STRUCTURAL ANALYSIS OF TROPOMYOSIN TACTOIDS

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Received December 8,1975

Summary

A refined computer graphics approach to correlation of molecular sequence with electron micrographs of tropomyosin tactoids is presented. It is shown that antiparallel structures with molecular chains in phase, 21 or 14 residue overlap and C or N terminal overlap are consistent with the morphology. The C terminal overlap structure previously postulated gives the best direct correlation but chemical evidence appears to support the N terminal overlap structure. The parallel tactoid form appears more complicated and no adequate structure has yet been elucidated.

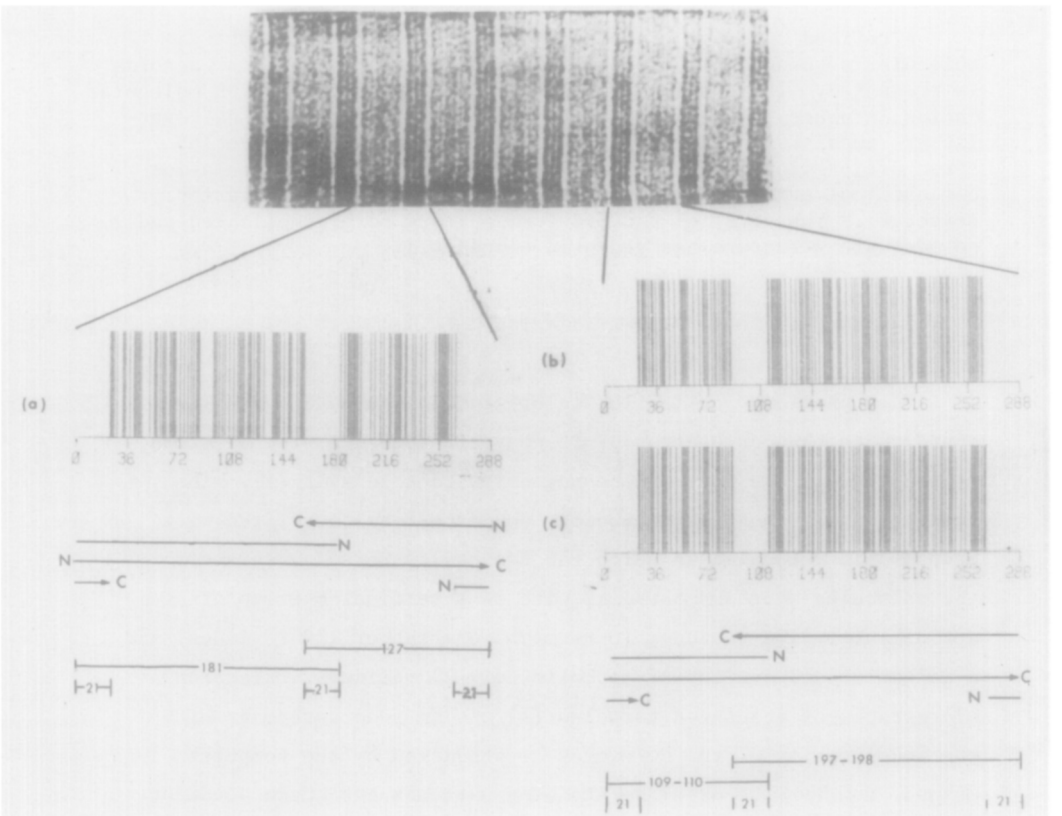
Introduction

In a previous publication we presented a computer graphics approach to the correlation of tropomyosin sequence with electron micrographs of one form of the magnesium tactoid salt (1). The results showed that the morphology could best be correlated with tropomyosin molecules in which the two chains were in register, the molecules were antiparallel with an N terminal overlap of approximately 178 residues (C terminal overlap of 130 residues) and a molecular end overlap of approximately 21 residues. Electron micrographs of fractured tactoids (2) are in good agreement with the end location of the molecules as predicted by the computer method, however the detailed staining patterns and chain position were not accessible by the previous method which involved summation of negatively charged residues over a nine residue resolution. In this paper we present a more detailed study using line graphics representation, similar to that used by Miller et. al. in the solution of collagen structures (3,4).

Method

As before, it is assumed, that uranyl acetate staining, fingerprints glutamate and aspartate residues, that there are two parallel

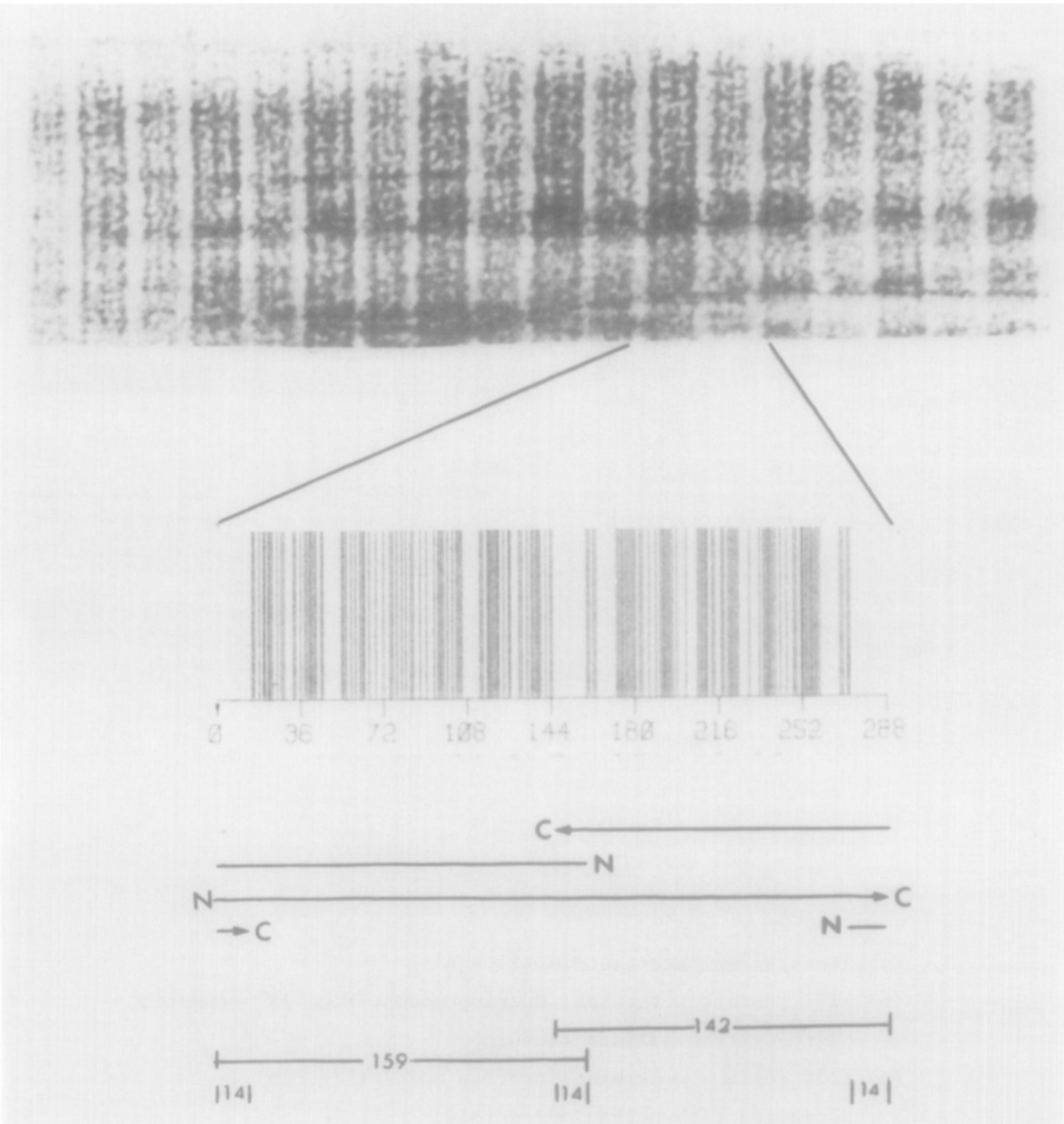
chains in each molecule and that various tactoid forms correspond to various molecular phasing and overlap. In the present approach, lines are drawn corresponding to each Asp and Glu residue (5). Because of the great sensitivity of this method (which corresponds to one residue resolution) shifts of one residue in molecular phasing produce significant changes in the predicted morphological pattern. We have therefore made two further assumptions not inherent in the



1. Magnesium tactoid (9) compared with
   (a) C terminal overlap of 127 residues, N terminal overlap of 181 residues;
   (b) N terminal overlap of 110 residues; and
   (c) N terminal overlap of 109 residues.

   The molecular end overlap is 21 residues in all cases. All numbers are in residues $\pm$ 3 based on a rational 287 residue sequence (1).

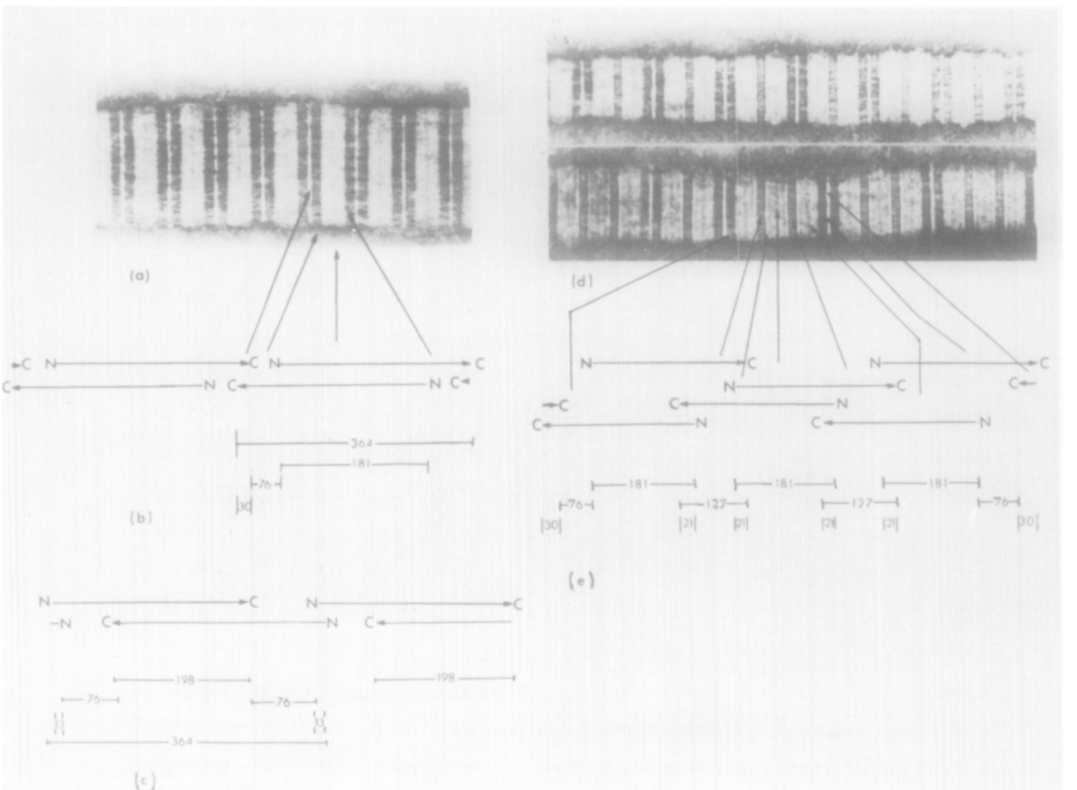
previous paper: a) there is no chain stagger within the molecules (6, 7) and b) the stain does not penetrate the molecular end overlap region. Even so, a complete study of all molecular phasing and feasible end overlap leads to prohibitive computer usage. Consequently



2. Mg^{2+}/Ba^{2+} tactoid (9) compared with graphics output for model having C terminal overlap of 142 residues, end overlap of 14 residues.

we have begun with transparent overlays of the computer-predicted staining patterns for the single chain, located approximate molecular phasing and then refined these positions by computer graphics.

The sensitivity of the method is demonstrated in Figure 1 where one form of the Mg^{2+} tropomyosin tactoid is compared with three graphic outputs. The first (a) corresponds approximately to the C terminal overlap previously postulated of 127 residues. Output b) and c) correspond to N terminal overlaps 109 and 110 residues varied by one



3. (a) Dextran sulfate tactoid (2).
   (b) 181 residue N-terminal overlap model (Fig. 1a) compared with dextran sulfate tactoid.
   (c) 110 residue N-terminal overlap model (Fig. 1b) is compared with polyanion tactoid.
   (d) Mg^{2+} pattern and dextran sulfate pattern combined in one tactoid (2).
   (e) Two dimensional representation of model for (d) using 181 residue N-terminal overlap.

residue phasing. In both cases the molecular end overlap is 21 residues.

Figure 2 shows a second form of Mg^{2+} tactoid (also formed with Ba^{2+}) the molecular overlap depicted in the graphics output is 142 residues antiparallel (counted from the C terminus) and the molecular end overlap is 14 residues. (The tactoid repeat period in this latter electron micrograph is 7 residues longer than in Figure 1).

In Figure 3, models for the C and N terminal overlap structures are compared with the dextran sulfate tactoid and a second tactoid type which combines the dextran sulfate and Mg^{2+} patterns. This second form (Figure 3d) seems to indicate that the molecular overlap should be the same for both the dextran sulfate form and the Mg^{2+} tactoid. The gray region of the polyanion tactoid measures approximately 180 residues, which fits closely with the 181 residue overlap of the model depicted in Figure 1(a). Models (b) and (c) would produce too large a gray region. We attribute the dark banding to a lower chain density in these regions.

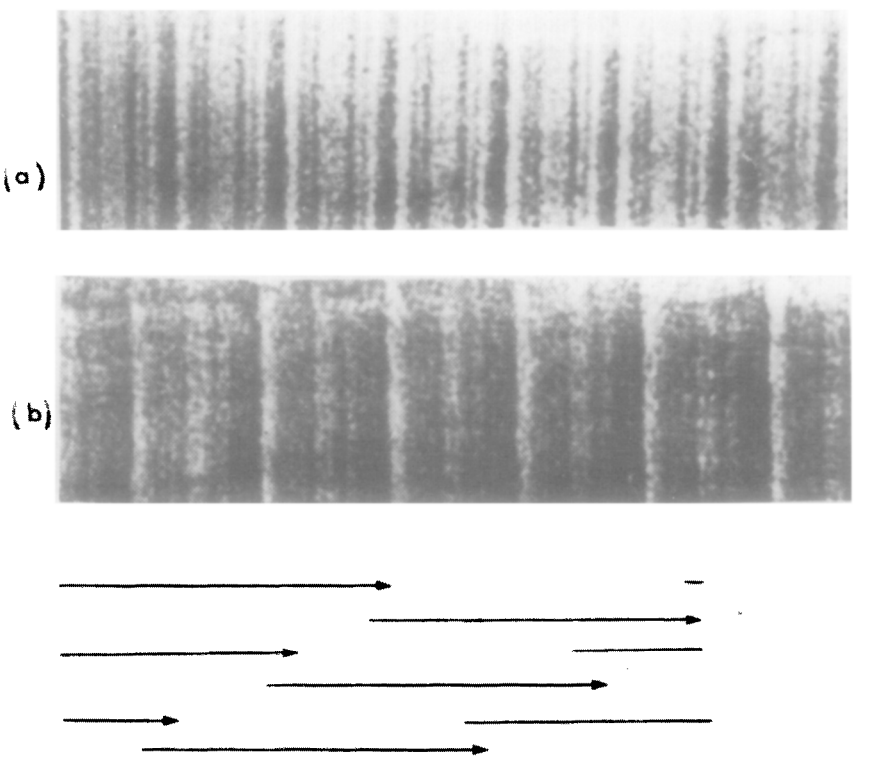
All of the preceding tactoid structures clearly have antiparallel molecules. The solution of the parallel structure, which relates to that found natively, appears to be much more difficult. Comparison with electron micrographs of the lead tactoid suggests that there should be a molecular overlap of approximately 110-120 residues. We have used the computer to scan both C and N terminal overlap regions and though there are low resolution similarities, there is no good correlation at the high resolution level with only two molecules. The negative stain pattern of lead tactoids suggests that there may be more than two molecules in a unit cell, in which case our computer/modelling method could not be expected to work with current limitations. Based on preliminary studies, the three molecule arrangement shown in Figure 4 gives an approximate correlation with the tactoid.

Discussion

The new computer graphics methodology shows good correlation of the divalent salt tropomyosin tactoids with an antiparallel arrangement of two molecules in a unit cell. It is, however, difficult to decide whether there is a C or N terminal overlap on the basis of correlation of the graphics output with the Mg^{2+} pattern alone. In Figure 1 it appears that the C terminal overlap gives the best spacing as correlated with electron micrographs of the Mg^{2+} salt and of the

order described previously. However the stagger of 127 residues places the single Cys group in the outer stain bands of the triplet (as previously noted). A recent paper (6) suggests that the Cys in adjacent molecules lies in the center of the gray region which would correspond to the N terminal stagger (110 residue overlap). This pattern does not quite match with the micrographs since the dark triplet band structure is slightly narrow. It is conceivable that some negative stain extends the region slightly.

Troponin binds to the center of the gray region of the Mg^{2+} tactoid (2). Of the three troponin components, only TN-T binds to tropomyosin (8). Since TN-T contains no cysteine, it is unlikely that the Cys in position of 190 of tropomyosin molecule is itself the binding site. Therefore placement of Cys near the troponin binding site alone is not sufficient for choice of the N



4. Possible three molecule model for Pb^{2+} tactoid paired with (a) positive and (b) negative staining patterns (9). End overlap equals 21 residues.

terminal pattern over the C terminal one. Ohtsuki (2) has indicated that troponin does not bind to the polyanion paracrystal. This might suggest troponin normally binds to basic residues. Model 1a places the TN-T binding site approximately between residues 86 and 96, shown by Stone et. al. (5) to be an acid poor region containing the sequence Arg-Arg (residues 90-91). Model 1b would place TN-T binding between residues 179 and 186; the only base in this region is Arg 182.

Acknowledgements

We wish to thank H. J. R. Weintraub for help with computer programming used in this study, and the authors and publishers for permission to reproduce published electron micrographs, as indicated.

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