Tropomyosin Lysine Reactivities and Relationship to Coiled-Coil Structure†

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ABSTRACT: We have carried out a detailed analysis of tropomyosin structure using lysines as specific probes for the protein surface in regions of the molecule that have not been investigated by other methods. We have measured the relative reactivities of lysines in rabbit skeletal muscle α,α-tropomyosin with acetic anhydride using a competitive labeling procedure. We have identified 37 of 39 lysines and find that they range 20-fold in reactivity. The observed reactivities are related to the coiled-coil model of the tropomyosin molecule [Crick, F. H. C. (1953) *Acta Crystallogr. 6*, 689–697; McLachlan, A. D., Stewart, M., & Smillie, L. B. (1975) *J. Mol. Biol. 98*, 281–291] and other available chemical and physical information about the structure. In most cases, the observed lysine reactivities can be explained by allowable interactions with neighboring amino acid side chains on the same or facing α-helix. However, we found no correlation between reactivity and helical position of a given lysine. For example, lysines in the outer helical positions included lysines of low as well as high reactivity, indicating that they vary widely in their accessibility to solvent and that the coiled coil is heterogeneous along its length. Furthermore, the middle of the molecule (residues 126–182) that is susceptible to proteolysis and known to be the least stable region of the protein also contains some of the least and most reactive lysines. We have discussed the implications of our results on our understanding the structures of tropomyosin and other coiled-coil proteins as well as globular proteins containing helical regions.

Tropomyosin is a regulatory protein found in muscle and nonmuscle cells, and it functions in association with actin and troponin to confer calcium sensitivity in vertebrate skeletal and cardiac muscle (Ebashi & Endo, 1968; Weber & Murray, 1973; Leavis & Gergely, 1984). In addition to its role in regulation and associated cooperativity, tropomyosin interests biophysicists as a model protein because of its α-helical coiled-coil structure (Crick, 1953; Cohen & Szent-Gyorgyi, 1957; Wu & Yang, 1976). Although all tropomyosins have the same fundamental structure, rabbit muscle tropomyosin has been studied in the greatest detail. It is a coiled coil of two parallel polypeptides that are in register (Johnson & Smillie, 1975; Lehrer, 1975; Stewart, 1975). Analysis of the published amino acid sequence of α-tropomyosin (Sodek et al., 1978; Stone & Smillie, 1978) has revealed the presence of periodicities due to the α-helical repeat, gene duplication, and nonhelical periodicities postulated to be related to the periodic binding of tropomyosin to actin (McLachlan et al., 1975; McLachlan & Stewart, 1975, 1976; Parry, 1975; Smillie et al., 1980). A detailed theoretical molecular model has resulted from these studies.

Tropomyosin is a coiled coil except for the extreme ends of the molecule. However, the stability is not uniform along the length of the molecule as heat and salt denaturation studies, calorimetric measurements of the intact molecule as well as peptides, motions of tropomyosin in the crystal lattice, and preferential sites of proteolytic digestion show (Ooi, 1967; Wood, 1969; Pont & Woods, 1971; Eckard & Cowgill, 1976; Gorecka & Drabikowski, 1977; Pato & Smillie, 1978; Ueno & Ooi, 1978; Phillips et al., 1979, 1981; Edwards & Sykes, 1980; Pato et al., 1981; Williams & Swenson, 1981; Potekhin & Privalov, 1982; Stewart & Roberts, 1983; Ueno, 1984). The results of many experiments indicate that the COOH-terminal half of the molecule is less stable than the NH_2-terminal half.

α-Tropomyosin contains a single cysteine per chain that can be modified without greatly altering biological activity, depending on the reagent. Until now, cysteine-190 has served as the major structural probe for tropomyosin. A region of preferential instability surrounds Cys-190, interpreted to be due to localized chain separation (Betcher-Lange & Lehrer, 1978; Lehrer, 1978; Betteridge & Lehrer, 1983). The state of oxidation or type of modification of the cysteines can have long-range effects: it can alter the susceptibility to proteolysis in the region 60 residues toward the NH_2-terminus (Gorecka & Drabikowski, 1977; Ueno, 1984); the NMR resonance of histidine-153, 5.5 nm toward the NH_2-terminus, is changed (Edwards & Sykes, 1980), and the degree of polymerization that involves the extreme ends of the molecule is affected (Graceffa & Lehrer, 1980).

Experimental investigation of tropomyosin structure has been limited by difficulties in obtaining high-resolution crystals and by the presence of few side chains suitable for specific modification. In the present study we have used the reactivities of lysine residues as a probe for the surface topography of tropomyosin. Tropomyosin contains 39 lysines distributed throughout the sequence, allowing us to probe the surface structure in regions of the molecule that have not been investigated by using other methods and to test the theoretical model. Lysines were labeled with acetic anhydride by using the competitive labeling method of Kaplan et al. (1971), modified for the present application by Hitchcock et al. (1981). The observed lysine reactivities were interpreted in terms of the coiled-coil model of tropomyosin, as well as structural and dynamic features of the protein. Our results with tropomyosin

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relate to models of other coiled coils as well as topography of non-coiled-coil proteins. A preliminary report on part of this research was presented elsewhere (1984).

Materials and Methods

Preparation of Tropomyosin for Labeling. Tropomyosin was prepared from the back and thigh muscles of New Zealand white rabbits. The precipitate following isoelectric fractionation during troponin preparation (Hitchcock et al., 1981) was resuspended in 1 M NaCl, 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.5, and 0.5 mM dithiothreitol, adjusted to pH 7 with 1 M Trizma base, and dialyzed against the same buffer. Insoluble material was removed by centrifugation for 15 min at 20000 rpm in an SS 34 rotor. The protein precipitating between 40% and 60% ammonium sulfate saturation (Schwarz/Mann: 40% = 243 g/L, 60% = 132 g/L) was collected by centrifugation as before and dialyzed against 1 M KCl, 1 mM phosphate buffer, pH 7, and 0.5 mM dithiothreitol. α,α- and α,β-tropomyosins were separated on hydroxylapatite (Bio-Gel HT, Bio-Rad) (Eisenberg & Keilley, 1974) with a 1–200 mM linear phosphate gradient. Only α,α-tropomyosin was used in the present study. Purity was monitored on 12% sodium dodecyl sulfate (SDS)–polyacrylamide gels (Laemmli, 1970). All operations were carried out at 0–4 °C. On occasion, the hydroxylapatite column was run at room temperature.

Labeling of Lysine Residues with Acetic Anhydride. A detailed description of trace labeling of lysines with acetic anhydride has been published (Hitchcock et al., 1981).

We labeled tropomyosin in two different ionic conditions. The first, close to physiological ionic strength, was 0.1 M NaCl, 10 mM Tris-HCl, pH 7.5, 2 mM $MgCl_2$, 0.01 mM $CaCl_2$, 0.5 mM dithiothreitol, and 0.1 mM NaN_3. The second was the same buffer containing 0.6 M NaCl. The tropomyosin was dialyzed against the buffers prior to labeling and centrifuged for 2 h at 28000 rpm in a Beckman 30 rotor to remove any aggregated material. Additional dithiothreitol (0.5 mM) was added to each sample immediately prior to labeling to ensure complete reduction of cysteines; 0.24 μmol of tropomyosin at a concentration of 24 μM was used for each labeling condition.

Freshly opened [^{3}H]acetic anhydride (10 Ci/mmol; Amersham) was resuspended in acetonitrile. Two 5-μL aliquots were added at 10-min intervals, a total of 0.5 μmol acetic anhydride per tropomyosin sample. Labeling was carried out at 25 °C with shaking. Following labeling, the tropomyosin was dialyzed exhaustively against 20 mM NH_4HCO_3 to remove unreacted reagent and lyophilized. By use of this procedure, only 0.1–0.2% of the lysines were modified.

Tropomyosin was labeled in the denatured state with [^{14}C]acetic anhydride. Lyophilized unlabeled tropomyosin was dissolved in 6 M guanidine hydrochloride (Schwarz/Mann) and 20 mM triethanolamine, pH 9, and dialyzed against the above buffer. Dithiothreitol was added to 5 mM, and the protein was incubated for 1 h at 45 °C to ensure complete reduction of cysteines. Tropomyosin (86 μM, 0.36 μmol) was labeled with 10 μmol of [1-^{14}C]acetic anhydride (29 mCi/mmol; Amersham). The [^{14}C]acetic anhydride was resuspended in 250 μL of acetonitrile and added in 25-μL aliquots at 30-min intervals with stirring at room temperature. Following labeling, the tropomyosin was dialyzed exhaustively against 50 mM NH_4HCO_3 to remove unreacted isotope. ^{14}C allows comparison of the reactivity of the peptides in the native state (^{3}H) to that in the denatured state when all the lysines should have the same reactivity. For this comparison to be valid, the specific activities of all lysines should be the same in 6 M guanidine hydrochloride. We have shown this to be the case in our studies of other proteins, and in 6 M guanidine hydrochloride tropomyosin is known to be completely random coil (Woods, 1969; Pont & Woods, 1971).

The [^{3}H]tropomyosin was resuspended in 50 mM NH_4HCO_3 and combined with [^{14}C]tropomyosin to give a convenient ^{3}H/^{14}C ratio (>5). It was freeze-dried and resuspended in 6 M guanidine hydrochloride 5 mM EDTA, 20 mM triethanolamine, pH8, 0.1 mM TPCK [L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; Sigma), 0.1 mM TLCK (N^{α}-*p*-tosyl-L-lysine chloromethyl ketone; Sigma), 0.1 mM PMSF (phenylmethanesulfonyl fluoride; Sigma). Six milligrams of unlabeled tropomyosin was added to each sample to increase the total amount of tropomyosin. The tropomyosin was reduced and alkylated, maintaining the pH at 8 during alkylation (Hirs, 1967). The tropomyosin was then completely chemically labeled with an excess of unlabeled acetic anhydride (6600 molar excess) while maintaining the pH between 8.5 and 9.5 by addition of 10 N NaOH. The chemically modified protein was desalted on a Sephadex G-25 course column in 50 mM NH_4HCO_3 and lyophilized.

Enzymatic Digestion of Acetylated Tropomyosin. [^{14}C,^{3}H]-Acetylated tropomyosin was resuspended in 50 mM NH_4HCO_3 to give a concentration of 1 mg/mL. TPCK-treated trypsin (Sigma) was added at a 1:100 molar ratio, and the mixture was incubated for 4 h at 37 °C and then lyophilized. Trypsin does not cleave after acetylated lysines. We experienced significant chymotryptic activity in the trypsin as some of the observed cleavages of isolated peptides indicate.

Major peptides that contained multiple lysines were redigested with *Staphylococcus aureus* V8 protease (Miles) or TLCK-treated chymotrypsin (Sigma). For *S. aureus* protease digestion, the peptide was suspended in 50 mM NH_4HCO_3, pH 7.8, and 2 mM EDTA, enzyme was added at a ratio of 1:30 to peptide, and the mixture was incubated at 37 °C for 16 h. The resulting digest was frozen or lyophilized. Digestion with chymotrypsin was performed in 50 mM NH_4HCO_3 for 4 h at 37 °C at a ratio of 1:50 to peptide. The sample was frozen or lyophilized after digestion.

Separation of Peptides. Trypsin digests were chromatographed on a DE-52 cellulose column (Whatman, 1 × 15 cm), equilibrated, and washed with 10 mM NH_4HCO_3 before initiation of linear gradient of from 10 to 1000 mM NH_4HCO_3. Aliquots of each fraction were analyzed by thin-layer chromatography (Polygram Sil G plates, Macherey-Nagel in 1-butanol/H_2O/acetic acid, 1/1/4), and peptide-containing fractions were pooled and lyophilized.

The peptides were further fractionated by using reverse-phase high-performance liquid chromatography (HPLC) on a C3 column (Beckman RSPC). Peptides were eluted with an acetonitrile gradient in 0.05% trifluoroacetic acid (Pierce). The gradient used depended on the peptides separated, but a typical gradient was linear, 0–50% acetonitrile, at 1 mL/min for 100 min. The gradient was generated by using a Waters Model 720 system controller with M45 and M6000 pumps. The effluent was monitored at 214 nm on a Beckman 160 detector and 1-min fractions were collected. Fractions were counted and analyzed by thin-layer chromatography. Fractions that contained single peptides, as judged by staining with fluorescamine (Sigma) and autoradiography, were counted for ^{3}H/^{14}C and analyzed for amino acid composition. Redigested peptides were chromatographed on a C3 or C18 (Bio-Rad, RP318) column. Peptides that did not bind to the C3 column or that were poorly separated were rechromatographed on a C18 column (Waters, μBondapak, or Bio-Rad, RP318). Pure

peptides that were identified in the published amino acid sequence are listed in Table I, and the chromatographic history of each peptide is indicated.

Analysis of Peptides. Acetylated peptides were hydrolyzed in 6 N HCl and 1% phenol at 110 °C in sealed, evacuated tubes for 16 h. Amino acid analyses were carried out on a Durrum D500 amino acid analyzer. The NH_2-terminal residues of the peptides were determined by using thin-layer chromatography on micropolyamide sheets (Analtech) of hydrolysates of dansylated peptides (Hartley, 1970). The results were used to confirm the position of the peptide in the sequence based on amino acid composition.

Identification of Lysine-Containing Peptides. We were able to identify 37 of 39 lysines in 22 peptides. Fourteen lysines were isolated in peptides containing single lysines; nine lysines were present in four peptides containing multiple lysines because the lysines are adjacent to each other in the sequence and were not separable by our digestions. Nine lysines were isolated in four peptides separated by one or more amino acids but were not cleaved enzymatically in our experiments. The $^3H/^{14}C$ ratios of five lysines in three peptides were calculated as described in Figure 1.

Table I shows the amino acid compositions of peptides we used in our analysis of tropomyosin labeled in 0.1 M NaCl. Common contaminants include Asp, Glu, and occasionally Gly. In most cases the observed amino terminus agreed with that expected. Unfortunately we lost several analyses due to failure of the dansyl procedure, and occasionally no result was obtained because the amount available for analysis was too low for detection. We frequently failed to detect Glu as the NH_2-terminus, a difficulty we have experienced previously (Hitchcock-DeGregori, 1982). In most cases the peptides were pure and identification was straightforward. Lys-51 is contaminated with Asp and Ala, but these fractional amounts are high because the overall analysis was rather low. We are confident of this identification and that of Lys-48 and -49 because they came from a chymotryptic digestion of a peptide containing all three lysines. In addition, the NH_2-terminal residues agree with those expected. For Lys-118, the lysine was not used for normalization of the composition because of a large NH_3 peak which interfered with lysine and is responsible for an artifactually high calculation for lysine. In Lys-136 and -140, there is lower than the expected amount of methionine, due to oxidation. The Asp is high due to a leading shoulder which we presume to be due to methionine sulfone. The identification of the peptide we believe to contain Lys-189 was difficult because the dansyl analysis failed and because cleavage after Glu is not one expected for trypsin or chymotrypsin. We have identified it as residues 188–193 for two reasons. First, it contains a stoichiometric amount of Gly, an unusual residue in tropomyosin, and the analysis is too high for it to be present as a contaminant in the observed amount. Second, the material identified as (carboxymethyl)cysteine eluted slightly earlier than Asp in the analysis of standards, where (carboxymethyl)cysteine would be expected. In addition there is a smaller peak following the main peak which we assume to be contaminating Asp. Lys-198,205 is contaminated by Asp and is low in Thr. The overall analysis is low, and it is possible that some Thr and Ser were lost during hydrolysis. Lys-264 is low in Ala, and Lys-268 is contaminated by Glu. In general, we are confident with the identifications, and many of these peptides were isolated and identified more than once.

Isotope Counting. Radioactive samples were counted in ACS aqueous counting scintillant (Amersham) in a Traycor Mark III liquid scintillation counter equipped to do dpm calculations. Samples were counted so the error was less than 1%.

Chemicals. All chemicals were of reagent grade and special suppliers are indicated in the text. The pH of all the buffers was adjusted at room temperature. Water used was house deionized passed through an organic removal cartridge and a high purity ionic exchange resin and then glass distilled. Water used for the HPLC was collected in a glass container shortly before use.

Results and Discussion

Rationale. We have used the reactivities of tropomyosin lysines with acetic anhydride as a probe for the surface topography of the molecule and to allow analysis of regions of the structure that have not been studies previously. Lysine is a good probe: the 39 lysine residues are well distributed in the sequence and they occupy all but one of the helical positions in the coiled coil (Crick, 1953). The profile of reactivities can be related to the coiled-coil model for tropomyosin (McLachlan & Stewart, 1975). Although McLachlan and Stewart recognized the helicity was not perfect along the length of the molecule, their model describes tropomyosin as a perfect coiled coil. Can individual lysine reactivities be understood in terms of the coiled-coil structure? Does the profile of reactivities reflect the known regions of disorder and instability in the tropomyosin coiled-coil as well as the nonhelical ends involved in head-to-tail overlap? Is there any relationship between the pattern of lysine reactivities and the postulated periodic actin binding sites (McLachlan et al., 1975; McLachlan & Stewart, 1975, 1976; Parry, 1975; Smillie et al., 1980)? We have addressed these questions in the analysis of our results.

Since the basic elements of tropomyosin structure are relatively simple and fundamental, our observations have relevance for the structure of other coiled coils as well as for helical regions within globular proteins. Is there a relationship between helical positions and reactivity? Are lysines on the outside of the molecule more reactive than those in or near the helical interface? Surprisingly, the answer to both these questions is no.

We have modified lysines with acetic anhydride because it is a small uncharged reagent accessible to large numbers of groups that specifically acetylate the ϵ-amino group of lysine in the conditions of our experiments. The principle of the method is that in the presence of a trace chemical amount of radioactive label amino groups will compete for the acetic anhydride. Reactivity of a group depends on steric factors as well as its pK_a and nucleophilicity (Kaplan et al., 1971). On the basis of previous studies using acetic anhydride to modify proteins whose structures have been solved crystallographically, it is reasonable to assume that reactivity reflects properties of the structure and is related to the accessibility of amino groups to the solvent (Kaplan et al., 1971; Kaplan, 1972; Bresciani, 1977).

The importance of competitive trace labeling with [^{3}H]acetic anhydride is that the protein is not significantly chemically modified and that the reactivities of the tritiated lysines reflect the native conformation of the protein. The chance of modifying more than one lysine in an individual protein molecule is statistically negligible. Therefore, structural changes that have occurred as a result of modification will not be detected by using the procedure. We assume that differences observed in acetylation of lysines in the native state (as reflected by the $^3H/^{14}C$ ratio) are due to the secondary, tertiary, and quaternary structure of the native protein since the tropomyosin is in a random coil when labeled with [^{14}C]acetic anhydride.

Table I: Amino Acid Compositions of Tropomyosin Peptides[a]

amino acid	Lys-5,6,7 res 1–8		Lys-12,15 res 12–21		Lys-29,30 res 22–35		Lys-37 res 36–39		Lys-48,49 res 47–50		Lys-51 res 51–53	
	obsd	seq	obsd	seq	obsd	seq	obsd	seq	obsd	seq	obsd	seq
Asp	1.3	1	2.9	3	2.0	2					0.6	
Thr											0.9	1
Ser							0.8	1	1.1	1		
Glu			1.5	1	4.2	4	1.1	1				
Pro												
Gly	0.3										1.1	1
Ala	1.2	1	1.2	1	4.1	5					0.6	
Val												
Met	1.9	2										
Ile	1.0*	1										
Leu			1.9*	2			1.0*	1	1.1*	1		
Tyr			0.4									
Phe												
His												
Lys	3.0*	3	1.9*	2	2.0*	2	1.0*	1	1.8*	2	1.0*	1
Arg			1.1*	1	1.0*	1						
NH_2-term	failed	acet	Lys	Lys	Ala	Ala	failed	Ser	Glu	Gln	Lys	Lys
digestion	tryp, chymo		tryp, chymo		tryp		tryp		tryp, chymo		tryp, chymo	
chrom	C3		C3		C18				C3		C3	

amino acid	Lys-59 res 57–62		Lys-65 res 64–68		Lys-76,77 res 72–80		Lys-112 res 106–115		Lys-118 res116–124		Lys-128 res 126–133	
	obsd	seq	obsd	seq	obsd	seq	obsd	seq	obsd	seq	obsd	seq
Asp	0.7	1	0.9	1	1.5	1	0.9	1	1.2	1		
Thr					0.8	1						
Ser	0.9	1	0.2						0.9	1	1.0	1
Glu	1.6	1	1.3	1	2.7	3	3.2	3	3.6	3	1.2	1
Pro												
Gly											1.2	1
Ala			0.8	1			2.0	2				
Val											0.8	1
Met											1.1	1
Ile											0.7*	1
Leu	1.0*	1	1.0*	1	1.0*	1	3.0*	3	3.0*	3		
Tyr	0.7	1										
Phe												
His												
Lys	1.0*	1	1.0*	1	2.0*	2	1.0*	1	1.3	1	1.2*	1
Arg											1.2*	1
NH_2-term	Leu	Leu	low	Leu	nothing	Glu	Leu	Leu	Ala	Ala	Gly	Gly
digestion	tryp, *S. aur.*		tryp		tryp, *S. aur.*		tryp, *S. aur.*		tryp, *S. aur.*		tryp	
chrom	RP318		C3		C3		C3		C3			

amino acid	Lys-136,140 res 134–141		Lys-149 res 146–150		Lys-152 res 151–160		Lys-161 res 161–167		Lys-189 res 188–193		Lys-198,205 res 194–207	
	obsd	seq	obsd	seq	obsd	seq	obsd	seq	obsd	seq	obsd	seq
CM-Cys									1.4	1		
Asp	1.3	1			2.1	2					1.5	1
Thr											0.5	1
Ser											0.8	1
Glu	3.2	3	2.0	2	1.1	1	2.0	2	0.9	1	2.6	3
Pro												
Gly									1.0	1		
Ala	1.6	1			3.0	3	1.0	1	0.8	1	1.2	1
Val							1.0	1			0.8	1
Met	0.6	1										
Ile			1.0*	1	0.9*	1						
Leu			1.0*	1			1.0	1	0.7	1	1.9*	2
Tyr												
Phe												
His					1.0	1						
Lys	2.0*	2	1.0*	1	1.0*	1	1.0*	1	1.0*	1	2.2*	2
Arg					1.0*	1	1.0*	1				
NH_2-term	failed	Ala	Ile	Ile	Ala	Ala	Lys	Lys	failed	Glu	failed	Glu
digestion	tryp		tryp, *S. aur.*		tryp, *S. aur.*		tryp		tryp		tryp	
chrom	C18		C3		C3		C18					

amino acid	Lys-213,217,220, 226,231,233 res 211–238		Lys-226,231,233 res 225–234		Lys-248 res 245–249		Lys-248,251 res 245–256		Lys-248, 251,264, 266,268 res 245–274		Lys-264 res 261–265		Lys-268 res 268–271	
	obsd	seq	obsd	seq	obsd	seq	obsd	seq	obsd	seq	obsd	seq	obsd	seq
Asp	2.6	2	1.1	1			2.1	2	3.7	3				
Thr	1.0	1			0.9	1	0.9	1	1.3	1				

Table I (Continued)

amino acid	Lys-213,217,220, 226,231,233 res 211–238 obsd	seq	Lys-226,231,233 res 225–234 obsd	seq	Lys-248 res 245–249 obsd	seq	Lys-248,251 res 245–256 obsd	seq	Lys-248, 251,264, 266,268 res 245–274 obsd	seq	Lys-264 res 261–265 obsd	seq	Lys-268 res 268–271 obsd	seq
Ser	2.1	2	0.9	1	1.0	1	1.8	2	2.2	3			0.8	1
Glu	8.8	8	1.2	1	0.2		1.4	1	6.4	6	1.2	1	0.4	
Pro														
Gly					0.2									
Ala	1.8	2			0.2				1.9	2	0.6	1	1.0	1
Val	1.1	1	1.1	1			0.9	1	1.1	1				
Met														
Ile	1.0*	1	0.9*	1			1.0*	1	2.0	2			0.9*	1
Leu	2.7	2	2.2	2	1.0*	1	2.1*	2	4.9*	5	1.0*	1		
Tyr	2.0	2							2.0	2	0.9*	1		
Phe														
His														
Lys	6.0*	6	2.9	3	1.0*	1	2.0*	2	5.1*	5	1.0*	1	1.1*	1
Arg	0.9	1												
NH_2-term	low	Lys	Ile	Ile	failed	Ser	low	Ser	Ser	Ser	failed	Tyr	Lys	Lys
digestion	tryp		tryp, *S. aur.*		tryp		tryp		tryp		tryp		tryp	
chrom	RP318		RP318		RP318									

[a] The amount of each amino acid was normalized to an average, taking the values of one or more amino acids as integral in the composition. Those amino acids are indicated with an asterisk. Values of less than 0.2 after normalization were not included in the table. The observed NH_2-terminus was determined by dansylation; the expected is that in the published sequence. All peptides are the result of tryptic digestion followed by chromatography on DEAE and C3 columns. Further digestion with chymotrypsin (chymo) or *S. aureus* V8 protease and/or additional chromatographic separation are indicated. Abbreviations: res, residues; NH_2-term, NH_2-terminus; chrom, chromatographic method; tryp, trypsin; *S. aur.*, *S. aureus*; chymo, chymotrypsin.

Lysine Reactivities. Tropomyosin was labeled in two ionic conditions, as described under Materials and Methods. We obtained our most complete data set for tropomyosin labeled in 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.5, 2 mM $MgCl_2$, 0.01 mM $CaCl_2$, 0.5 mM dithiothreitol, and 0.1 mM NaN_3, isolating 37 lysines in 22 peptides. The amino acid compositions are in Table I and are discussed under Materials and Methods. A less complete analysis was made of tropomyosin labeled in the same buffer containing 0.6 M NaCl. Since the profile of reactivities was similar to that labeled at physiological ionic strength, we did not search exhaustively for the remaining lysines.

The profile of the relative reactivities of the lysines of tropomyosin labeled with [^{3}H]acetic anhydride in 0.1 M NaCl is shown in Figure 1. The $^3H/^{14}C$ ratio is an arbitrary number (see Materials and Methods) that reflects the reactivity of lysines in the native structure. There is a wide range of reactivities, with the most reactive lysine being 20 times as reactive as the least reactive, the widest of any protein we have studied using this method (Hitchcock et al., 1981; Hitchcock, 1981; Hitchcock-DeGregori, 1982; Hitchcock-DeGregori et al., 1982). Since tropomyosin is largely α-helical along its length (except for the extreme ends), we were surprised to see such a wide range of reactivities, given that most of the lysines are on the outside of the helix. We suggest that many of these differences in reactivities could be due to local interactions such as formation of salt bridges with neighboring carboxyl groups or hydrophobic interactions via the hydrocarbon side chain (see below). Most of the observed reactivities can be explained in these terms without invoking longer range effects such as differences in helical stability.

The profile of relative reactivities of tropomyosin labeled in 0.6 M NaCl is similar to that of tropomyosin labeled in 0.1 M NaCl (Table II). Since the $^3H/^{14}C$ ratio is an arbitrary number, we calculated the ratio of relative reactivities of tropomyosin labeled in the two ionic conditions. The ratios of the different peptides are in a narrow range, indicating that the profile of lysine reactivities is similar in the two labeling conditions. The greatest difference is seen in Lys-5,6,7 which is less reactive relative to other lysines in 0.6 M NaCl, although it is still among the most reactive peptides. The increase in reactivity of lysines in the helical region relative to those at the NH_2 end is consistent with the idea that ionic strength affects the strength of salt bridges stabilizing the coiled coil. In addition, there could be a general effect of ionic strength on reactivity. Woods (1969) showed that salt increases the thermal stability of tropomyosin, but the effects were minimal at the pH and temperature of our experiments.

Table II: Comparison of Tropomyosin Labeled at Different Salt Concentrations

lysine	salt concentration 0.1 M ($^3H/^{14}C$)	0.6 M ($^3H/^{14}C$)	0.1/0.6 M
5,6,7	12.7	12.9	0.98
12,15	5.5	9.1	0.60
29,30	1.5	2.1	0.71
37	3.7	7.1	0.52
76,77	0.8	1.2	0.67
112	2.9	3.8	0.76
118	1.6	2.4	0.67
128	1.8	4.5	0.40
136,140	2.8	4.7	0.60
149	9.0	16.1	0.56
161	1.8	3.9	0.46
226,231,233	2.0	5.4	0.37
264	7.1	15.8	0.45

The high reactivity of Lys-5,6,7 is in agreement with the results of Johnson & Smillie (1977), who observed that Lys-7 was the most reactive lysine in tropomyosin. Lys-5 and -6 were also quite reactive. It is not possible to compare our results in detail because their extent of chemical modification with acetic anhydride was much greater, and their method of data analysis did not include a measure of specific or relative reactivity. However, given these limitations our results seem to be in general agreement for most residues they identified (14 lysines).

Analysis of Data in Relation to the Theoretical Model of Tropomyosin. We have analyzed the observed relative reactivities in terms of the coiled-coil structure proposed for

Table III: Reactivities of Individual Lysines in Different Helical Positions

b		*c*		*e*		*f*		*g*	
Lys	$^3H/^{14}C$	Lys	$^3H/^{14}C$	Lys	$^3H/^{14}C$	Lys	$^3H/^{14}C$	Lys	$^3H/^{14}C$
37	3.7	59	1.5	152	7.7	118	1.6	112	2.9
51	2.3	128	1.8	264	7.1	251	0.7	161	1.8
65	16.1	248	8.6	266	6.7			189	14.7
149	9.0								
268	6.5								

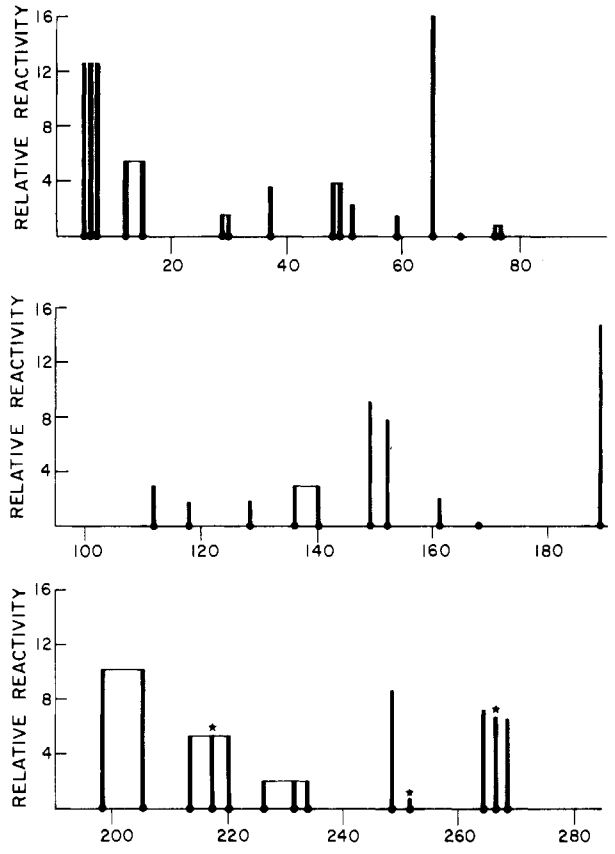

FIGURE 1: Relative reactiviites of tropomyosin lysines. The relative reactivities ($^3H/^{14}C$ ratio) of 37 of 39 tropomyosin lysines are shown. The position of each lysine in the amino acid sequence is indicated. Lysines whose bars are connected across the top were isolated in a single peptide. The ratios of those with stars were calculated. For example, we isolated Lys-251 in a peptide with Lys-248. In addition, Lys-248 was isolated individually. This allowed calculation of a $^3H/^{14}C$ ratio for Lys-251 = $(R_{248,251})(2) - R_{248} = R_{251}$ ($R = {}^3H/^{14}C$ ratio). The $^3H/^{14}C$ ratios for Lys-213,217,220 and Lys-266 were also calculated in this manner.

tropomyosin by McLachlan & Stewart (1975). Figure 2 shows the amino acid sequence of tropomyosin drawn on a helical net following the notation of McLachlan and Stewart. In general, the observed reactivities can be explained by the interhelical and intrahelical interactions allowable in the model. We constructed CPK models of lysine-containing regions of tropomyosin to aid in the analysis.

Relationship between Reactivity and Helical Position. Lysine residues are present in all helical positions, except *d*, and there is no obvious relationship between position and reactivity (Table III). Lysines in the helical interface (*a*) were not isolated individually. The *e* and *g* lysines at the helical interface vary widely in reactivity as do those in the outer helical positions, *b*, *c*, and *f*. From this, we can make two generalizations: lysines in a given helical position do not necessarily have similar environments, and although the basic structure is a coiled coil, the character of the surface is nonuniform along the length. Lysines present on the outsides of proteins are usually expected to be accessible to solvent. It

Table IV: Interhelical Interactions[a]

lysine	position	$^3H/^{14}C$	facing residues on other α-helix[b]
5(6)	1*e* (1*f*)	12.9	—, Ala, Ile, Lys
7	1*g*		Lys, Met, Gln, Lys
12	2*e*	5.5	Lys, Leu, Met, Asp
15	3*a*		Leu, Lys, Ala, Asp
29(30)	5*a*(5*b*)	1.5	Ala, Lys, Ala, Asp
49(48)	7*g*(7*f*)	3.8	Glu, Leu, Lys, Gln
77(76)	11*g*(11*f*)	0.8	Glu, Ala, Thr, Glu
112	16*g*	2.9	Glu, Leu, Glu, Leu
140(136)	20*g*(20*c*)	2.8	Glu, Met, Glu, Glu
152	22*e*	7.7	Gln, Ala, Glu, Ile
161	23*g*	1.8	Ala, Tyr, Glu, Asp
189	27*g*	14.7	Glu, Cys, Ala, Glu
217(213) (220)	31*g*(31*c*) (32*c*)	5.4	Glu, Glu, Asp, Ser
231(226) (233)	33*g*(33*b*) (34*b*)	2.0	Glu, Leu, Lys, Ser
264	38*e*	7.1	Glu, Gln, Ala, Lys
266	38*g*	6.7	Ser, Tyr, Lys, Lys

[a] Lysines in the *a*, *e*, and *g* helical positions are included in this table. The possible interactions are based on the model of McLachlan & Stewart (1975). Primary interactions are underlined. Lysines isolated in peptides containing more than one lysine are indicated. [b] a = n*a*, n*d*, n*e*, n + 1*a*; e = n − 1*g*, n*c*, n*d*, n*g*; g = n + 1*e*, n + 1*a*, n + 1*b*, n*e*.

is surprising that the lysines in the outer helical positions vary widely in reactivity; one would expect them to be accessible (reactive) and minimally influenced by interactions with the opposite helix. However, they must be affected by interactions with neighboring side groups and reflect nonuniform features of the coiled coil. It is unlikely that the less reactive lysines are deeply buried in this structure. We anticipate these generalizations will be applicable to other coiled coils as well as globular proteins as chemical information of this type becomes available for proteins whose three-dimensional structures are known.

Interhelical Interactions. In tropomyosin, there is a high concentration of basic residues (Arg and Lys) at *g* and Glu and Asp at *e* (McLachlan & Stewart, 1975). In the coiled coil, the *e* and *g* positions face each other, and McLachlan & Stewart (1975) suggest that acidic and basic residues in these positions could be important in stabilizing the coiled coil through salt bridges.

In tropomyosin, 16 of 39 lysines are in *e* (4) or *g* (12) positions of the helix, and in all but four cases there is a Glu or Asp available for interaction in the ng (n*e*) or n + 1*g* (n + 1*e*) position of the other helix. (n is the number of the seven residue helical period, 1–41. See Figure 2.) The lysines present at the interface between the α-helices of the coiled coil and the facing residues on the opposite α-helix are listed in Table IV. Lysines with Asp or Glu residues available for interaction tend to have low relative reactivities. In contrast, when Lys or Gln and a hydrophobic residue are present in the primary interaction positions, the relative reactivity is higher. The most striking exception is Lys-189 which has a high

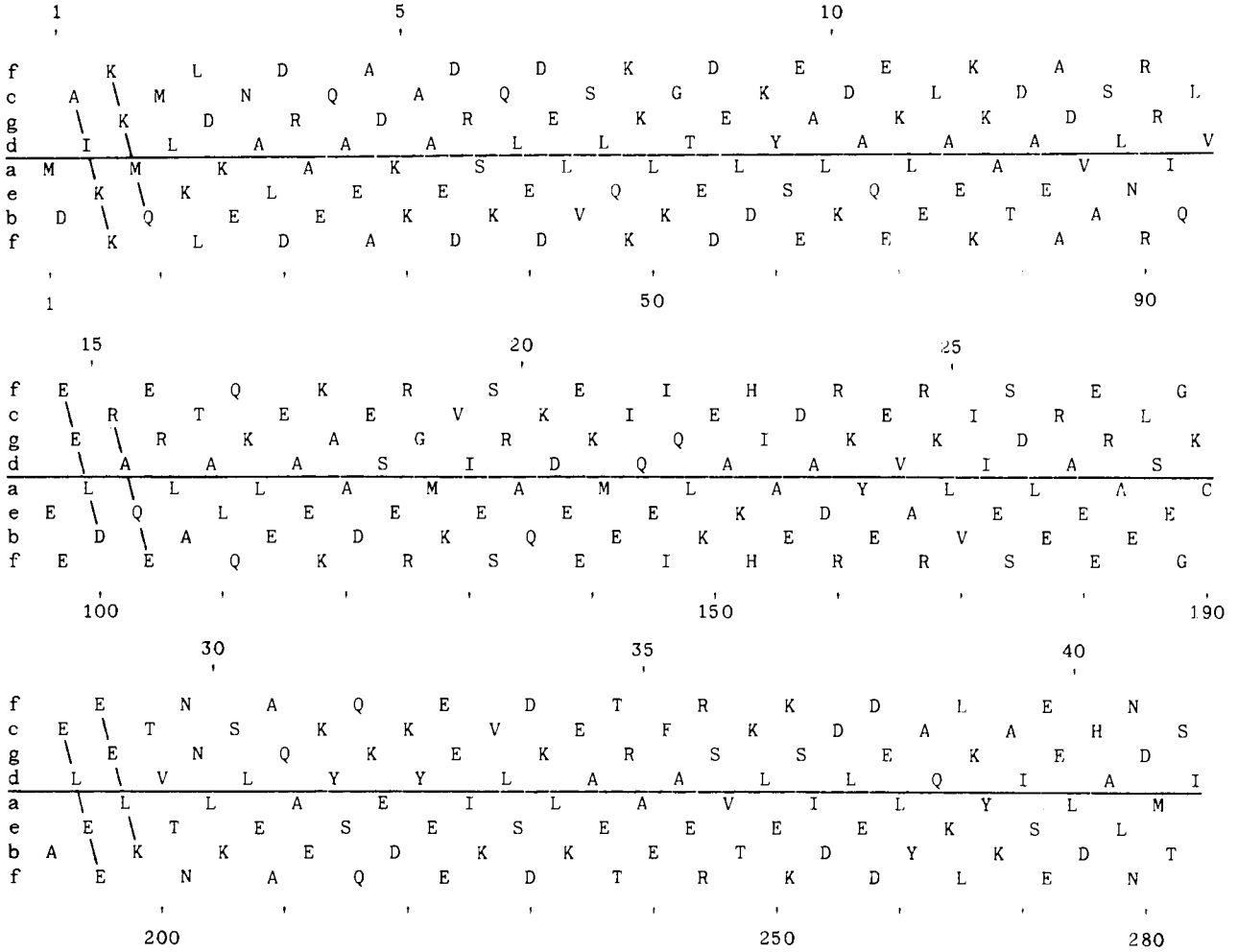

FIGURE 2: Amino acid sequence of α-tropomyosin (Sodek et al., 1978; Stone & Smillie, 1978) drawn on a helical net with 3.5 residues per turn according to the model of McLachlan & Stewart (1975). The sequence runs downward from left to right as indicated by the oblique lines. The numbers underlying the sequence refer to the residue numbers in the published sequence (1–284). The numbers overlying the sequence are the seven residue periods, beginning at position *a* (1–41). The line indicates the interface between the two helices.

relative reactivity even though there are two Glu residues for interaction. Lys-189 is next to Cys-190 which should be reduced in the conditions of labeling. Although reduced tropomyosin is a more stable structure than oxidized or cross-linked tropomyosin, there is still preferential unfolding in the region of Cys-190 (Lehrer, 1978) which may contribute to the high reactivity of Lys-189. While attractive *e* and *g* pairs may be involved in maintenance of tropomyosin structure, apparently they are not a general feature of coiled coils since relatively few such pairs are found in nematode myosin rod (McLachlan & Karn, 1982, 1983) or streptococcal M protein (Manjula et al., 1983).

From study of space-filling models we noticed that the interhelical *e*–*g* pairs were not always favorable, and in some cases intrahelical interactions seemed more likely to be important in determining lysine reactivity. On occasion, the bulky hydrophobic groups in the helical interface seemed to make the interaction of *e* or *g* lysines with a Glu on the other helix difficult. For example, Leu-50 (8*a*) and Thr-53 (8*d*) appeared to restrict the mobility of Lys-49 (7*g*). The bulky Tyr-163 (24*a*) seemed to prevent interaction of Lys-161 (23*g*) with Asp-159 (23*e*) and Glu-163 (24*b*). Similarly, Tyr-213 (31*d*) and Tyr-267 (39*a*) restrict interaction of Lys-213 (31*c*) and Lys-266 (38*g*) with residues on the opposing helix. However, space-filling models may not accurately reflect the steric aspects of hydrophobic interactions.

Two lysines are present in the predominantly hydrophobic *a* position (Lys-15 and -29). Because of lysine's long side chain, the amino group is outside the hydrophobic interface, and the hydrocarbon side chain might even stabilize the coiled coil. The lysines are relatively low in reactivity, implying they do not disrupt the structure, but whether the low reactivity is due to ionic interactions or inaccessibility by being close to the interface, we cannot say. Interpretation is difficult because the lysines were not isolated individually.

Intrahelical Interactions. Lysines in all helical positions have allowable polar or ionic interactions with other side groups on the same chain, summarized in Table V. In addition, in some instances hydrophobic interactions of the hydrocarbon side chain could affect the accessibility of the lysine amino group to solvent. As discussed above, there is no correlation between a particular helical position and reactivity. In general, the observed reactivities can be understood in terms of local interactions with other side groups.

The relative reactivities of certain lysine-containing peptides will now be discussed. For Lys-5,6,7, in addition to the absence of interhelical interactions, the large number of repulsive intrahelical side groups explains the high reactivity of this peptide. These lysines are in the end-to-end overlap region, and Johnson & Smillie (1977) have shown that acetylation of Lys-7 results in loss of tropomyosin polymerizability. In our labeling conditions the degree of end-to-end overlap would be minimal and we expected these lysines to be among the most reactive. Lys-12,15 has repulsive as well as attractive interactions. The restricted accessibility of Lys-15 in position 3*a* may also help explain the intermediate reactivity of this peptide. The low reactivity of Lys-28,30 can be explained by the neighboring acidic residues and lower accessibility of

Table V: Intrahelical Interactions[a]

lysine	position	$^3H/^{14}C$	neighboring charged and polar residues
5	1*e*		Lys-6, Gln-9
6	1*f*	12.7	Asp-2, Lys-5, Lys-7, Gln-9
7	1*g*		Lys-6
12	2*e*	5.5	Lys-15, Glu-16, Gln-9
15	3*a*		Lys-12, Glu-16
29	5*a*	1.5	Glu-33, Glu-36
30	5*b*		Glu-26, Glu-33, Asp-34
37	6*b*	3.7	Glu-33, Glu-40, Asp-41
48	7*f*	3.8	Lys-49, Lys-51, Gln-47
49	7*g*		no polar groups
51	8*b*	2.3	Lys-48, Glu-54, Asp-55
59	9*c*	1.5	Asp-55, Glu-56, Asp-58, Glu-62
65	10*b*	16.1	Glu-62, Asp-66, Glu-69
76	11*f*	0.8	Glu-72, Asp-80
77	11*g*		no polar groups
112	16*g*	2.9	no polar groups
118	17*f*	1.6	Glu-114, Glu-115, Glu-122
128	19*b*	1.8	Glu-124, Arg-125, Glu-131
136	20*c*	2.8	Glu-139
140	20*g*		Asp-137
149	22*b*	9.0	Glu-145, Glu-150
152	22*e*	7.7	Glu-156
161	23*g*	1.8	Asp-157, Glu-164
189	27*g*	14.7	Glu-192
198	29*b*	10.3	Glu-194, Glu-195
205	30*b*		Glu-208
213	31*c*		Glu-212
217	31*g*	5.4	next to Tyr-221
220	32*c*		Glu-223,224
226	33*b*		Glu-222, Asp-230
231	33*g*	2.0	Glu-230, Glu-234
233	34*b*		Asp-230, Glu-234, Glu-236
248	36*c*	8.6	Arg-244, Lys-251
251	36*f*	0.7	Glu-250, Asp-254, Asp-255
264	38*e*	7.1	no polar groups
266	38*g*	6.7	no polar groups
268	39*b*	6.5	Glu-272

[a] Lysines in all helical positions are included in this table, and those present in the same peptide are grouped. Information concerning neighboring residues is from the McLachlan and Stewart model as well as our own analysis with space-filling models.

Lys-29 in position 5*a*. Lys-48,49 is unusual in that there are no attractive interactions on the same coil. Lys-48 would be expected to have a high reactivity if isolated alone. The lower reactivity of this peptide may be explained by interaction of Lys-49 with Glu-54 on the other helix and by restricted accessibility due to the bulky hydrophobic groups Leu-50 and Thr-53 on the same chain. The high reactivity of Lys-65 is surprising since there are neighboring acidic groups and it is in a stable region of the helix. The low reactivity of Lys-76,77 can be explained by ionic interactions: Lys-76, on the same helix, and Lys-77 could form a salt bridge with Glu-82 on the other helix. Lys-112 could possibly interact with Glu-115 on the same chain, but the low reactivity is more likely explained by interaction with Glu-117 on the other helix. The low reactivity of Lys-136,140 can be due to ionic interactions on the same chain as well as Lys-140–Glu-138 on the other helix. In addition, Met-141 could stabilize Lys-140 through hydrophobic interaction with the hydrocarbon side chain. The relative reactivities of Lys-149 and -152 are high despite the presence of Glu side chains in the vicinity. One explanation may be that these lysines are surrounded by hydrophobic groups making the surface open to the solvent. Since acetic anhydride is a small, uncharged molecule, it would be accessible to hydrophobic regions on the surface of the protein. Lys-161 has neighboring acidic side chains, but an additional reason for the low reactivity may be that Tyr-162 restricts the accessibility of the lysine side chain. Lys-189 has the highest reactivity in spite of nearby Glu on the same and facing chains. Lys-189 is surrounded by small hydrophobic residues (Gly-188, Leu-185, and Leu-193), making the long lysine side chain unusually accessible. The same situation is found for Lys-198,205, another quite reactive peptide. Lys-213,217,220 is in an unusual region where two tyrosines and one Glu are present at the helical interface. The high reactivity of Lys-248 can be due to nearby basic residues as well as small hydrophobic groups on the outer helix. Lysines-264 and -266 do not have acidic groups available for interaction, and they are in highly hydrophobic surroundings. Although Lys-264 faces a Glu on the other helix, Tyr-261 may prevent formation of a salt bridge. Lys-268 has only one neighboring Glu.

In analyzing the periodicities of the amino acid sequence, McLachlan & Stewart (1976) proposed alternating actin binding sites, α and β, each with positive and negative zones. Smillie et al. (1980) suggested that the nonpolar positive zones of the α-regions may be less ordered and more flexible than those in the β-regions. We have seen no correlation between lysine reactivity and localization in a paricular zone.

Relationship between Reactivity and Stability. We have interpreted our results in terms of what is known about the stability of the tropomyosin coiled coil.

Except for the extreme ends of tropomyosin that are involved in head-to-tail overlap, the molecule is largely α-helical coiled coil, although there are localized regions that depart from this structure. Crystallographic studies show that the COOH-terminal half is less stable than the NH_2-terminal half (Phillips et al., 1979, 1981). Studies of tropomyosin modified at Cys-190 indicate the unstable region is in the vicinity of the cysteine (cf. Lehrer, 1978; see introduction). Further indication that the least stable region is close to Cys-190 comes from experiments showing preferential sites of proteolysis in this region (Pato & Smillie, 1978; Ueno & Ooi, (1978; Pato et al., 1981; Ueno, 1984). Pato et al. (1981) showed that the initial sites of tryptic and chymotryptic digestion were Arg-133 and Leu-169, respectively. Further digestion resulted in the formation of proteolytic resistant fragments, residues 13–125 and 183–284, indicating that the NH_2-terminal overlap region and residues 126–182 are more susceptible to digestion. Ueno (1984) has shown that the middle region of tropomyosin becomes more susceptible to trypsin at low ionic strength, at higher temperature, and with formation of an interchain disulfide bond at Cys-190.

In general the lysines in the NH_2-terminal half tend to have lower reactivities than those in the COOH-terminal region, but there is no clear correlation between reactivity of a given lysine and the stability of that region of the molecule. For example, Lys-128 and Lys-136,140 are close to Arg-133, the site of initial tryptic digestion, yet they are low in reactivity. Similarly, Lys-161 is low yet close to Leu-169. The lysines following the protease sensitive region are among the most reactive in the protein. In the condition of our experiment, the helix should be maximally stable.

Given the heterogeneity of the surface of tropomyosin indicated by the results of our experiments, and knowing that tropomyosin is largely helical, one might ask what is the re-

lationship between helical stability and lysine reactivity or susceptibility to proteolysis. While reactivity appears to be related to accessibility, small changes in dynamic regions of the molecule detected by enzyme analysis or by covalent modification with larger reagents may not be visible to a small uncharged molecule such as acetic anhydride. This is probably why in all our studies using acetic anhydride we have never found a lysine that is unreactive. In contrast, enzymes cleave at a few specific sites, and it is possible that the rate of cleavage can be influenced more dramatically by small structural changes than is lysine reactivity with acetic anhydride. In analyzing space filling models of the region of tropomyosin susceptible to proteolysis, we noted that there were an unusual number of small hydrophobic groups on the surface (see above). This surface topography may contribute to making the backbone of the protein susceptible to proteolytic cleavage without being related only to the stability of the α-helix.

CONCLUSION

In this paper we have presented a detailed structural study of tropomyosin using lysines as specific probes for the protein surface in regions of the molecule that have not been investigated using other methods. Analysis of the reactivities in terms of the coiled-coil model of tropomyosin shows that the reactivities of surface lysines vary widely and indicates that the surface topography of tropomyosin is nonuniform along its length. In addition to the new information about tropomyosin and the implications of our findings about the structure of other coiled coils and helical structures, the study provides the groundwork for study of actin and troponin binding sites on tropomyosin. Until now, determination of binding sites has also been limited by the presence of specific probes only at Cys-190 and the overlap region. Experiments are now in progress to analyze tropomyosin lysine reactivities in the presence of troponin. Our knowledge of actin binding sites is from analysis of sequence periodicities. The approach we have described here would allow a more discrete description of binding sites.

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Effect of Various Anions on the Stability of the Coiled Coil of Skeletal Muscle Myosin†

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ABSTRACT: The stability of skeletal myosin rod was studied by following the dependence of both papain digestion kinetics and helix–coil transition temperatures on the concentration of neutral salts. The rate of papain-catalyzed digestion of rod to form subfragment 2 and light meromyosin was strongly dependent on chloride concentration but essentially independent of acetate concentration up to 2.0 M. The rod exhibited a biphasic melting curve in 0.6 M NaCl, 5 mM phosphate, and 0.1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.3, with transitions at 45 and 53 °C. In 0.6 M CH_3COONa, 5 mM phosphate, and 0.1 mM EDTA, pH 7.3, the transitions occurred at 50 and 58 °C, respectively. Transition temperatures were obtained with a novel curve-fitting method. The effect of increasing chloride ion concentration on melting profiles was 2-fold. Below 0.6 M salt, the two transition temperatures, $T_{m,1}$ and $T_{m,2}$, depended on salt concentration such that increasing NaCl concentration caused a small stabilization of the helix while increasing acetate concentration caused the helix to become markedly more stable. Between 0.6 and 1.0 M, variation of chloride concentration had almost no effect on the thermal stability of the rod while increasing acetate concentration increased its stability considerably. Above 1.0 M NaCl, the melting profiles became broad with a third transition being observed (e.g., at 3.0 M, $T_{m,3}$ = 38 °C), indicating the existence of a region which has a tendency to be destabilized by chloride. The third transition was not observed at comparable concentrations of acetate. This effect of chloride was not expected on the basis of its position in the Hofmeister series. Taken together, these observations suggested that while increasing salt concentration stabilizes the major portion of the coiled coil, chloride ion destabilizes a domain near the subfragment 2/light meromyosin junction with a concomitant increase in its susceptibility to proteolysis by papain.

Myosin consists of two high molecular weight polypeptides (heavy chains, M_r 200000) assembled into two morphologically distinct regions; it has a rod-shaped tail composed of two α-helical polypeptide chains wrapped in the coiled-coil configuration. At the N-terminus of each chain is a globular head which contains the ATPase and actin binding sites. Each myosin head, in turn, has bound to it two different low molecular weight subunits (light chains, M_r ~20000). Cleavage of myosin with various proteolytic enzymes produces distinct subfragments, each of which retains specific functions of the parent molecule (Lowey et al., 1969). The proteolytic susceptibility of two regions, one between the head and tail and the other between subfragment 2 and light meromyosin, has been taken to indicate flexibility in them. The flexibility of myosin and myosin rod has been investigated through a number of independent measurements of viscosity (Burke et al., 1973; de la Torre & Bloomfield, 1980), electrical birefringence (Highsmith et al., 1977), polarized light scattering (Highsmith et al., 1982), fluorescence depolarization (Harvey & Cheung, 1977), viscoelastic properties (Rosser et al., 1977; Hvidt et al., 1982; 1983), and electron microscopy (Elliott & Offer, 1978).

The effect of neutral salts on the stability of protein structure is well-known (Von Hippel & Scleich, 1969). Effects of salts on myosin ATPase activity and structure have been investigated (Warren et al., 1960; Brahms & Brezner, 1961; Tonomura et al., 1962; Seidel, 1969) and were found to follow the Hofmiester series (Hofmiester, 1888) for most ions studied. In a study by Stafford & Margossian (1982), the salt concentration dependence of the rate of papain-catalyzed proteolysis of myosin rod was found to depend on the type of anion according to the Hofmeister series (vis., citrate > sulfate > phosphate > acetate). It was observed subsequently that the concentration dependence of the rate constant in chloride, which was expected to be less than that of acetate, was out of order. This observation led to the investigation described below in which the effects of chloride on both the papain digestion kinetics and the thermal stability of myosin rod were studied.

Since chloride ion is used extensively to control ionic strength in in vitro experiments with myosin, it seemed worthwhile to

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