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Amino-Acid Sequence of Rabbit Skeletal Tropomyosin and Its Coiled-Coil Structure

(COOH-terminal/α-helix/three-dimensional structure)

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ABSTRACT **A tentative amino-acid sequence for the COOH-terminal half of rabbit skeletal tropomyosin is reported. These studies confirm our previous conclusions that this tropomyosin consists of several different but similar polypeptide chains. In the sequence, nonpolar residues occur in two series at intervals of seven residues. Amino-acid residues in series I are three residues on the NH_2-terminal side of, and four residues on the COOH-terminal side of, residues in series II. The presence of occasional charged or ambivalent residues in the positions of series I or II does not lead to a disruption of this long-range pattern. The majority of residues located between the nonpolar residues are charged or polar amino acids. Two highly similar or identical α-helices with the reported sequence can be packed together in parallel in a coiled-coil structure. These may be in register or staggered by seven residues or some multiple of it. The observation that groups of small hydrophobic side chains appear to alternate with groups of bulky side chains suggests that a staggered arrangement of the two α-helices would maximize the regularity and hydrophobic interactions of the coiled-coil. Model building considerations show that this would occur with a stagger of 14 residues. Such an arrangement could account for the end-to-end aggregation of tropomyosin in solution, and in crystal and tactoid filaments. However, a structure in which the two polypeptides are in register cannot be ruled out.**

Preparations of rabbit skeletal tropomyosin of molecular-weight about 70,000 dissociate in denaturing medium to monomers of 35,000 daltons (1–6). The highly helical polypeptide chains are believed to be arranged in a two-stranded coiled-coil (7, 8) or segmented rope (9) whose structure is stabilized by hydrophobic interactions between amino-acid residues situated in the core of the molecule. In such an arrangement, the regular interlocking of amino-acid side chains in a two-stranded coiled-coil predicts a regular occurrence of alternating polar and nonpolar residues in tropomyosin. To test this prediction and to facilitate the interpretation of x-ray and electron microscopic studies of tropomyosin crystals and tactoids (10–12), we undertook the amino-acid sequence analysis of this protein. These studies (13–15) have demonstrated that, although the constituent polypeptide chains of preparations of rabbit skeletal tropomyosin are not chemically identical, they must be very similar in amino-acid sequence. Although it has not yet been possible to isolate these chains (of which there are at least four) in a pure state, it seemed feasible to perform a sequence analysis of the mixed population. Towards this end, cyanogen bromide cleavage of *S*-carboxymethylated tropomyosin was done, and a fragment with a minimum molecular weight of 17,000 was isolated by ion-exchange chromatography on QAE-Sephadex in 8 M urea (16, 17). This fragment, which dimerizes to a molecular weight of 35,000 and assumes a relatively high degree of α-helical content (50–60%) in the absence of urea, contained both histidine residues of native tropomyosin. Since one of these residues was known from previous studies to be close to the COOH-terminus of the protein (14), it was concluded that the fragment represented the bulk of the COOH-terminal half of the tropomyosin molecule. Although the component is heterogeneous in that it contains at least four different polypeptide chains corresponding to the parent chains of the intact tropomyosin molecule(s), it is homogeneous in that it is free of cyanogen bromide fragments arising from other regions of the polypeptide chains. In this communication we report a tentative, but almost final, amino-acid sequence for this fragment that shows a regular repeat of hydrophobic amino-acid residues interrupted only occasionally by polar or charged groups. From considerations based on model building studies, we propose that the two strands of the coiled-coil of tropomyosin are assembled in a slightly staggered and parallel arrangement.

MATERIALS AND METHODS

The preparation of the [*carboxymethyl*-^{14}C]cyanogen bromide fragment and its amino-acid composition and physical properties have been described (17). Chymotryptic and α-lytic protease digests of the fragment were done in the usual way. Peptides arising from tryptic digestion of the maleylated fragment were fractionated by gel filtration on Sephadex G-75 columns and ion-exchange chromatography on Dowex-50. These were, in some cases, subjected to further degradation with trypsin or α-lytic protease after demaleylation. Amino-acid analysis, amino-acid sequence determination, high voltage paper electrophoresis, and Dowex-1 and Dowex-50 chromatography were performed as described (14, 15). Model building studies were with CPK space-filling components.

RESULTS AND DISCUSSION

The tentative sequence of the COOH-terminal half of rabbit skeletal tropomyosin is presented in Fig. 1. In this representation a regular right-handed α-helix with 3.6 residues per turn is shown as a cylinder that has been opened up and laid flat on the paper. The position of each amino-acid residue in the sequence is then plotted on the cylinder in a position corresponding to its position in the α-helix. The open squares and circles represent two different series of hydrophobic residues, each of which repeats at every seventh residue in the amino-acid sequence. The residues of series I occur at positions 7, 14, 21, 28 . . . ., while the residues of series II occur at

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positions 10, 17, 24, 31 . . . . . In this pattern an amino acid in series I is always found 3 residues on the NH_2-terminal side and 4 residues on the COOH-terminal side of a residue in series II. Thus, considering the two series together, there is an alternating 3 to 4 pattern of hydrophobic residues that appears to be essentially continuous throughout the sequence of this half of the tropomyosin molecule. A line may be drawn midway between the positions occupied by the residues in series I and those in series II. This line is not parallel to the axis of the cylinder because of the nonintegral nature of the α-helix. Nonpolar residues that do not occur in either series I or II are circled by broken lines. The great majority of the hydrophobic residues in the sequence are found along the band formed by the residues of the two series and the majority of residues outside this region are polar and charged residues.

At several positions in either series I or series II there occur amino acids that are not ordinarily classified as hydrophobes. Thus, glutamic acid or glutamine occurs at positions 77 and 122, serine as a replacement for alanine is found at positions 38 and 45, and cysteine is located at position 49. Four tyrosines are found at positions 21, 73, 80, and 126. All of these residues, with the exception of glutamic acid, can be considered as ambivalent hydrophobes and are in fact found in the interior of globular proteins in certain circumstances. On the other hand, it is possible that their occurrence at certain positions in the hydrophobic pattern leads to a local disruption of the regularity of the coiled-coil and may be of importance in the interaction of tropomyosin with actin or one or more members of the troponin complex. Irrespective of their significance it is clear that their occurrence does not lead to a disruption of the long-range pattern of repeating hydrophobes in the two series.

If two such projected cylinders or helical nets are superimposed face-to-face at an angle, so that the hydrophobic band of one helix lies over that of the other, the side chains of one helix fall between those of the other helix. This can only be done if the side chains of residues in series I or II are aligned with residues of the same series. Thus, in the coiled-coil, the two identical or very similar α-helices can only be packed together in a knobs-into-holes pattern in a manner such that the two chains are in register or out of register by seven amino-acid residues or some multiple of it.

Inspection of the sequence represented in Fig. 1 shows that there appears to be an alternation of groups with bulky and less-bulky side chains in the hydrophobic pattern. Thus, three alanines are grouped close together at positions 10, 14, and 17; three alanines and cysteine occur at positions 38, 42, 45, and 49, and a further three alanines at positions 94, 98,

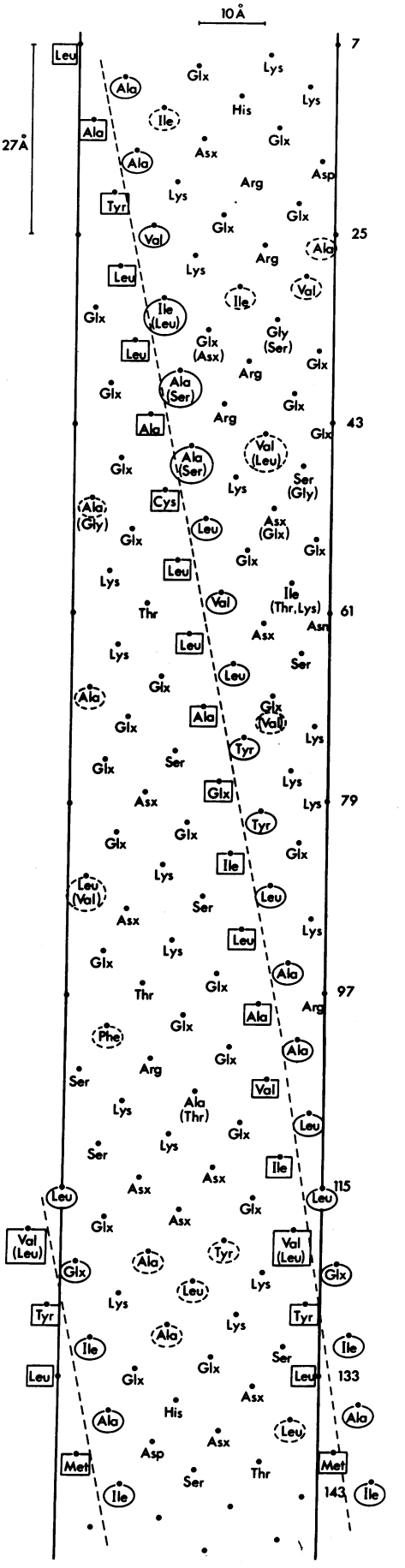

FIG. 1. Tentative amino-acid sequence of the COOH-terminal half of rabbit skeletal tropomyosin plotted on an α-helical net. The radius of the α-helix is taken as 5 Å, with 3.6 residues per turn and a residue translation of 1.5 Å. The NH_2-terminal to COOH-terminal direction is from *top* to *bottom*. The cyanogen bromide fragment whose sequence was determined is from residues 7 to 140. The sequence of the six residues at the NH_2-terminal end has not been determined. Residues 128–143 had been previously determined (14). An unambiguous overlap is still required in the region of residues 124–129. A regular pattern of nonpolar residues occurs in two series, I and II. Series I positions are in *squares;* series II positions are in *circles*. Other nonpolar residues are enclosed in *broken circles*. Amino-acid substitutions have been observed in at least 15 positions and indicate a minimum of 4 different polypeptide chains.

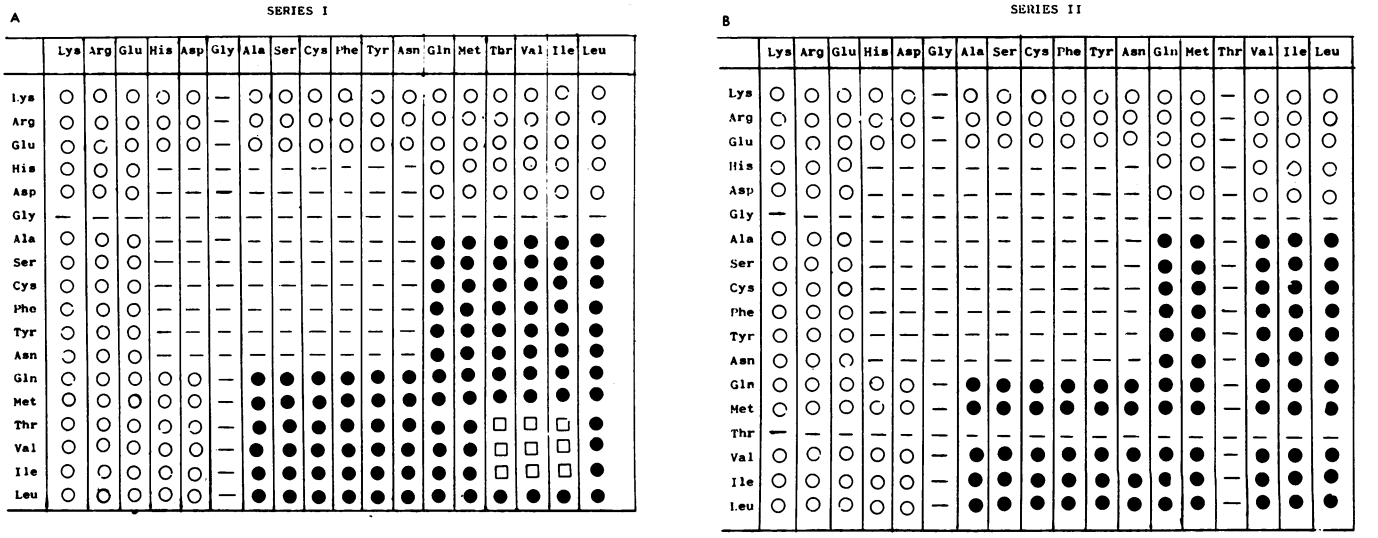

FIG. 2. Amino-acid pairs in series I (*A*) and series II (*B*).——, Stereochemical fit; no β- to γ- or δ-carbon and hydrogen van der Waal's contacts. □, Not stereochemically permissible. ○, van der Waal's contacts between β- and γ- or δ-carbon and hydrogens but involve charged residue(s). ●, van der Waal's contacts between β- and γ- or δ-carbons and hydrogens and do not involve charged residues.

and 101. These regions of small hydrophobes are separated by regions rich in bulky side-chains such as leucine, valine, and isoleucine. The significance of this pattern may be to bring the chains into appropriate alignment. Thus, if the parallel chains are in register there would be interaction between the bulky hydrophobes of one chain with the same bulky hydrophobes of the other. Alternatively, the parallel chains may be staggered by 7, 14, 21, or 28 residues such that bulky hyrodphobes of one chain interact with the less bulky of the other. In an attempt to test which of these two possibilities seemed more likely, various model systems of the coiled-coil were built with CPK space-filling components.

Two parallel and identical chains in register result in 9 valine-to-valine or isoleucine-to-isoleucine interactions. The

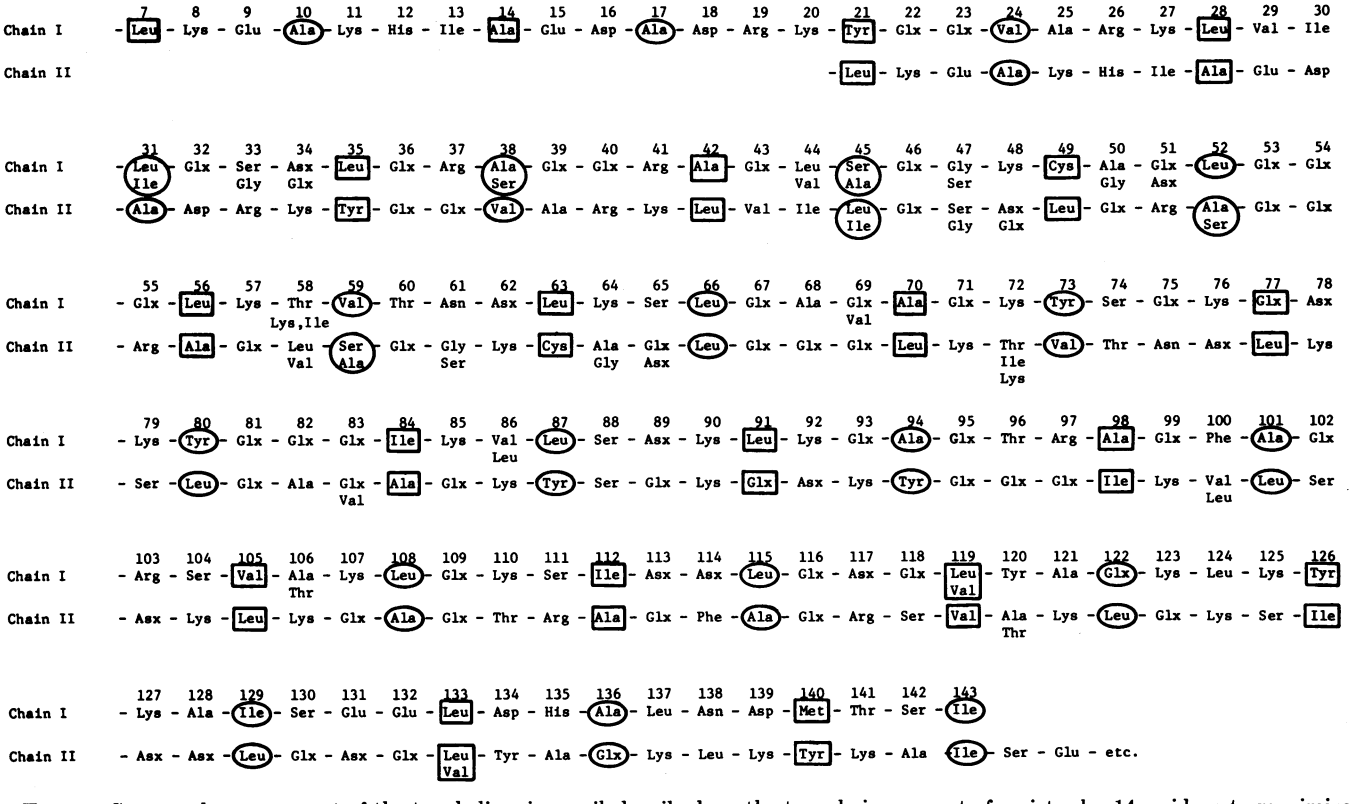

FIG. 3. Staggered arrangement of the two helices in a coiled–coil where the two chains are out of register by 14 residues to maximize hydrophobic interaction and regularity of the coiled-coil.

minimum radius of the coiled-coil at these positions occurs when the γ-positions of the side chains of these residues are in van der Waal's contact. The major hydrophobic bonding then results from γ- to γ-carbon and hydrogen interaction. However, such an arrangement would prevent interaction between the alanine residues unless the radius of the coiled-coil was variable and constricted in the regions of these residues.

A more attractive arrangement would appear to be one in which the radius of the coiled-coil is intermediate between a situation in which two valines or isoleucines interact and that in which two alanines are paired through β- to β-carbon and hydrogen interaction. This structure will occur, for example, when an alanine is paired with an isoleucine through β- to γ-carbon and hydrogen interaction or when two leucines are paired through their β- and δ-carbons and hydrogens. To represent this intermediate situation, we constructed a coiled-coil in which all hydrophobic positions were occupied by leucines and in which the two α-helices were running in the same direction. Pairs of leucines were removed from positions corresponding to series I and II and replaced with all combinations of amino-acid pairs. It was necessary to do this for positions in both series I and II since residues in the two series are not stereochemically equivalent in the coiled-coil. Each of the amino-acid pairs was tested for its stereochemical fit without disruption of the coiled-coil established by the leucine-to-leucine interactions. In this way it was possible to construct matrices for all possible combinations of amino acids for both series. These are shown in Fig. 2*A* and *B*. Amino-acid pairs fall into one of four categories in series I and into one of three categories in series II positions. For series I the situation is more restricted since residues involving β-carbon branched side chains (i.e., isoleucine, valine, and threonine) cannot be sterically accommodated without an increase in the radius of the coiled-coil. However, each of these residues can be paired with any other residue that does not have a branched β-carbon side chain. Of the remaining three categories in the matrices, the one most favorable to the stabilization of the coiled-coil is that which provides β- to γ- or δ-carbon and hydrogen van der Waal's contacts and does not involve charged residues. We refer to this as the favorable pairing category.

If the two chains of tropomyosin sequence are staggered by 14 residues (see Fig. 3), then the regions of small hydrophobes tend to pair with the bulkier hydrophobes in a satisfactory manner. All pairs now fall into the favorable pairing category with only two exceptions. Thus, at position 94 of chain I, the pairing of alanine with tyrosine provides no β- to γ- or δ-interaction but is stereochemically permissible without a change in the radius of the coiled-coil structure. It has also been observed that the aromatic ring of tyrosine (or phenylalanine) can interact with other side chains in the immediate vicinity to perhaps compensate for the absence of β- or γ- or δ-interaction. An apparently more serious discrepancy occurs at position 119, where the pairing of two valines in series I is stereochemically not permissible without an increase in radius of the coiled-coil. However, the valine at position 119 of chain I was found only as a substitution for leucine and in much lower yield. Thus, it probably arises from the minor tropomyosin component (18, 19), and it is possible that a compensating substitution for valine occurs in chain II at this position but has not yet been detected in our sequence

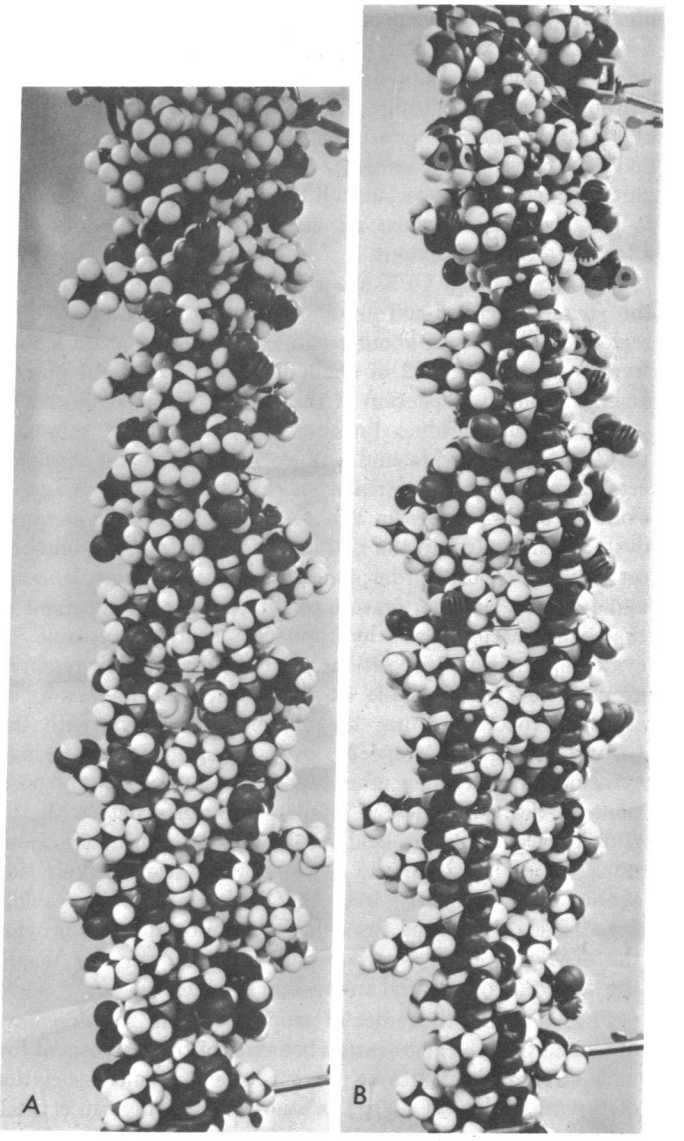

FIG. 4. Photographs of a CPK space-filling model of a portion of the staggered coiled-coil of tropomyosin. The region shown is from about residues 28–86 of chain I and from residues 14–72 of chain II (see Fig. 3). The direction of the chain (NH_2-terminal to COOH-terminal) is from *top* to *bottom*. (*A*) The sulfhydryl of cysteine-49 of chain II can be seen just below the middle of the photograph. (*B*) Taken from the opposite side of the structure: many of the side chains have been removed to illustrate the pairing of the hydrophobic side chains.

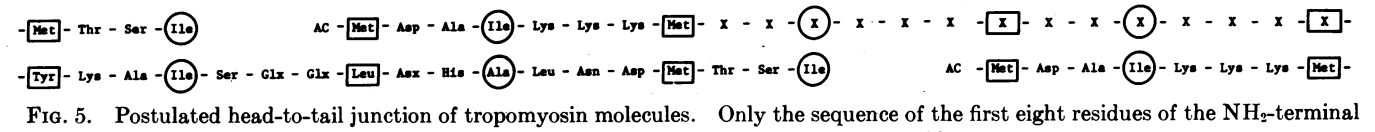

FIG. 5. Postulated head-to-tail junction of tropomyosin molecules. Only the sequence of the first eight residues of the NH_2-terminal region of the chains has been determined (17). *Squares*, series I residues; *circles*, series II residues.

analysis. The staggering of the two chains by 7, 21, or 28 residues leads to a less satisfactory packing arrangement since 8, 6, and 10 amino-acid pairs, respectively, do not fall within the favored pairing category as detailed in the matrices of Fig. 2. In these comparisons, the residues at positions 77 and 122 have been assumed to be glutamines rather than glutamic acids. However, even if this is not so, the comparison is not invalidated since all staggering arrangements are affected to the same extent.

Photographs of a CPK space-filling model of a portion of the staggered coiled-coil are shown in Fig. 4*A* and *B*. The region shown is from about residues 28 to 86 of chain I and from residues 14 to 72 of chain II (Fig. 3). Several points emerge from an inspection of the details of the structure. A portion of the residues forming the hydrophobic core are turned to the outside and will be exposed at the surface, creating patches or streaks of hydrophobicity. A good example of this is seen in Fig. 4*A*, where below the exposed cysteine sulfhydryl in the middle of the photograph, one observes in descending order; leucine, leucine, tyrosine, leucine, and tyrosine. Another feature of the packing arrangement is that where ambivalent hydrophobes, such as tyrosine or cysteine, occur in the positions of series I or II, their reactive groups are still available at the surface for ionization or chemical reaction. Thus, the model is consistent with the titration data of Lowey (20) even though four of the five tyrosines of the cyanogen bromide fragment occur in the positions of the two series. A similar situation exists with the glutamine or glutamic acid residues that occur at two positions (77 and 122) in the hydrophobic pattern. Their side chains are sufficiently long that the amidated or acidic moieties can be turned towards the exterior and still provide the necessary β- to γ-carbon and hydrogen van der Waal's contact with their paired amino-acid residues.

Physicochemical studies of tropomyosin in solution have indicated that the aggregation behavior of tropomyosin at low ionic strength is explicable in terms of end-to-end association of the molecules (21, 22). The study of tropomyosin crystals by x-ray diffraction and electron microscopy has shown that there is a specific head-to-tail bonding of tropomyosin molecules in the filaments of these kite-like structures (10, 11). The observations of Higashi-Fujime and Ooi (12) on the edges of negatively stained crystals suggest that the molecules end near the middle of the short arms of these kites. However, there appears to be no discontinuity or appreciable overlap in this region between the ends of the two-stranded coiled-coil molecules (11). Our observation that the amino-acid sequence of tropomyosin can be best accommodated in a staggered coiled-coil may provide an explanation for these observations. If the two polypeptide chains are of equal length, there will be a 14-residue segment of single-stranded structure at each end of the molecule. With monomeric tropomyosin in solution these segments presumably exist in a nonhelical or disordered form. In an aggregated state or in tactoids and crystals, however, these "sticky ends" may overlap and provide the end-to-end attachment sites such that the coiled-coil structure could be continuous along the length of the tropomyosin filaments. Although our knowledge of the amino-acid sequence at the NH_2-terminal end of tropomyosin is still fragmentary, available evidence (17) is consistent with such a hypothesis. Thus, the NH_2-terminal end of tropomyosin does show the repeating pattern of hydrophobic residues in two series and can be aligned with the COOH-terminal sequence such that there is no interruption of the amino-acid pairing pattern between the two strands (Fig. 5). There would be only a gap of three amino-acid residues of single-stranded structure at the junction points. It is probably also significant that the NH_2-terminal end is acetylated, thus reducing the local disruption that might otherwise occur to the order of the structure with a charged NH_2-terminal group.

Although a structure in which the two α-helices of the coiled-coil are arranged in a staggered conformation provides an attractive explanation for the head-to-tail association of tropomyosin molecules in solution, in crystals, and in the thin filaments of muscle, the possibility that the two chains are in register cannot be excluded at this time. Indeed, the more irregular structure provided by changes in the radius of the coiled-coil arising from the two chains in register could be of importance in the interaction of tropomyosin with actin and one or more members of the troponin complex in the myofibril.

It is predicted that a similar pattern of repeating hydrophobic residues as we have observed in tropomyosin will be found in the rod region of the myosin molecule and in paramyosin. However, because of their different association properties, the longer-range pattern of bulky and small nonpolar residues and the distribution of these and ionic and polar residues on the surface of the coiled-coil may be quite different.

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