined, and the protein was concentrated by isoelectric precipitation at pH 4.7. The hydroxylapatite-chromatographed gizzard tropomyosin was purified further by ion-exchange chromatography according to Sanders and Smillie (1984), except that DEAE-Sephadex A-50 was substituted for DEAE-cellulose. The tubes of the main peak were combined, and the protein was concentrated by isoelectric precipitation. In agreement with Sanders and Smillie (1984), this ion-exchange chromatography resulted in a drop in the A_{260}/A_{280} value, indicating removal of nucleic acid. Rabbit skeletal tropomyosin was prepared by the method of Bailey (1948) as modified by Greaser and Gergely (1971). Tropomyosin was stored in 0.5 M NaCl, 2.5 mM Mops, 1 mM EDTA, and 0.01% NaN_3, pH 7 at 0 °C. The concentration of gizzard tropomyosin was determined by the Lowry method using skeletal tropomyosin as a standard. The concentration of skeletal tropomyosin was determined from the absorbance at 277 nm (Lehrer, 1978).

N-TM chains were dissociated by adding 8 M guanidinium chloride (Schwarz/Mann Ultra Pure) to a final concentration of 4–5 M (Pont & Woods, 1971; Woods, 1976) to N-TM at 1–5 mg/mL in 1 M NaCl, 5 mM Mops, and 1 mM EDTA, pH 7.5 at room temperature, and incubated at 0 °C for between 5 min and 2 h. The chains were then allowed to reassemble by dialysis at 4 °C against 1 M NaCl, 5 mM 2-(*N*-morpholino)ethanesulfonic acid, and 1 mM EDTA, pH 6.0, and then against 1 M NaCl, 5 mM Mops, and 1 mM EDTA, pH 7.5. This dissociated–reassociated gizzard tropomyosin will be referred to as DR-TM. In some cases, DR-TM, at a final concentration of 1–2 mg/mL, was dissociated in 4–5 M guanidinium chloride, 10 mM Mops, and 1 mM EDTA, pH 7.5 at room temperature, and then reassociated by dialyzing, stepwise, against decreasing concentrations of guanidinium chloride with 10 mM Mops and 1 mM EDTA, pH 7.5, always present and at room temperature. The dialysis against 3 M, then 2 M, and then 1 M guanidinium chloride was performed for 2 h (at each guanidinium chloride concentration) in a large, continuously rotating test tube. The sample was then dialyzed at 4 °C against 1 M NaCl, 5 mM Mops, and 1 mM EDTA, pH 7.5. Dissociated–reassociated DR-TM will be referred to as DRDR-TM.

Viscosity was determined at 24 °C, unless otherwise stated, in an Ostwald-type viscometer (Cannon 150/A554) which had a buffer outflow time (t_{buffer}) of 31 s. Viscosity was measured for tropomyosin adjusted to 0.8 mg/mL after exhaustive dialysis against a low-salt buffer containing 2 mM Mops and 0.1 mM EDTA, pH 7.5. Salt was varied by adding small volumes of a concentrated NaCl solution to the viscometer. Specific viscosity = $(t_{tropomyosin}/t_{buffer}) - 1$. Low-salt viscosity refers to that measured in 2 mM Mops and 0.1 mM EDTA, pH 7.5, unless otherwise stated. Successive measurements on a sample in the viscometer sometimes led to small monotonic decreases in the viscosity, particularly with the more viscous solutions. In these cases, we took the first value as the viscosity since it is known that a capillary viscometer of this type can generate high shear rates which can orient, deform, and break polymers, all leading to decreased viscosity (Cooper & Pollard, 1982).

The helix unfolding profile of gizzard tropomyosin, in a 1-cm cell, was obtained by measuring the circular dichroism ellipticity at 222 nm as a function of temperature, at 0.2 °C intervals, on an updated Cary 60 spectropolarimeter (Aviv Associates). Temperature was controlled with the Hewlett-Packard HP 89100A thermoelectric accessory which includes a probe which was placed in the sample for temperature measurement.

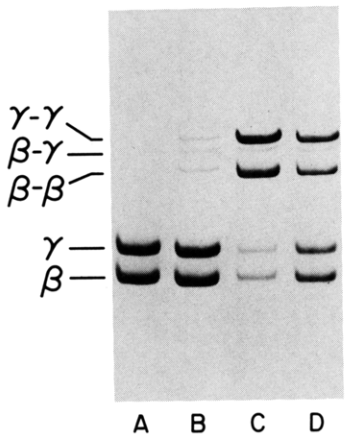

FIGURE 1: Gel electrophoresis pattern of N-TM (lane A) and oxidized N-TM (lane B), oxidized DR-TM (lane C), and oxidized DRDR-TM (lane D). Dimer band assignments are according to Strasburg and Greaser (1976).

In order to form disulfide bridges, gizzard tropomyosin was oxidized by Cu^{2+}-catalyzed $K_3Fe(CN)_6$ oxidation as performed previously on skeletal tropomyosin (Graceffa & Lehrer, 1984), except that the reaction was carried out for only 2 h. Gizzard tropomyosin at 1–2 mg/mL, which had been exhaustively dialyzed against 1 M NaCl and 5 mM Mops, pH 7.5, to remove EDTA, was incubated at room temperature (22 °C) in the presence of 5 mM $K_3Fe(CN)_6$ (freshly prepared) and 1–2 μM $CuSO_4$. After 2 h, samples were dialyzed against 1 M NaCl, 5 mM Mops, and 1 mM EDTA, pH 7.5 at 4 °C. EDTA complexes the Cu^{2+} and precipitiously reduces the oxidation reaction rate (Bridgart & Wilson, 1973).

Electrophoresis of gizzard tropomyosin, first dialyzed against 50 mM NaCl, 5 mM Mops, and 1 mM EDTA, pH 7.5, and unfolded in 1% sodium dodecyl sulfate, was carried out on a 7.5% polyacrylamide slab gel in 0.1 M Tris, 0.1 M *N*,*N*-bis-(2-hydroxyethyl)glycine, pH 8.4, and 0.1% sodium dodecyl sulfate. The protein bands were stained with Coomassie brilliant blue R-250 and quantified by scanning on a Joyce-loebl Chromoscan 3 densitometer interfaced with a Packard Bell IS/VT 286 computer. Tropomyosin dimer band assignments on these gels are according to Strasburg and Greaser (1976).

Results

Disulfide Cross-Linking. We have found it difficult to disulfide-cross-link native gizzard tropomyosin (N-TM), in agreement with previous work (Strasburg & Greaser, 1976; Lehrer et al., 1984). However, we now demonstrate that gizzard tropomyosin can be easily disulfide-cross-linked, if it is first dissociated by 4–5 M guanidinium chloride and then reassociated by dialysis against 1 M NaCl (DR-TM). Unoxidized N-TM (Figure 1) and DR-TM (not shown) showed only monomer bands of the β and γ chains with close to 50% contribution of each band. Oxidized N-TM showed only a small amount of dimer formation (Figure 1) with the dimer bands comprising only 8% of the total protein (Table I). Oxidized DR-TM showed much higher dimerization (Figure 1), with the two homodimer bands, of roughly equal contribution, together accounting for 82% of the total protein (Table I). These results show that gizzard tropomyosin dis-

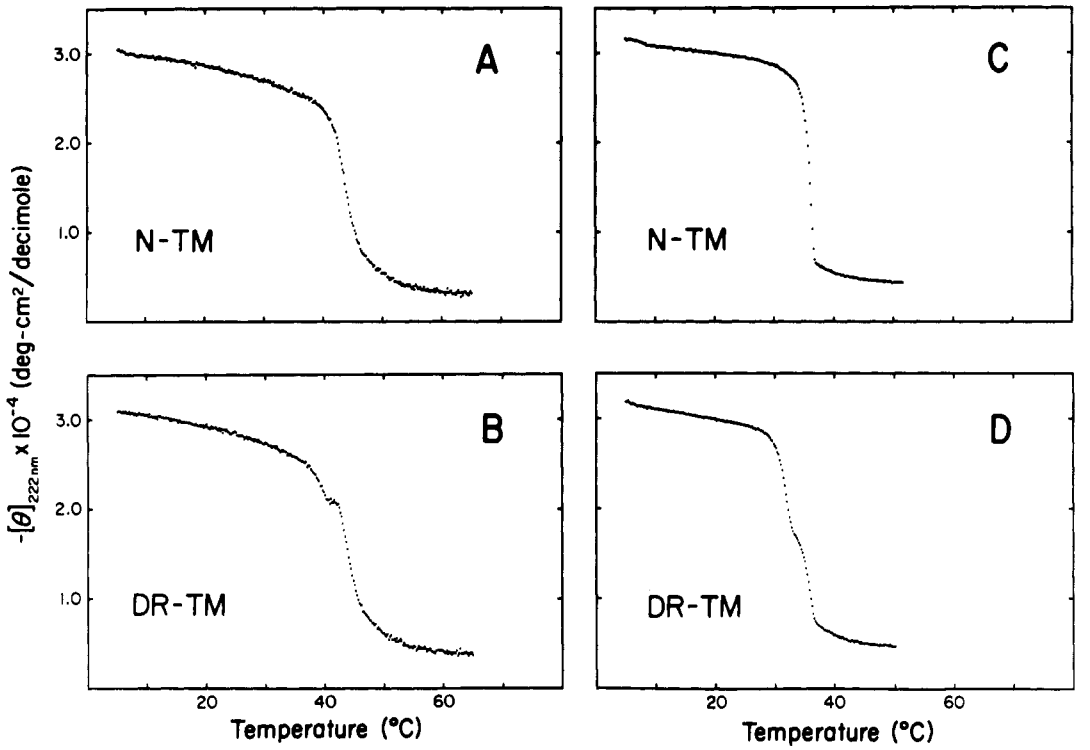

FIGURE 2: Helix unfolding profile of N-TM (A and C) and DR-TM (B and D) at 0.04 mg/mL in 1 M NaCl, 5 mM Mops, and 1 mM EDTA, pH 7.5 (A and B), or in 5 mM NaCl, 2 mM Mops, and 0.1 mM EDTA, pH 7.5 (C and D). Mean residue ellipticity ($[\theta]_{222nm}$) vs temperature.

Table I: Quantification of Densitometric Scans of Figure 1 Gels of Oxidized N-TM, Oxidized DR-TM, and Oxidized DRDR-TM

	% of total protein on gel		
gel band	N-TM	DR-TM	DRDR-TM
$\gamma\gamma$ dimer	2.5	41.2	26.4
$\beta\gamma$ dimer	2.1	2.0	2.2
$\beta\beta$ dimer	3.0	40.8	24.4
γ monomer	45.9	6.0	21.2
β monomer	46.5	10.0	25.8

sociated by guanidinium chloride reassembles to predominantly form $\beta\beta$ and $\gamma\gamma$ parallel, in-register homodimers. [The small amount of cross-linked heterodimer observed for oxidized N-TM and DR-TM (Figure 1, Table I) may be due to intermolecular disulfide formation (Sanders et al., 1986).]

Circular Dichroism. The circular dichroism thermal melting profiles of N-TM and DR-TM were compared at high (Figure 2A,B) and low salt (Figure 2C,D). N-TM and DR-TM have the same high circular dichroism ellipticity at ≤35 °C in high salt or at <30 °C in low salt, making it clear that DR-TM has reassembled properly to form the coiled-coil, α-helical structure characteristic of the tropomyosin molecule. However, this structure unfolds differently for the two tropomyosins. N-TM unfolds in one step at 44 °C in 1 M NaCl and at 36 °C in 5 mM NaCl, in agreement with previous work (Woods, 1976; Lehrer et al., 1984). On the other hand, DR-TM unfolds in a two-step process with the transitions taking place at 40 and 44 °C in 1 M NaCl and at 32 and 36 °C in 5 mM NaCl. Since DR-TM is composed of a mixture of two types of homodimers, we cannot yet assign the transitions. Therefore, one, or both, of the parallel, in-register homodimers of gizzard tropomyosin differs from the native tropomyosin with respect to the thermal stability of the α-helical structure.

Viscosity. N-TM and DR-TM also differ considerably with respect to their low-salt viscosity and thus their end-to-end interaction. When N-TM was dissociated by guanidinium chloride and reassociated by dialysis to form DR-TM, the

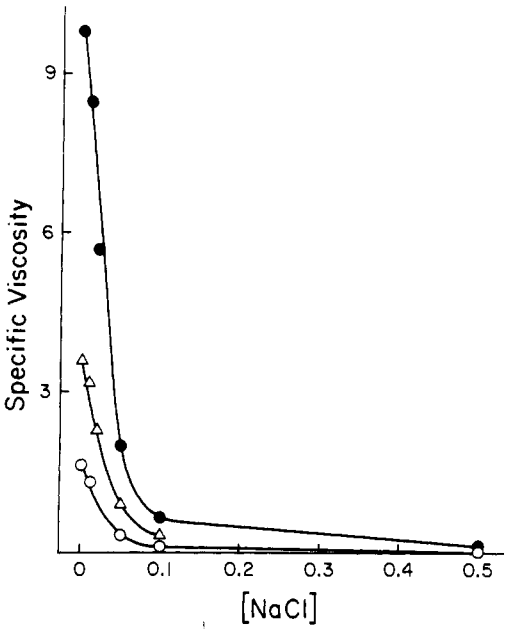

FIGURE 3: Salt dependence of the specific viscosity of tropomyosin at 0.8 mg/mL in varying concentrations of NaCl, 2 mM Mops, and 0.1 mM EDTA, pH 7.5, 24 °C. (●) N-TM, (△) DR-TM, (○) skeletal tropomyosin.

Table II: Low-Salt Viscosity of Skeletal Tropomyosin (SkTM) and Native (N-TM) and Dissociated–Reassociated (DR-TM) Gizzard Tropomyosins

tropomyosin at 0.8 mg/mL in 2 mM Mops and 0.1 mM EDTA, pH 7.5	specific viscosity at 24 °C
N-TM	8–14 (10)[a]
DR-TM	3–5 (6)
SkTM	1.1–1.6 (>10)

[a] Numbers in parentheses indicate the total number of determinations covering three to four different tropomyosin preparations.

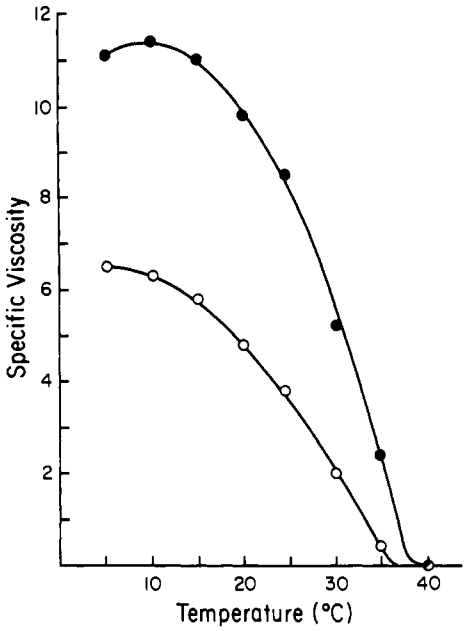

FIGURE 4: Temperature dependence of the specific viscosity of (●) N-TM and (○) DR-TM at 0.8 mg/mL in 5 mM NaCl, 2 mM Mops, and 0.1 mM EDTA, pH 7.5.

viscosity dropped dramatically but still remained greater than that of skeletal tropomyosin, over a wide range of ionic strengths (Table II, Figure 3). If N-TM was incubated at 0 °C in guanidinium chloride for varying times between 5 min and 2 h before reassociation, the low-salt viscosity of DR-TM always fell within the range shown in Table II. This indicates that the change in viscosity is most likely due to the process of dissociation–reassociation rather than to some time-dependent chemical reaction. Skeletal tropomyosin shows little or no change in viscosity when it is similarly dissociated and reassociated (Graceffa & Lehrer, 1986), indicating that guanidinium chloride has no general deleterious effects on tropomyosin.

The high end-to-end interaction of gizzard tropomyosin and its reduction by dissociation–reassociation do not appear to be due to an impurity. First of all, gel electrophoresis of gizzard tropomyosin shows no bands other than those of tropomyosin (Figure 1). In particular, these gels show no indication of caldesmon, which we have previously shown to interact with and thereby increase the low-salt viscosity of gizzard tropomyosin (Graceffa, 1987, 1988). Furthermore, when gizzard tropomyosin, which had a low-salt specific viscosity of 9.0, was purified by hydroxylapatite and ion exchange chromatography, the viscosity remained essentially unchanged with a value of 8.7. After a dissociation–reassociation cycle, the viscosity of this purified gizzard tropomyosin dropped to a value of 3.6, the same result we obtained with unchromatographed tropomyosin.

The low-salt viscosity of tropomyosin was generally measured at 24 °C (Figure 3, Table II). In order to be sure that the differences which we observed were not peculiar to that temperature, the viscosities of N-TM and DR-TM were measured as a function of temperature (Figure 4). The large difference in viscosity between the two forms of gizzard tropomyosin was maintained over the entire temperature range of 5–40 °C. Thus, the difference in end-to-end interaction between N-TM and DR-TM extends over a wide range of ionic strength (Figure 3) and temperature (Figure 4).

In some cases, the polypeptide chains of DR-TM were dissociated in 4–5 M guanidinium chloride and reassociated by a stepwise dialysis against decreasing concentrations of guanidinium chloride before dialysis against 1 M NaCl, yielding DRDR-TM. In this experiment, the initial decrease in low-salt specific viscosity from 9.5 to 4.5, corresponding to the transition from N-TM to DR-TM, was largely reversed to yield a value of 8.5 for the final product, DRDR-TM. This was accompanied by a reduction in the fraction of parallel, in-register homodimers from 82% for DR-TM to 50% for DRDR-TM (Figure 1, Table I). The two-step helix thermal unfolding profiles of DR-TM were also partially reversed upon formation of DRDR-TM (not shown). These results provide further evidence for a qualitative correlation between increased parallel, in-register homodimer population and decreased end-to-end interaction and an increased tendency to unfold with temperature in a two-step process. This ability to reverse the reduction in end-to-end interaction resulting from the formation of DR-TM also indicates that the low end-to-end interaction of DR-TM is not due to an irreversible alteration of tropomyosin by the guanidinium chloride treatment but to a reversible assembly of β and γ chains into in-register homodimers.

Discussion

We have shown that, although native gizzard tropomyosin does not readily form disulfide cross-links, gizzard tropomyosin dimer chains dissociated by guanidinium chloride and reassembled by dialysis against 1 M NaCl easily form disulfide cross-links, indicating the predominant formation of parallel, in-register homodimers. These homodimers have a much lower end-to-end interaction and a different thermal stability than the native tropomyosin. A recent chemical cross-linking study has provided evidence that native gizzard tropomyosin is predominantly composed of heterdimers (Sanders et al., 1986), which cannot form disulfide cross-links since the cysteines on the β and γ chains are in widely separated residues. Our results, together with this evidence, suggest that the physical properties, including end-to-end interaction and thermal stability, of the heterodimer and homodimers of gizzard tropomyosin differ considerably. This is a first step toward an understanding of the molecular basis of the high end-to-end interaction of smooth muscle tropomyosin.

Our ability to easily (i.e., oxidation for 2 h or less at 22 °C) form large amounts of disulfide-cross-linked homodimers in reassembled tropomyosin and only very small quantities in the native protein suggests that parallel, in-register homodimers are present in only minor amounts in native gizzard tropomyosin. This is consistent with the view that native gizzard tropomyosin is composed predominantly of heterodimers (Sanders et al., 1986). Previous studies by others and by us on native gizzard tropomyosin have reported only a fraction of disulfide-cross-linked dimers formed only after prolonged oxidation for 24–48 h (Strasburg & Greaser, 1976; Lehrer et al., 1984) and at a somewhat elevated temperature of 35 °C (Lehrer et al., 1984). However, such long periods and higher temperatures for oxidation may result in disulfide-cross-linked dimers which form via chain exchange and/or intermolecular cross-linking, as also suggested by Sanders et al. (1986), and which would thus not necessarily reflect the subunit composition of the native molecule.

Since the reassembled gizzard tropomyosin is composed of a mixture of homodimers, we cannot assign the two helix unfolding transitions which we have observed. However, a recent study has presented evidence that $\gamma\gamma$ homodimers are less thermally stable than $\beta\beta$ homodimers (Sanders et al., 1986), suggesting the possibility that the $\gamma\gamma$ homodimer is responsible for the lower temperature transition which we observed. Skeletal tropomyosin also unfolds in a two-step

process with temperature (Woods, 1969b; Satoh & Mihashi, 1972; Lehrer, 1978) which has been described as an equilibrium between the native molecule, a partially unfolded intermediate state, and the completely denatured tropomyosin (Woods, 1976; Graceffa & Lehrer, 1980, 1984; Betteridge & Lehrer, 1983). It is not certain whether this type of equilibrium contributes to the two-step unfolding of the reassembled gizzard tropomyosin.

The reassociation of dissociated gizzard tropomyosin chains appears to depend on the conditions during the reassociation. The reassociation of gizzard tropomyosin chains by stepwise dialysis against decreasing concentrations of guanidinium chloride results in about 25% of the $\beta\beta$ homodimer, 25% of the $\gamma\gamma$ homodimer, and about 50% of un-cross-linkable material (DRDR-TM; Figure 1, Table I). One possibility is that this un-cross-linkable material corresponds to heterodimer, since the heterodimer cannot form disulfide cross-links. If this is the case, then this would indicate that the chains reassociate in a random fashion. This contrasts to a direct dialysis against no guanidinium chloride where the chains preferentially form homodimers (41% $\beta\beta$ and 41% $\gamma\gamma$) (DRTM; Figure 1, Table I). Thus, the reversible assembly of tropomyosin chains appears to result in tropomyosin molecules of variable composition depending on the path taken to reassociate the chains. It is presently unclear why this should be the case, but this appears to be a general phenomenon of tropomyosin since it has been shown that dissociated skeletal tropomyosin chains also reassociate to form dimers of different compositions depending on the manner in which the molecules are refolded (Brown & Schachat, 1985; Holtzer et al., 1984). An understanding of this phenomenon may have important implications for the assembly of tropomyosin during its synthesis in vivo.

Our previous work has shown that gizzard tropomyosin, labeled at cysteine residues with pyrenylmaleimide, did not exhibit excimer fluorescence, for which it is necessary that two pyrene moieties be in close proximity (Lehrer et al., 1984). Since the protein was labeled in the dissociated state in guanidinium chloride before being reassembled by dialysis, we would expect that the tropomyosin would be present predominantly as in-register homodimers and that the pyrenes would thus be aligned and easily form excimers as is the case for skeletal tropomyosin (Betcher-Lange & Lehrer, 1978; Graceffa & Lehrer, 1980). One possible reason for the lack of excimer fluorescence is that there may be some conformational restraint on the ability of the pyrenes to properly approach one another [see discussion by Lehrer et al. (1981)]. Another possibility is that the labeled tropomyosin chains do not refold in the same manner as the unlabeled chains. Finally, the buffer conditions used to reassemble the pyrene-labeled dissociated chains were not exactly the same as those used in this work and possibly do not favor in-register homodimer formation.

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Differential Behavior of Two Cysteine Residues on the Myosin Head in Muscle Fibers†

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ABSTRACT: We have previously shown that the orientation of (iodoacetamido)tetramethylrhodamine labels on SH_1 thiol of S-1 moieties changes when MgADP is added to the fibers in rigor [Borejdo, J., Assulin, O., Ando, T., & Putnam, S. (1982) *J. Mol. Biol. 158*, 391–414. Burghardt, T. P., Ando, T., & Borejdo, J. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 7515–7519]. Here we report the results of experiments in which the SH_2 of S-1 was labeled with maleimidorhodamine. The specificity of modification of thiols was checked by measuring the stoichiometry of attached dye, by determining the extent of the decrease in EDTA(K^+)- and Ca^{2+}-ATPase activities, and by the localization of the dyes on peptides containing SH_1 and/or SH_2. Labeled S-1 was diffused into single glycerinated fibers of rabbit psoas muscle, and the orientation of chromophores was measured by fluorescence detected dichroism. The dye attached to SH_1 was oriented at 65° with respect to the fiber axis in rigor and at 51° in the presence of MgADP, regardless of whether SH_2 was modified or not. The dye on SH_2 was oriented near 42° both in the presence and in the absence of ADP, regardless of whether SH_1 was modified or not. Our results show that rhodamine oriented differently when attached to SH_2 compared with when attached to SH_1 and that in the former placement it was not sensitive to MgADP. We think this indicates that the SH_2-containing region has a mobility different from that of the SH_1-containing region, i.e., that this is evidence for internal flexibility of S-1.

The central problem in muscle contractility is the question of how myosin heads (S-1[1] moieties of myosin) interact with actin. One approach in investigating this problem has been to measure the orientation of fluorescent or spin-labeled molecules attached to a reactive cysteine residue on S-1 of myosin and to follow the S-1 orientation at different physiological states [for reviews see Morales et al. (1982) and Cooke (1986)]. By use of the fluorescent dipoles of (iodoacetamido)tetramethylrhodamine (IAR) (Borejdo et al., 1982; Burghardt et al., 1983) or of *N*-[[(iodoacetyl)amino]ethyl]-5-naphthylamine-1-sulfonic acid (1,5-IAEDANS) (Ajtai & Burghardt, 1987) on the SH_1 residue of a myosin head to detect the polar angle of the absorbance dipole, it was found that the angle in the rigor state is different from that in the presence of nucleotides. The same change in angle also occurs when the temperature is reduced from >2 to <−10 °C (Ajtai & Burghardt, 1986). On the other hand, Thomas and Cooke (1980) reported that the EPR signal of a spin label [maleimido spin label (MSL)] on SH_1 is insensitive to MgADP. This raises the possibility that some probes (e.g., IAR, 1,5-IAEDANS) rotate through a favorable angle for detecting angular motion, while others (e.g., MSL) rotate through an unfavorable angle. Alternatively, the ring system of one probe could rest on a structural niche (or domain) that moves, while the ring system of another could rest on a niche (or domain) that does not move.

In this paper, we modified SH_1 and SH_2 with several reagents in an attempt to compare the angular reorientations of SH_1- or SH_2-labeled regions of S-1 in response to the addition of nucleotides. In both cases, modified S-1 retained actin- and ATP-binding abilities. IAR was located almost exclusively on the SH_1 residue and maleimidotetramethylrhodamine (MLR) on the SH_2 residue of S-1. Labeled S-1 was diffused into single glycerinated rabbit psoas fibers in rigor solution and bound to the I-bands. Following addition of MgADP there was a change in dipole orientation for a dye attached to SH_1 but not for a dye on SH_2. The dipoles were found to be firmly attached to the protein, and hence we

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[1] Abbreviations: S-1, myosin subfragment 1; SH_1, reactive cysteine residue 1; SH_2, reactive cysteine residue 2; FDNB, 2,4-dinitrofluorobenzene; IAA, iodoacetamide; NEM, *N*-ethylmaleimide; IAR, (iodoacetamido)tetramethylrhodamine; 1,5-IAEDANS, *N*-[[(iodoacetyl)-amino]ethyl]-5-naphthylamine-1-sulfonic acid; MSL, maleimido spin label; MLR, maleimidotetramethylrhodamine; ϵ-ATP, 1,N^6-ethenoadenosine triphosphate; DTT, dithiothreitol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; TES, 2-[[tris(hydroxymethyl)methyl]amino]ethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; kDa, kilodaltons; LD, linear dichroism; FP, fluorescence polarization = polarization of fluorescence.

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