# TROPOMYOSIN: EVIDENCE FOR NO STAGGER BETWEEN CHAINS

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## 1. Introduction

Tropomyosin, a component of the regulatory system of vertebrate skeletal muscle, is a rod-shaped molecule about 40 nm long, constructed from two parallel α-helical chain tightly bound into a coiled-coil configuration [1]. Chemical analysis of rabbit skeletal tropomyosin has indicated two types of chain, designated α and β, with the α being about four times as abundant [2]. These chains have similar amino-acid compositions [2] and sequences [3], but they differ in cysteine content with the α-chain have one such residue compared with two for the β [2]. A pseudo-repeat of 7 residues has been demonstrated in the sequence, which produces a strip of hydrophobic residues down one side of each chain [3]. In the coiled-coil, these strips form the faces of mutual contact between the chains. On the basis of the stacking geometry of these hydrophobic residues, it has been proposed that the two chains in the tropomyosin molecule are staggered by some multiple of 7 residues and most probably by 14 [3]. If such a stagger occurs, the formation of intramolecular disulphide bridges between the two chains would be sterically impossible. This article demonstrates the production of such an intramolecular bond and so suggests that the two polypeptide chains are in register.

## 2. Materials and methods

Troponin-free tropomyosin was prepared from rabbit back and thigh muscles as described by Cummins and Perry [2] and its concentration measured by ultraviolet absorption taking $E^{1\%}_{278} = 2.9\ cm^{-1}$ [4]. Oxidised tropomyosin was prepared by stirring tropomyosin in 1 M NaCl, 25 mM sodium borate, 25 mM $CuCl_2$ pH 9.3 at 20°C under air until no free sulphydryl groups could be detected. Reduced tropomyosin was prepared by dialysing tropomyosin against 1 M NaCl, 25 mM sodium borate, 5 mM dithiothreitol pH 9.3 at 4°C for 48 hr. Sulphydryl concentration was measured by the DTNB procedure of Cowgill [5]. Gel electrophoresis was performed on 10% polyacrylamide slabs with a 0.1% SDS, 1.0 M Tris–bicine pH 8.1 running buffer and, to prevent oxidation, 1 mM *N*-ethylmaleimide was added to the sample buffer in place of 2-mercapto-ethanol [5]. Gels were stained with Coomassie Brilliant Blue. Low-speed sedimentation equilibrium runs were performed in a MSE analytical ultracentrifuge at 9000 rev/min with a 6 sample rotor. To overcome the tendency of tropomyosin to aggregate at low ionic strength, the solvent employed was 1 M NaCl, 25 mM Tris–HCl, pH 7.5 at 5°C with 1 mM dithiothreitol added for reduced tropomyosin. Magnesium paracrystals of tropomyosin were prepared and examined negatively stained as described by Yamaguchi et al. [6] using a Philips EM 300 electron microscope operated at 80kV.

## 3. Results

The cysteine residues of tropomyosin are easily oxidised as illustrated by the rapid decrease in sulphydryl content observed when the protein was stirred in the presence of air and a trace of $CuCl_2$ (fig.1). Gel electrophoresis (fig.2), indicated that, while reduced tropomyosin was dissociated into its constituent chains in the presence of SDS and ran as a monomer (defined here as a single chain), the majority of oxidised tropomyosin was not dissociated and ran as a dimer (defined as two chains). This established that

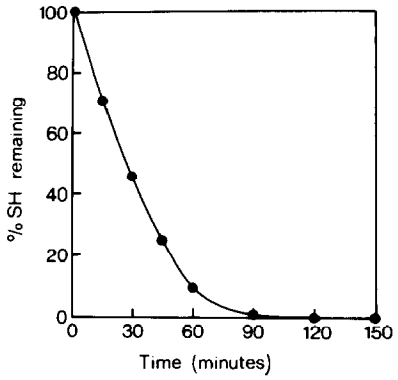


Fig.1. Decrease in sulphydryl content of reduced tropomyosin (freed of dithiothreitol by dialysis against 5 mM HCl [5]) with time when exposed to air as described in the Methods section.

most of its sulphydryl groups had been oxidised to disulphides. The minor component of oxidised tropomyosin running as a monomer was probably due to its cysteines being oxidised further (e.g. to cysteic acid) and there was also evidence that a small quantity of material of higher mol. wt had been formed (indicating some oligomer formation involving the two sulphydryls of the β-chains). When oxidised tropomyosin was reduced, it behaved similarly to reduced tropomyosin, confirming that the dimer formation was due to disulphide bond formation.

Mol. wt determination in a non-denaturing solvent by the low-speed sedimentation method established that these disulphide bonds were primarily intramolecular. The apparent mol. wt at zero concentration of reduced tropomyosin was found to be 69 000 ± 4000 (3 runs) which compares well with the accepted

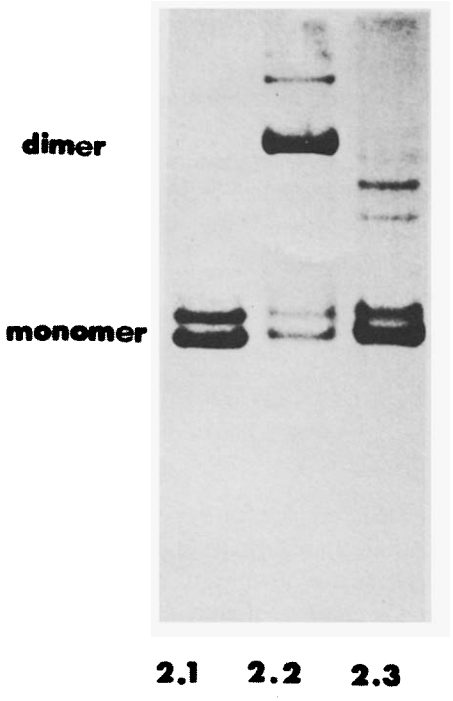


Fig.2. SDS gel electrophoresis of tropomyosin. 2.1 reduced (the two bands are the α- and β-chains [2]). 2.2 oxidised 2.3 oxidised and then reduced as described in the Methods section.

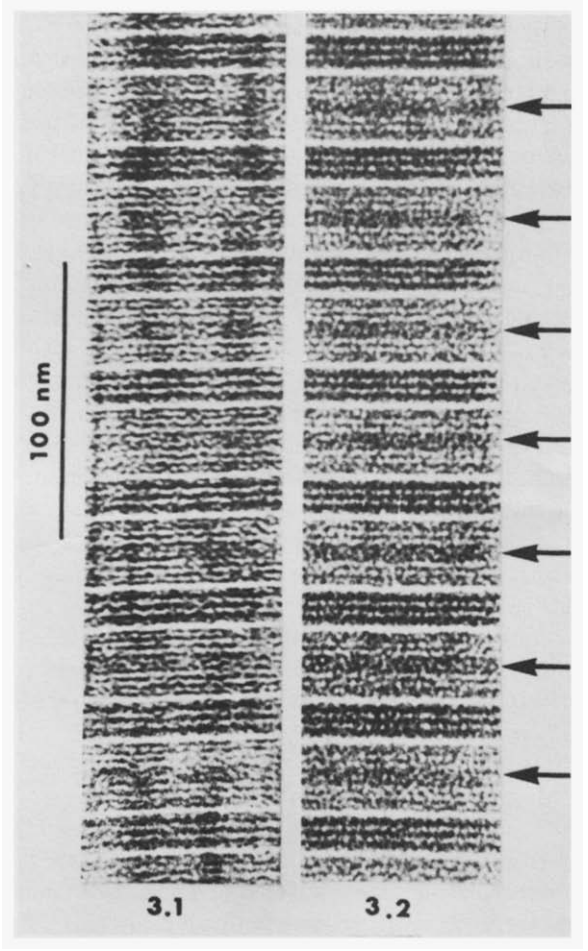


Fig.3. Magnesium paracrystals of tropomyosin. 3.1 reduced. 3.2 oxidised. Note the general similarity of the patterns and also the increased density at 40 nm intervals (arrowed) in 3.2.

value of 68 000 [7]. That of oxidised tropomyosin was found to be 74 000 ± 3000 ( 5 runs) which is the result expected if the disulphide bonds were primarily intramolecular (producing no increase in mol. wt) with a small number between molecules producing a slightly higher mol. wt. The result is clearly inconsistent with the formation of only intermolecular linkages since, in this case, the expected mol. wt of the oxidised form would be in excess of 136 000.

Additional evidence for the presence of intramolecular disulphide linkages was obtained from a study of paracrystals formed from oxidised and reduced tropomyosins. If these linkages were formed between molecules, one, would not expect the polymeric material so produced to be able to form highly ordered aggregates. However, both oxidised and reduced tropomyosins formed highly ordered aggregates (fig.3) which gave virtually identical banding patterns, indicating that the molecular structure was not altered during the oxidation procedure. The one difference observed was a slight increase in density at 40 nm intervals in the paracrystals produced from oxidised tropomyosin (arrows in fig.3). Since the two paracrystals differ only as a result of the oxidation state of their cysteine residues, this observation suggests that these residues are located at the position of increased density. The increased density is interpreted as being due to the disulphide bond formation causing a local disordering of the coiled-coil (to be expected as the cysteine residues are located on the faces of mutual contact the chains [3]) allowing the negative stain to penetrate the structure more easily at this point.

## 4. Discussion

The manner in which the chain interact to form a coiled-coil [8] makes it sterically impossible to form intramolecular disulphide bonds if the chains are staggered. Therefore, these results argue most strongly for a molecular model for tropomyosin in which the chains are in register. This has two important consequences. First, to achieve maximum hydrophobic interaction between the two chains in the coiled-coil, the radius of the coiled-coil cannot, as is usually assumed, be constant, but must vary with the size of the residue to be accommodated: expanding for bulky residues and contracting for small. Second, it has been proposed that the end-to-end association of tropomyosin occurs by the intertwining of the single chains which would project from the molecular ends as a result of chain stagger [3]. Such an explanation cannot apply when chains are in register and instead an overlapping of molecular ends would appear to be likely. As this would effectively double the thickness at the overlap point, such a model would explain the observation [9] of stain-excluding bands (which are indicative of a higher protein density) at the positions corresponding to the molecular ends in negatively stained paracrystals.

The position of the cysteine residues deduced from the difference in paracrystal staining corresponds to the position where troponin binds to these paracrystals [9]. This suggests that the troponin binding site is located close to the cysteine residues in the tropomyosin sequence.

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## References

[1] Caspar, D. L. D., Cohen, C. and Longley, W. (1969) J. Mol. Biol. 41, 87–107.
[2] Cummins, P. and Perry, S. V. (1973) Biochem. J. 133, 765–777.
[3] Sodek, J., Hodges, R. S., Smillie, L. B. and Jurasek, L. (1972) Proc. Natl. Acad. Sci. U.S. 69, 3800–3804.
[4] Hartshorne, D. J. and Mueller, H. (1969) Biochem. Biophys. Acta 175, 301–319.
[5] Cowgill, R. W. (1974) Biochem. 13, 2467–2474.
[6] Yamaguchi, M., Greaser, M. L. and Cassens, R. G. (1974) J. Ultrastructure Res. 48, 33–58.
[7] Woods, E. F. (1967) J. Biol. Chem. 242, 2859–2871.
[8] Crick, F. H. C. (1953) Acta Crystallogr. 6, 689–697.
[9] Ohtsuki, I. (1974) J. Biochem. 75, 753–765.