# STRUCTURAL STUDIES ON THE TROPOMYOSIN/TROPONIN COMPLEX OF VERTEBRATE SKELETAL MUSCLE

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Received January 4,1974

Summary:- It has been shown that there is a 29Å periodicity in the C-terminal half of the sequence of rabbit tropomyosin. This period, which is found in the surface apolar residues and in some of the positively and negatively charged residues, may be related to the axial separation of actin molecules in the thin filaments of vertebrate skeletal muscle. The types of interactions between tropomyosin and actin during the contraction/relaxation process are suggested. It has also been shown that the two chains of tropomyosin are most probably staggered with respect to one another by either 7 or 14 residues. Troponin appears to bind to tropomyosin either 100 or 150Å from the N terminus of tropomyosin indicating that the homologous cysteine sequence is not implicated directly in the tropomyosin/troponin interaction.

Rabbit tropomyosin has been well characterised as a result of extensive physical and structural studies (1-6). It may form crystals or tactoids and in most cases, the molecules bond head to tail to a form a continuous polar filament. It has been shown (7-10) that troponin (consisting of three chains known as TN-T (MW ∿37,000), TN-I (MW ∿23,000) and TN-C (MW ∿18,000)) binds to one tropomyosin molecule and that the head to tail assembly of this regulatory complex is located in the grooves of the actin helix (11-13). Also in vertebrate skeletal muscle, the tropomyosin may move in the groove of the actin helix (14-16) depending on the amount of $Ca^{2+}$ bound by the TN-C component of troponin.

Results have been obtained (2,16) which indicate that the antiparallel pair of molecules comprising the basic unit in the Mg-tactoid has a 28Å axial period. This infers that each molecule has an axial (or psuedo axial) period of about 28Å ($^1/_{14}$th the length of the molecule) or 56Å ($^1/_7$th the length of the molecule). The axial periodicity between actin monomers in the thin filaments is about 27Å. These results suggest that a 28Å periodicity may be present in the rabbit tropomyosin sequence (6).

About half of the apolar residues not in Series I or Series II (6) show a periodicity of about 19½ residues (29Å) which extends along the entire length of the chain so far sequenced (Table I). All of the remaining "surface"

Table I

Calculated and Observed Positions of Some Residue Types

Showing 19½ residue Periodicity

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| APOLAR | Calc. | 11 | - | 30½ | - | 50 | - | 69½ | - | 89 | - | 108½ | - | 128 |
| | Obs. | 13 | - | 29<br>30 | - | 50 | - | 68 | - | 86 | - | 106 | - | 128 |
| APOLAR | Calc. | 23 | - | 42½ | - | 62 | - | 81½ | - | 101 | - | 120½ | - | 140 |
| | Obs. | 25 | - | 44 | | | | | | 100 | - | 120<br>121 | - | 137 |
| NEGATIVELY | Calc. | 17 | - | 36½ | - | 56 | - | 75½ | - | 95 | - | 114½ | - | 134 |
| CHARGED | Obs. | 16<br>18 | - | 36 | - | 55 | - | 75 | - | 95 | - | 114 | - | 134 |
| POSITIVELY | Calc. | 7½ | - | 27 | - | 46½ | - | 66 | - | 85½ | - | 105 | - | 124½ |
| CHARGED | Obs. | 8 | - | 27 | - | 48 | - | 64 | - | 85 | - | 103 | - | 125 |

Residue numbering is the same as that given by Hodges et al (1972)

apolar residues fit closely on another 19½ residue periodicity which is displaced with respect to the other by 12 residues. This periodicity is continued into the single strand overlap region. In addition, the scheme predicts an apolar residue close to the middle of the first seven unordered residues of the sequence. An extra isoleucine is found in this heptapeptide. There is also a 19½ residue periodicity (Table I) in some of the negatively charged residues (ASP, GLU, ASX, GLX) and the positively charged residues (LYS, ARG). Other periodicities may exist in the sequence but these have not been sought in the present work.

As the length of tropomyosin is 410 ± 4Å (5) and the average residue translation of coiled-coils of $\alpha$-helices is 1.485Å, the equivalent number

of residues per chain is (410 ± 4)/(1.485 ± 0.015) = 276 ± 5.5. Hodges et al (6) have postulated that Series I and Series II interactions are continuous across the region where the single stranded chains overlap. It follows that the length of 410Å must correspond to an integral multiple of seven residues, i.e. number of residues should be either 273 or 280. The period of 19½ residues is 1/14th of 273. The number of residues per tropomyosin chain will be 3 less than 273 or 280 due to the 3 residues single strand region. The molecular weight of rabbit tropomyosin is therefore either 62,100 or 63,710 (mean residue weight 115).

It would be surprising if this 19½ residue (29Å) periodicity bore no relationship to the same axial period between actin molecules in the thin filaments of muscle. It is possible that tropomyosin may interact with actin molecules along each of the two strands of the actin helix or that the tropomyosin molecules in each groove of the actin helix are in axial register (18). In the latter case, the two sets of tropomyosin/actin interactions will only be quasi equivalent. It is also possible that the 29Å period represents a psuedo period of a longer repeating unit but there is not sufficient data yet to test this possibility.

Parry and Squire (16) have shown that there is a relative movement of about 15Å between actin and tropomyosin on activation of vertebrate skeletal muscle. The role of conserved and variable linkages in protein interactions in general and of the tropomyosin in the groove of the actin helix in particular has been discussed (5,17,18). Equal periodicities along the actin helix and the tropomyosin mean that quasi-equivalent salt linkages could be formed between actin and seven (or fourteen) points along tropomyosin. Since these would be formed between long and flexible side chains, they could be maintained even over a 15Å displacement. On the other hand, apolar interactions are non specific and the periodicity of these residues on tropomyosin might correspond to an apolar stripe on the surface of actin. Relative movement may occur without grossly changing the strength of the interactions.

The C-terminal half of the tropomyosin sequence can be considered as psuedo repeating heptapeptides with Series I and Series II residues occupying positions 2 and 6. Charged residues in positions 3 and 5 of different chains are close enough to interact. Studies on the $\alpha_1$ chain of collagen (19) have shown that polar interactions are extremely important in determining the relative stagger between tropocollagen molecules even in an aqueous environment. Table II shows that the greatest number of negatively charged residues

Table II

| | Position in Heptapeptide | | | | | | |
|---|---|---|---|---|---|---|---|
| Residue | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| GLU, GLX | 3½ | 1 | 10 | 5 | 5 | 1 | 6 |
| ASP, ASX | 2½ | 0 | 1 | 2 | 2½ | 0 | 3 |
| LYS | 3⅓ | 0 | 2 | 1 | 6 | 0 | 6 |
| ARG | 1 | 0 | 0 | 3 | 2 | 0 | 0 |
| SER | 2 | 1 | 3 | 1 | 2 | 0 | 0 |
| THR | ⅓ | 0 | 1 | 1 | 0 | 0 | 1½ |
| APOLAR | 6⅓ | 18 | 2 | 3 | 1½ | 19 | 3½ |

(including GLX and ASX) are found in position 3 and the largest number of positively charged residues found in position 5. Confirmation that the charged residues determine (in part) the relative stagger of the chains must await the complete sequence and the acid/amide assignment of the GLX and ASX residues. However, assuming charge interactions and "graded" apolar interactions (6), the total number of interactions has been calculated for relative chain staggers of 0, 7, 14, 21 and 28 residues (Table III). Staggers of 7, 14 and 21 residues appear equally good though staggers of 0 and 28 residues appear less satisfactory.

Table III

Interactions

| Type of Interactions | | Stagger (Residues) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 7 | 14 | 21 | 28 |
| Apolar Series I | A | 8½ | 13½ | 15½ | 12 | 8 |
| | B | 7 | 2 | 0 | 2 | 5 |
| | C | 4½ | 3½ | 2½ | 3 | 3 |
| Apolar Series II | A | 10½ | 13½ | 15 | 12 | 11½ |
| | B | 8½ | 3½ | 1 | 3 | 3½ |
| | C | 1 | 2 | 2 | 2 | 1 |
| Polar | | 8 | 14 | 10 | 11 | 9 |
| Total Apolar and Polar | A | 27 | 41 | 40½ | 35 | 28½ |
| | B | 15½ | 5½ | 1 | 5 | 8½ |
| | C | 5½ | 5½ | 4½ | 5 | 4 |
| Hydrophilic | | 6 | 8 | 5 | 5½ | 6 |

A: - Van der Waals contact between $\beta$ and $\gamma$ or $\delta$ carbons and hydrogen and do not involve charged residues.

B: - No $\beta$ to $\gamma$ or $\delta$ carbon and hydrogen Van der Waals contact but stereochemically permissable.

C: - Not stereochemically permissable or involving charged residue (GLX taken as GLU).

These definitions follow closely those put forward by Hodges et al (19' Hydrophilic interactions are defined as those between SER and THR with THR, ASX or GLX. Similarly polar interactions are defined as those between LYS or ARG with ASP, GLU, ASX, GLX and must be an overestimate the number of interactions.

X-ray diffraction patterns from both fibres of tropomyosin and tropomyosin complexed with parachloromercuribenzoate (pcmb) were obtained by Caspar et.al (2). Intensity measurements along the meridian were made by Cohen and Parry

who located the cysteine residues by means of a difference Patterson function (Cohen and Parry - in preparation). Fourier transforms of models in which the two chains of tropomyosin were staggered by 0, 7, 14, 21 and 28 residues with respect to one another have now been calculated. One dimensional Pattersons were calculated from the model data and compared with that obtained experimentally. Relative staggers between chains of 0, 7 or 14 residues were compatible with the data though staggers of 21 or 28 residues were not. Since (a) there is no evidence that two stranded regions of tropomyosin molecules overlap in the crystals (5, 18) and (b) it is difficult to visualise how head to tail interactions could be made if the chains were not displaced with respect to one another allowing single strand overlap, it seems that the possible stagger between chains is restricted to either 7 or 14 residues. The latter value was chosen by Hodges et al (6) from packing considerations.

There are three indications that the broken end of the Mg tactoids (5,7) may correspond to the C-terminal end of the tropomyosin molecules. Firstly, the "brush" end of the tactoid is often seen to terminate in a narrow white non staining band about 20-30Å wide. Even when this band is not seen due to imperfections in specimen preservation, the corresponding band related by the 2-fold axis at the centre of the brush region is always missing. The C-terminal end of the sequence is rich in uncharged residues. Of the last 18 residues (17Å), 6 are non acidic (a much higher proportion than average) and 6 occur in series I or Series II. The electron micrographs do not indicate any non staining band at a position corresponding to the other end of the molecule. Hodges et al (6) have sequenced the first 8 residues at the N-terminus of rabbit tropomyosin. This shows only 1 non acidic residue and 3 residues in Series I and Series II indicating that this region is not rich in non acidic residues.

Secondly, it has been found (20) that troponin may be dissociated from tropomyosin by the use of mercurial compounds. This may implicate the cysteine residues in some way with the binding to tropomyosin to troponin. If this conclusion is confirmed, it might be expected that there would be little

microheterogeneity in the sequence in the vicinity of the cysteine residue. However, the opposite is true. In the C-terminal half of the molecule (143 residues), there are 15 substitutions, 5 of which occur within an 8 residue span encompassing CYS-49. This does not eliminate the possibility of troponin binding near the homologous cysteine sequence but it does seem surprising that such a high percentage of the substitutions in the C-terminal half of the chain should occur at this point. Since troponin is known to bind to tropomyosin at a distance of about 25-40Å (5, 18) from the 2-fold axis distant from the brush end of the Mg-tactoids it follows from the initial assumption that the brush end corresponds to the C-terminus of the molecule and that the homologous cysteine sequence is not implicated directly in the troponin binding.

Thirdly, one interpretation of the meridional Patterson function places the average position of the homologous and "substituted" cysteine residues about 80Å and 165Å respectively from a 2-fold axis. Precise values for the homologous cysteine residues place them 80, 75 and 86, 71 and 92Å from a 2-fold axis for relative chain staggers of 0, 7 and 14 residues respectively. Since Hodges et al (6) have shown that the cysteine residue is found 95 residues ($\backsim$142Å) from the C-terminus and the 2-fold axis at the brush end of the tactoid is about 70 ± 8Å from the end of the molecules (18), the distance of the homologous cysteine residue from the end of the molecule can be calculated for each stagger (0, 7 and 14 residues) and shown to be consistent with the sequence data. Consequently, both the substituted cysteine residue and the troponin must be about 25-40Å from the 2-fold axis distant from the "brush" end of the tactoid. It is not known whether the "substituted" cysteine and the troponin binding site are the same side of the 2-fold axis though the dissociation data (20) suggests that this might be the case. Once again, this implies that the C-terminus of the molecule may be at the brush end of the Mg-tactoids. It should be noted that an alternative solution to the Patterson function places the homologous cysteine sequence at or very close to a 2-fold axis. This position

is compatible with the sequence data providing that the brush end of the Mg-tactoid corresponds to the N-terminus of the tropomyosin molecule.

Several workers have shown that troponin binds to tropomyosin at a position about one third the distance from the end of the molecule (5,7). Greaser et al (8) have suggested that troponin binds at the end of tropomyosin but this is clearly inconsistent with studies on the Mg tactoids complexed with troponin. Ebashi et al (7) believe that troponin is about 130Å from the C-terminal end of tropomyosin assuming that a derivative of dextran sulphate (a polyanion) links the N-terminal ends of the molecules. It is now known (6) that the N-terminus is acetylated which throws some doubt on the validity of this assumption. From similar reasoning, it might be predicted that divalent cations would bridge C-terminal ends of tropomyosin molecules giving a head to head interaction in the tactoids. It is known that this situation does not occur (5,7). The results presented here suggest (but do not prove) that the troponin is about 100 or 150Å from the N-terminal end of tropomyosin.

The findings may be summarised as follows:
(a) the "surface" apolar residues and some of the positively and negatively charged residues show a periodicity (or psuedo periodicity) of about 29Å. This indicates the type of interactions that might occur as tropomyosin slides across actin during the contraction/relaxation process (b) the polar residues are probably implicated in determining the relative stagger between the chains of tropomyosin (c) total apolar and polar interactions favour staggers of 7, 14 and 21 residues between chains (d) one dimensional Patterson data limits the possible stagger between chains to 0, 7 or 14 residues (e) the troponin binding site is located 100 or 150 Å from the N-terminus of tropomyosin and the homologous cysteine sequence is not involved directly in the interaction.

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