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Renaturation of skeletal muscle tropomyosin: Implications for *in vivo* assembly

(protein refolding/heterodimer formation/coiled coil)

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ABSTRACT **The observation that the $\alpha\beta$ heterodimer is the predominant species of tropomyosin in rabbit skeletal muscles has led to the suggestion that this species assembles preferentially. To understand the molecular basis of this assembly process, we have studied renaturation under conditions that favor heterodimer formation. When skeletal muscle tropomyosin composed of equal amounts of α and β subunits is renatured either by cooling or by dialysis a distribution that favors homodimers is generated. In contrast, rapid renaturation by dilution from urea favors the heterodimer. Further analysis of this latter renaturation procedure with cysteine-cleavage fragments of tropomyosin using circular dichroic measurements shows that as few as 30 residues in the NH_2-terminal third of each tropomyosin subunit are involved in the initial interaction that results in heterodimer formation. Based on the density of sequence substitutions between the α and β subunits, that region probably includes residues 36–64.**

Tropomyosin (TM) from rabbit skeletal muscle is a dimer composed of two different classes of subunits, α and β (1). Analysis of its subunit composition in rabbit fast, slow, and mixed skeletal muscles revealed that $\alpha\beta$, the heterodimer, is present at levels higher than expected from random association of the α and β subunits (2, 3). Because skeletal muscle TM has a low turnover rate (4) and its coiled-coil structure is stable (5), it was inferred that the abundance of the heterodimer was the outcome of the *in vivo* assembly process (2, 3).

The heterodimer-preferred distribution in rabbit skeletal muscle TM was unexpected because other extensively coiled-coil proteins assembled from pools of nonidentical subunits are homodimeric (6–9). Further, because it differed from the random distribution predicted by equilibrium thermodynamic analysis (10), it seemed likely that the *in vivo* distribution of skeletal muscle TM was the result of a kinetic trap.

To analyze this process, we took advantage of the ease with which TM renatures (1) and sought an *in vitro* renaturation procedure that generated heterodimers. Three denaturation–renaturation protocols were studied. Two of these, thermal denaturation followed by cooling and denaturation by chemical agents followed by dialysis, favored homodimer formation. The third procedure, denaturation with chaotropic agents followed by rapid dilution, favored heterodimer formation. Analysis of this last renaturation procedure indicates that the region involved in heterodimer formation *in vitro*, and most likely *in vivo*, is localized in the NH_2-terminal third of the TM sequence.

MATERIALS AND METHODS

Reagents and Buffers. Ultra-pure urea and guanidine·HCl (Gdn·HCl) were obtained from Schwarz/Mann, Sephadex G-100 was from Pharmacia, and 2-nitro-5-thiocyanobenzoic acid was from Kodak. 5,5′-Dithiobis(2-nitrobenzoic acid) (Nbs_2) was obtained either from Sigma or Aldrich. All other chemicals were reagent grade. Hydroxyapatite was prepared as described (3). The buffer used in renaturation experiments was 100 mM NaCl/30 mM sodium phosphate/0.5 mM dithiothreitol, pH 7.4.

TM Preparation. TM was prepared as described (3). $\alpha\beta$ fractions from hydroxyapatite were pooled and concentrated by dialysis against solid Sephadex G-200. To remove Triton X-100, TM was bound to hydroxyapatite in 100 mM NaCl/5 mM sodium phosphate, pH 7.4, washed with 8 column vol of that buffer, and eluted with 400 mM sodium phosphate (pH 7.4).

Crosslinking and Modification of TM. TM was crosslinked by oxidation of opposing cysteines with Nbs_2 by a modification of Lehrer's procedure (11). Samples in renaturation buffer were crosslinked by dialysis against a 150-fold volume excess of that buffer without dithiothreitol for 1 hr followed by dialysis against a 150-fold volume excess of the same buffer supplemented with 10 mM Nbs_2. Crosslinking in urea was performed by three additions of 5 mM Nbs_2 at 15-min intervals. Nbs_2 obtained from Aldrich gave a consistently higher yield of crosslinked dimer, though when >80% of the TM was crosslinked the crosslinked dimer distributions were independent of the source of Nbs_2. Cyanylation followed by cleavage at cysteine was performed as described (12), and the NH_2-terminal and COOH-terminal fragments of α TM were separated by chromatography on Sephadex G-100 in 6 M Gdn·HCl.

Gel Electrophoresis. $NaDodSO_4$/PAGE was performed as described by Laemmli (13); staining and densitometry were performed as described (3) at TM concentrations in a linear response range. Crosslinked TM dimers dissolved in "diluting buffer" (13) without reducing agent were resolved on a 6.5% gel. This gel system was also used to determine the fraction of crosslinked to total TM that was interpreted as the yield of dimers. TM subunits were resolved on a 10% gel.

Denaturation and Renaturation of TM. TM at 0.1 mg/ml was thermally denatured by heating for 5 min at 70°C in a sealed glass tube and renatured immediately afterwards by immersion of the tube in ice water. Renaturation from chemical agents was performed either by dialyzing TM at 0.1 mg/ml in either 6 M Gdn·HCl or 8 M urea, at pH 7, against renaturation buffer or by rapid 1:20 to 1:40 dilution of TM from 8 M urea or 6 M Gdn·HCl to 0.1 mg/ml with renaturation buffer. Thus, all renaturations were performed at 0.1 mg/ml.

Protein Determination and Circular Dichroism Measurements. TM concentrations were determined either by the

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Abbreviations: TM, tropomyosin; Nbs_2, 5,5′-dithiobis(2-nitrobenzoic acid); Gdn·HCl, guanidine·HCl.

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Bradford assay (14) or by amino acid analysis (15). The circular dichroic spectra of TM solutions were determined on a Jobin-Yvon Dichrographe III and the molar ellipticity at 208 nm, $[\theta]_{208}$, was used to determine the fraction α-helix by equation 1 of Greenfield and Fasman (16).

RESULTS

Identification of TM Dimers by Gel Electrophoresis Following Renaturation. Both one- and two-dimensional NaDodSO$_4$/PAGE have been used to resolve the dimeric species of TM after they are covalently crosslinked by oxidation with Nbs_2 (2, 3, 11). Nbs_2 crosslinks by promoting disulfide bridge formation at the apposed cysteines at position 190 in α_2 and $\alpha\beta$ and, most likely, at the apposed cysteines at position 36 or 190 in β_2. Commonly, after renaturation of rabbit skeletal muscle TM nine bands are resolved by NaDodSO$_4$/PAGE under nonreducing conditions (Figs. 1*A* and 2). On the basis of their molecular weights, six of the bands result from TM dimers (the three major species, b, c, and f, and the three minor species, a, d, and e) and three are the result of uncrosslinked subunits (g, h, and i). Because this pattern was more complex than those previously observed in one dimension (2, 3, 11), the subunit composition of each dimer band was determined by excision of the band, treatment with dithiothreitol to reduce the cystine crosslinks, and NaDodSO$_4$/PAGE (Fig. 1*B*). The three major dimeric species, b, c, and f, are $\alpha\beta$, α_2, and β_2, respectively, as previously identified (2, 3). The three minor dimer bands, a, d, and e, all result from β_2 species. The origin of this β_2 heterogeneity is unclear. Though there is evidence for β-subunit heterogeneity (17), the magnitude of the mobility differences between β_2 species (e.g., between a and f) makes it likely that differences in the extent of modification and/or oxidation by Nbs_2 of the apposed cysteines at positions 36 and 190 are responsible for the minor β_2 species. The composition of the uncrosslinked monomeric bands, g–i in Fig. 1*A*, was similarly determined. They are composed of β, α, and $\dot{\alpha}$, respectively (Fig. 1*B*). Having identified all of the dimeric species by NaDodSO$_4$/PAGE, this procedure was used to analyze the distribution of TM dimers following renaturation.

Rapid Dilution Favors Formation of the $\alpha\beta$ Dimer. Three denaturation–renaturation procedures were studied: heat denaturation followed by slow cooling, chemical denaturation using either 6 M Gdn·HCl or 8 M urea followed by dialysis against renaturation buffer, and denaturation with either 6 M Gdn·HCl or 8 M urea followed by dilution. In each experiment the denaturing conditions were severe enough to ensure complete subunit dissociation. This has been established for Gdn·HCl (18) and thermal denaturations (19, 20), and, in the case of urea denaturation, circular dichroic spectra between 3.5 M and 4 M urea show that the TM subunits assume a completely random coil configuration (unpublished observations) indicating they are not associated. The TM used in the studies was between 0.50 and 0.53 α subunit, and renaturations were carried out at a protein concentration of 0.1 mg/ml. After renaturation, NaDodSO$_4$/PAGE revealed that at least 80% of the TM was recovered as crosslinked dimer (the remainder was either monomeric or not crosslinked), and at least 80% of the α-helix content (determined from $[\theta]_{208}$) was recovered. The renatured TM also exhibited the chromatographic properties of native TM on hydroxyapatite. This was taken as evidence for native conformation in the renatured TM.

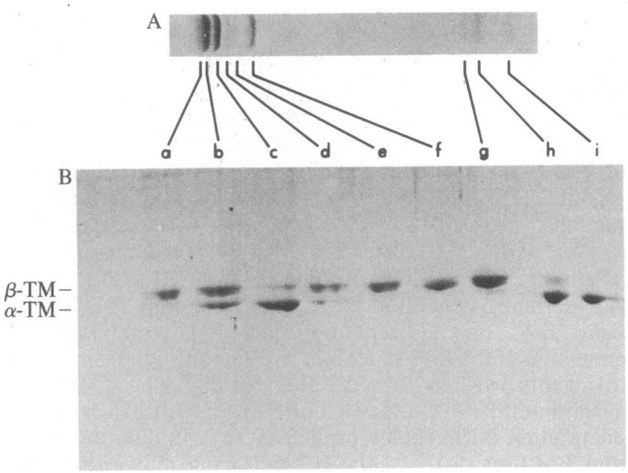

FIG. 1. Identification of dimeric and monomeric species of TM by NaDodSO$_4$/PAGE. (*A*) The results of a renaturation by cooling are seen after Nbs_2 oxidation on 6.5% NaDodSO$_4$/PAGE in the absence of reducing agent. (*B*) Identification of the subunit composition of all of the protein bands, a–i, on the gel in *A* by using 10% NaDodSO$_4$/PAGE in the presence of a reducing agent. The positions of the α and β TM subunits are labeled. The presence of trace amounts of β in bands c and h and of α in band d is due to cross-contamination. Comparison of the mobilities of the α and β TM subunits in *A* and *B* shows that the Nbs_2 treatment affects the position of uncrosslinked monomers.

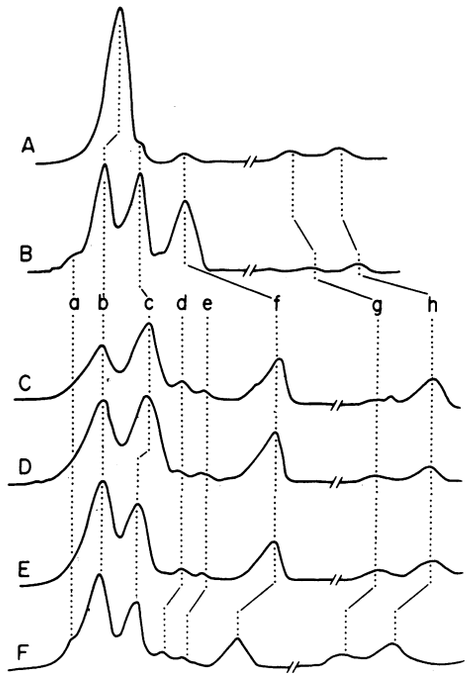

FIG. 2. Densitometric analysis of the dimeric species of native and denatured–renatured TM from the different renaturation protocols: A, native $\alpha\beta$ TM; B, thermal denaturation followed by cooling; C, Gdn·HCl denaturation followed by dialysis; D, urea denaturation followed by dialysis; E, Gdn·HCl denaturation followed by dilution; F, urea denaturation followed by dilution. The positions of the dimeric species and uncrosslinked subunits are labeled a–h below trace B as in Fig. 1*A*.

Fig. 2 shows densitometric tracings of dimer patterns resulting from these protocols. The distributions of α_2, $\alpha\beta$, and β_2 from these and other experiments are tabulated in Table 1. These results show that procedures more likely to result in an equilibrium distribution, renaturation by cooling or by dialysis, yield distributions at which the level of heterodimer (35–37%) is less than the 50% expected from random assortment of subunits. In contrast, renaturation from 8 M urea or 6 M Gdn·HCl by dilution yields higher levels of heterodimer. In particular, when TM is renatured by dilution following urea denaturation, the level of heterodimer is 57%. In other studies the effects of 5 mM Mg^{2+} and 37°C temperature on renaturation from urea by dilution were tested to approximate more closely the intracellular environment. Mg^{2+} has

Table 1. Dimer distributions following renaturation

Procedure	n	Dimer distribution, % α_2	$\alpha\beta$	β_2	t_s test, P*
Heating, cooling	8	34 ± 3	37 ± 2	29 ± 2	<0.001
Gdn·HCl, dialysis	5	34 ± 3	36 ± 4	30 ± 4	<0.02
Urea, dialysis	2	37 ± 5	35 ± 2	30 ± 2	>0.05
Gdn·HCl, dilution	3	34 ± 2	47 ± 1	19 ± 3	>0.05
Urea, dilution					
23°C	7	25 ± 3	57 ± 1	18 ± 2	<0.02
37°C	4	23 ± 1	61 ± 1	16 ± 0	<0.001
Serial dilution					
To 1 M	3	23 ± 1	61 ± 2	15 ± 1	<0.05
To 3 M	4	50 ± 2	18 ± 1	32 ± 2†	<0.001

Tabulation of the dimer distributions following renaturation protocols. The distribution of dimers was determined by quantitation of densitometric analyses like those presented in Fig. 2. All renaturations here were carried out at 0.1 mg/ml. n, The number of experimental trials. Studies showed that there was no concentration dependence on the dimer distributions in the range of 0.2–0.05 mg/ml. The dimer distributions were also independent of the extent of crosslinking when the extent of crosslinking was >80%. For this reason, the dimer distributions were taken as representative of the renatured dimers. With regard to the heterodimer bias, on dilution from urea at 37°C 97% of the TM was recovered as crosslinked dimer so, in that study, 59% of the total TM was heterodimeric.

*Student t_s test of the hypothesis that the levels of heterodimer are due to random assortment of subunits (21).

†The β subunit was underrepresented in the material recovered as dimer in this study. The reason for this is unclear. It probably reflects the difficulty of quantitating the three minor β_2 peaks. However, it does not affect the characterization of the distribution.

no effect; however, at 37°C formation of heterodimers is more favored (61% as opposed to 57%). *In vivo* the heterodimer is present at levels between 85% and 90% in rabbit skeletal muscles, where the levels of the α and β subunits are approximately equal (2, 3).

Comparison of the distributions following renaturation by dilution from urea with the 1:2:1 = α_2:$\alpha\beta$:β_2 distribution expected from random assortment (P values, Table 1) shows that the distributions from renaturation by cooling and dialysis favor homodimer formation. Those from dilution from urea favor heterodimer formation, like the *in vivo* distribution, with the greatest $\alpha\beta$ bias at 37°C and in serial dilution studies (presented below) in which the distribution is 1:3:1.

Characterization of the Region Involved in Heterodimer Formation. Because the level of heterodimer is dependent on the pathway of renaturation and is favored by a rapid procedure, dilution, we assumed that its formation, at least when favored, was not the result of an equilibrium process. Rather, it seemed that heterodimer formation was the result of a kinetic trap—that is, the first step in heterodimer formation is the interaction of specific segments of each subunit to form a stable coiled-coil region like that found in native TM—and that the rest of the coiled-coil structure "zippers" from that region, much as DNA renatures. To test that assumption and, if correct, characterize the region involved, the dimer distributions resulting from a "step renaturation" experiment were analyzed. Step renaturation was performed by rapidly diluting samples of TM denatured in 8 M urea with renaturation buffer to a protein concentration of 0.1 mg/ml at lower urea concentrations, incubating each sample at the new urea concentration for 5–10 min to allow the subunits to interact, dialyzing each against renaturation buffer, and assaying for dimers by Nbs_2 oxidation. We reasoned that, if heterodimer formation was dependent on the interaction of specific sequences, there would be a transition from the homodimer-favored distribution of the dialysis procedure to the heterodimer-favored distribution of the rapid dilution procedure when TM was diluted to a urea concentration

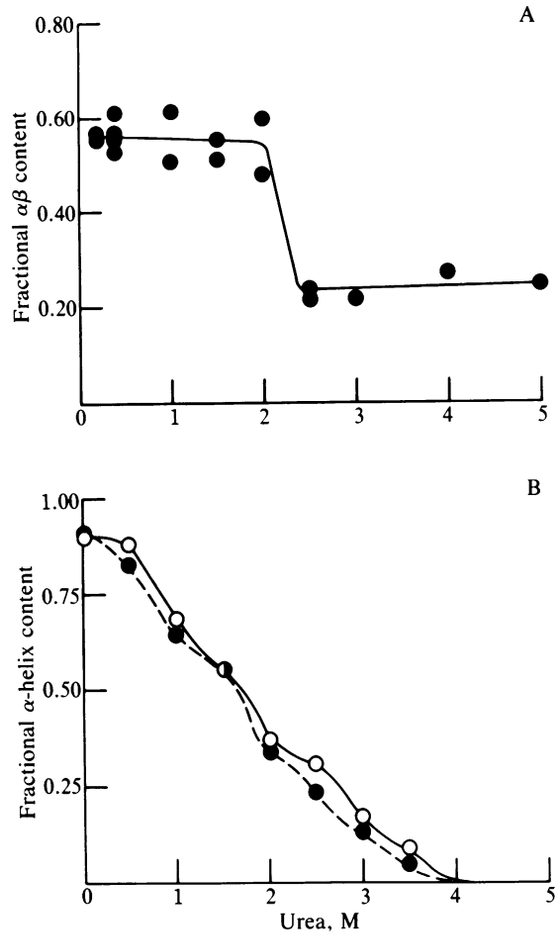

FIG. 3. Analysis of step renaturation and the stability of the region involved in heterodimer formation. (*A*) The fraction of the $\alpha\beta$ heterodimer resulting from the step renaturation protocol is plotted as a function of the final urea concentration to which the denatured TM subunits were diluted. Nbs_2 oxidation was performed after dialysis against renaturation buffer. (*B*) The fractional α-helix content of α_2 (○) and $\alpha\beta$ (●) TM after 1 hr at the indicated urea concentrations. The α-helix content was determined by using equation 1 of Greenfield and Fasman (16) from $[\theta]_{208}$. $[\theta]_{208}$ for native α_2 TM was −30,800 degree·cm^2/dmol. TM protein concentration was determined by amino acid analysis (15).

where those sequences assumed an undenatured conformation that allowed them to associate. In Fig. 3*A* the results of that study are presented, and there is a sharp transition to a heterodimer-favored distribution from a homodimer-favored distribution when TM is diluted from 8 M urea to between 2.0 and 2.5 M urea, dialyzed, and then oxidized.

Because these studies indicated that both the hetero- and homodimer-favored distributions were stable to dialysis after formation it appeared that there was very little exchange of TM subunits after association.‡ The absence of exchange reactions that significantly affect the nature of the distributions was confirmed by replacing the dialysis by a second dilution in the step renaturation procedure. This revealed that when diluted from 8 M urea to either 3.0 M or 1.0 M urea, then diluted again to 0.4 M, dialyzed, and oxidized, a homodimer-favored and heterodimer-favored distribution, respectively, are obtained (Table 1).

Knowing the urea concentrations at which the transition in

‡We have detected a subunit exchange reaction that occurs under mildly denaturing, nonphysiological conditions. It favors homodimer formation and occurs at urea concentrations between 0.4 and 2.0 M. However, because this reaction is slow, its effect is minimal in these experiments.

distributions occurs made it possible to characterize the region responsible for that transition by its resistance to urea denaturation. This was done by determining the change in the α-helix content by circular dichroic measurements of native αβ and α_2 TM at the urea concentrations that define the transition in distributions (β_2 was not studied because there is no identified rabbit skeletal muscle to use for its isolation in which it is a major species). The results are plotted in Fig. 3*B*. The curves for α_2 and αβ are almost superimposable, indicating they have similar structural stabilities. At the urea concentrations at which the transition occurs, the fraction of α-helix increases from 23% to 34%. So, only 1/4th to 1/3rd of TM exhibits a native conformation at urea concentrations at which heterodimer formation is favored.

Identification of the Region Involved in Heterodimer Formation. The extended, coiled-coil structure of TM is stabilized by a series of hydrophobic interactions that repeat regularly along the length (and primary sequence) of the molecule (5). Analysis of TM and its protein fragments by Gdn·HCl and thermal denaturation has shown that TM can be divided into three contiguous segments that differ in stability (18–20): the NH_2-terminal third, which is the most stable; the COOH-terminal third, which is less stable; and the central region including Cys-190, which is least stable. By assaying the stability to urea denaturation of each of the three segments of the molecule, it was possible to localize the region involved in generating the heterodimer-favored distribution.

The stability of the central region of the molecule was assessed by measuring the ability to crosslink the opposing cysteines at position 190 in αβ TM with Nbs_2. Fig. 4*A* shows that this region is easily denatured. By 1.2 M urea there is a 50% decrease in the ability to crosslink the subunits (closed circles). Since this region loses stability long before the 2.25 M urea concentration characteristic of the sequence involved in heterodimer formation (dashed line), it cannot be involved in heterodimer formation.

The stabilities of the COOH- and NH_2-terminal segments to urea denaturation were determined by measurements on the NH_2-terminal (positions 1–190) and COOH-terminal (positions 191–284) fragments prepared by cyanylation followed by cleavage at Cys-190 of α TM subunits. The α subunit was used because it contains only one site of cleavage and because our studies on the stability of αβ and α_2 in urea (Fig. 3*B*) and those of Williams and Swenson (19) indicate that the stabilities of dimers are not significantly different. As shown in Fig. 4*B*, in the region of the heterodimer transition (indicated by the dashed line in Fig. 4*A*) the α-helix content of the COOH-terminal fragment remains unchanged at 11%. This fraction of α-helix content is the equivalent of 10 residues, which studies with synthetic polypeptides show is too short to form a stable coiled-coil region (5). In contrast, the α-helix content of the NH_2-terminal fragment increases from 36% to 49% (from 70 to 95 residues) in that range of urea concentrations. Based on the circular dichroic analysis of fragments by Potekhin and Privalov (20) and our circular dichroic data, coupled with the study on the oxidation of Cys-190 (Fig. 4), the central region does not exhibit α-helical structure in the NH_2-terminal cyanylation fragment (that is why the NH_2-terminal fragment has a maximal α-helicity of 62%). So, although the NH_2-terminal fragment contains both the NH_2-terminal and central regions of the molecule, these results localize the region involved in heterodimer formation to the NH_2-terminal third of the TM subunits.

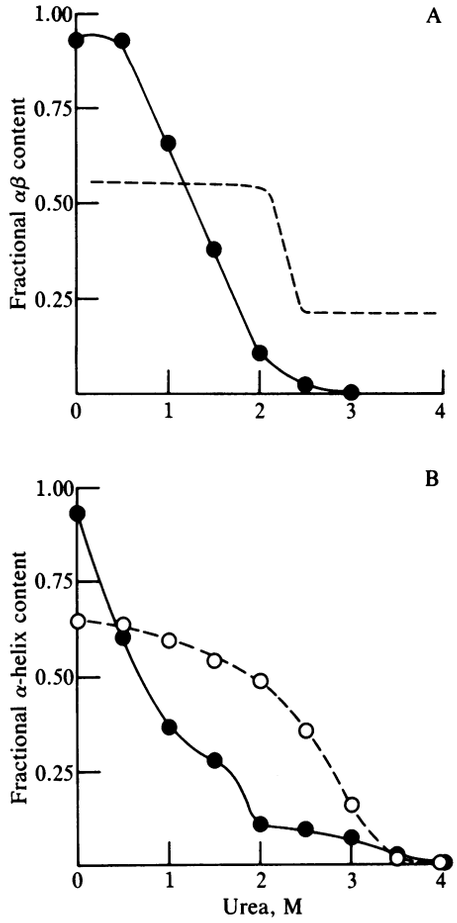

FIG. 4. Regional stability of TM. (*A*) The fraction of αβ crosslinked by Nbs_2 at the urea concentrations indicated is plotted (●). The dashed line indicates the position of the homodimer-to-heterodimer transition determined in the step renaturation experiment shown in Fig. 3. (*B*) The fractional α-helix content of the NH_2-terminal (○) and COOH-terminal (●) cysteine cleavage fragments was determined from the $[\theta]_{208}$ by using equation 1 of Greenfield and Fasman (16).

DISCUSSION

The prevalence of αβ TM in rabbit skeletal muscle *in vivo* means that despite their extraordinary sequence homology [2 nonconservative and 37 conservative substitutions in 284 residues (22)] the α and β TM subunits distinguish between each other as they assemble. Further, because of the absence of tertiary interactions in TM's coiled-coil structure, the region involved in heterodimer formation must be one in which the α and β subunits differ in sequence. The role of sequence in determining the *in vivo* distribution of coiled-coil proteins is evident from the difference in dimer distribution between rabbit skeletal muscle TM, which is predominantly heterodimeric, and *Caenorhabditis elegans* myosin heavy chains (6, 7) and bovine brain TM (7, 8), which are homodimeric.

Sequence differences between subunits in coiled-coil proteins can determine the distribution of dimers either through an equilibrium effect resulting from differences in free energies among the dimers or kinetically by forming a site or sites for assembly. Because the difference in free energy for any of the distributions observed in the reaction $\alpha_2 + \beta_2 = 2\alpha\beta$ is small [e.g., the difference in free energy between the homodimeric distribution from dialysis and the heterodimeric distribution *in vivo* is about 4 kcal·mole^{-1} (1 cal = 4.184 J)], the nature of the effect cannot be determined from the *in vivo* distributions. However, the results of these studies and our previous work (2, 3) indicate that the distributions are determined primarily by kinetic effects. We have found three distributions—the *in vivo* heterodimer, the dilution heterodimer, and the dialysis homodimer. The distributions are pathway dependent (at least in the case of the renaturation

studies) and do not interconvert or change under nondenaturing conditions. This implies that subunit exchange does not occur at a significant rate and that the distributions are not the result of an equilibrium process. Rather, the assembly appears to be kinetically determined. This behavior is similar to that found in DNA renaturation, which may not be surprising given the similar structure and periodicity of stabilizing interactions of TM (23) and DNA.

The step renaturation and serial dilution study localized the region involved in heterodimer formation *in vitro* to the NH_2-terminal third of TM. In that portion of the molecule, there is one region, positions 36–64, that is a candidate for the heterodimer-determining site. It is the only region in the NH_2-terminal third that exhibits a high density of sequence substitution (22), and those substitutions change the secondary structure potential as defined by Chou and Fasman (24) in a nonrandom manner. The substitutions at positions 43, 44, and 45 increase the probability of β-sheet in the α subunit, and those at positions 57, 58, 63, and 64 reduce the α-helical potential of the β subunit.

It is also likely that the homodimer-favored distributions arise from the interaction of a specific region of the TM molecule. There is significant α-helix content in TM at urea concentrations at which the homodimer-favored distribution is found in step renaturation (between 2.5 M and 3.5 M). Using arguments similar to those that localized the region responsible for heterodimer formation, we infer that the region responsible for the homodimer-preferred distribution is also in the NH_2-terminal third of the molecule. Because the homodimer-specifying site is more resistant to urea denaturation, in renaturation by dialysis this site probably associates stably before that specifying heterodimer formation, resulting in the homodimer bias. In fact, it is likely that the regions responsible for homodimer- and heterodimer-favoring reactions overlap as the region from positions 48 to 127, which includes part of the putative heterodimer-specifying region, appears to be the most stable portion of the molecule (20).

Comparison of the distributions after renaturation from urea by dialysis and by serial dilution to 3 M and then 0.5 M urea (Table 1) suggests that the homodimer and heterodimer reactions compete. Competing reactions might explain why there is not a greater bias towards the heterodimer on dilution from urea.

Although we believe that in characterizing renaturation from urea by dilution we are studying a reaction analogous to the *in vivo* assembly, it is important to remember that the heterodimer-specifying mechanism proposed here cannot be the only *in vivo* assembly pathway for rabbit skeletal muscle TM dimers. There are several fast skeletal muscles in which $\alpha\beta$ is not the major species [though it is always present at levels greater than expected by random association (2, 3)]. In those instances, TM homodimer probably forms because one or the other subunit is in excess (3); however, homodimer formation must be less efficient than heterodimer formation when both TM subunits are present. Also, there is an even greater bias towards the heterodimer *in vivo* than we find *in vitro*. This implies there are additional factors or effects that enhance heterodimer formation. With regard to this, the localization of the heterodimer-specifying region near the NH_2-terminal is very suggestive. It may be that in addition to specifying heterodimer formation, this region induces a metastable conformation during synthesis that inhibits its interactions between nascent or newly synthesized subunits that would generate homodimers.

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1. Cummins, P. & Perry, S. V. (1973) *Biochem. J.* **133,** 765–777.
2. Bronson, D. D. & Schachat, F. H. (1980) *J. Cell Biol.* **87,** 263a (abstr.).
3. Bronson, D. D. & Schachat, F. H. (1982) *J. Biol. Chem.* **257,** 3937–3944.
4. Zak, R., Martin, A. F., Prior, G. & Rabinowitz, M. (1977) *J. Biol. Chem.* **252,** 3430–3435.
5. Talbot, J. A. & Hodges, R. S. (1982) *Acc. Chem. Res.* **15,** 224–230.
6. Schachat, F. H., Harris, H. E. & Epstein, H. F. (1977) *Cell* **10,** 721–725.
7. Schachat, F. H., Garcea, R. L. & Epstein, H. F. (1978) *Cell* **15,** 405–411.
8. Bretscher, A. & Weber, K. (1978) *FEBS Lett.* **85,** 145–147.
9. Dabrowska, R., Sosinski, J. & Dabrikowski, W. (1980) *Muscle Plasticity* (de Gruyter, Berlin), pp. 225–239.
10. Crimmins, D. L. & Holtzer, A. (1981) *Biopolymers* **20,** 925–950.
11. Lehrer, S. S. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3377–3381.
12. Jacobson, G. R., Schaffer, M. H., Stark, G. R. & Vanaman, T. C. (1973) *J. Biol. Chem.* **248,** 6583–6591.
13. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
14. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
15. Spackman, D. H. (1967) *Methods Enzymol.* **11,** 3–15.
16. Greenfield, N. & Fasman, G. D. (1969) *Biochemistry* **8,** 4108–4115.
17. Mak, A. S., Smillie, L. B. & Stewart, G. R. (1980) *J. Biol. Chem.* **255,** 3647–3655.
18. Lehrer, S. S. (1978) *J. Mol. Biol.* **118,** 209–226.
19. Williams, D. L. & Swenson, C. A. (1981) *Biochemistry* **20,** 3856–3864.
20. Potekhin, S. A. & Privalov, P. L. (1982) *J. Mol. Biol.* **159,** 519–535.
21. Sokal, R. R. & Rohlf, F. J. (1969) *Biometry* (Freeman, San Francisco), pp. 145–147.
22. Mak, A. S., Lewis, W. G. & Smillie, L. B. (1979) *FEBS Lett.* **105,** 232–234.
23. Crick, F. H. C. (1953) *Acta Crystallogr.* **5,** 689–697.
24. Chou, P. Y. & Fasman, G. D. (1977) *Adv. Enzymol. Relat. Areas Mol. Biol.* **47,** 222–245.