PROPOSED ROLE FOR THE α2 CHAIN IN RESOLVING AN AMBIGUITY IN SELF - ASSEMBLY OF THE COLLAGEN FIBRIL

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Summary Analyses of the way in which the amino acid sequence of the α1 chain of a collagen molecule can specify the Hodge-Petruska staggered fibril structure have been unable to show how this sequence can specify the direction as well as the magnitude of stagger between neighbouring molecules. We propose that the role of the α2 chain in non-tissue-specific collagens is to introduce a heterogeneity into the interactions between molecules that enables this direction to be specified. Our proposal is supported by a simple one-dimensional theoretical treatment. We predict that fibrils made up of collagen molecules with three identical chains will not have regular three-dimensional lattices.

Introduction The formation of the collagen fibril from triple-helical molecules provides a simple model system for studying how amino acid sequences specify the assembly of protein molecules into more complicated biological structures. Non-tissue-specific collagen molecules contain two identical α1 chains and the non-identical, but in much of its length homologous α2 chain. Analysis of the amino acid sequence of the α1 chain has shown how it can specify a staggered array of parallel molecules in the fibril (1) but leaves an ambiguity as to the direction of the stagger. We propose that the role of the α2 chain may be to resolve this ambiguity by specifying a preferred stagger direction.

Hodge and Petruska's suggestion (2) for the axial relationship, or stagger, between the parallel molecules in the collagen fibril (Figure 1) is now not seriously contested; its correctness has recently been clearly confirmed by its ability to explain the positively stained banding pattern of electron micrographs (e.g. reference 3). Electron microscopy has so far told us very little about the three-dimensional arrangement of

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molecules which might give rise to this axial projection except that neighbouring molecules are axially displaced relative to one another by an integral multiple (n) of a distance D ≃ 67 nm, the axial repeat of the fibril. Hodge and Petruska originally limited their consideration to the case where n = 1 but electron microscopy can neither confirm nor disprove that the arrangement is more ordered than neighbouring molecules having random staggers with n = 1,2,3 or 4 (4). Several different models for the three-dimensional structure of the collagen fibril have been proposed, based on X-ray diffraction data, but none of them has yet been fully substantiated. Our considerations are fortunately independent of detailed models of this kind.

Ambiguity An understanding of the nature of the forces between adjacent parallel molecules may be gained from a one-dimensional treatment. Hulmes et al (1) showed how the α1 chain sequence led to a stable relationship between molecules when they were staggered by 1D, 2D, 3D and 4D, by calculating the self-convolution of a function representing the distribution of amino acid residues with charged and hydrophobic side chains. A way in which 1D might be specified, as opposed to any of the other staggers, has also been suggested (5).

These analyses (1, 5) leave an ambiguity in that they do not suggest how the direction of stagger is specified by the sequence. In order to make our point we consider a very schematic model for the structure of the collagen fibril, like that of Figure 1, where we consider molecules in a plane containing the fibril axis. Molecule A, in the figure, is related to molecule B by a translation of +1D while molecule C is related to B by a translation of -1D. It is not clear why, during the growth of the fibril, C should take up the axial stagger with respect to B that it does rather than that exemplified by A. Since analyses of the α1 sequence could not distinguish whether the sequence of B was being moved past that of C or vice versa, they could not answer this question.

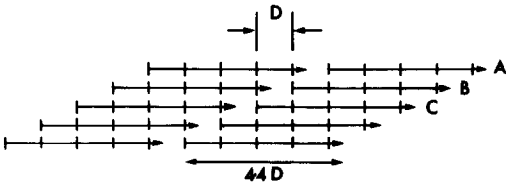

Figure 1 Schematic representation of Hodge and Petruska's model for the arrangement of molecules in the collagen fibril (2). The significance of molecules marked A, B and C is explained in the text. D is the axial repeat of the fibril.

Nevertheless in order for the sequence to specify the relationship between molecules in the fibril it must specify the direction as well as the magnitude of the stagger adopted by each molecule during self-assembly.

Resolution We propose that one function of the α2 chain in the triple-helical collagen molecule might be to enable the direction of stagger of adjacent parallel molecules to be specified by introducing a heterogeneity into their interaction. If the sequence of B were not identical to that of C the ambiguity of direction would be resolved. We believe that this non-identity could be achieved in practice by the interaction between the two molecules arising from the α2 sequence of one and the α1 of the other. Of course in three dimensions this interaction is between edges on the two molecules, rather than between one distinct chain in each (6) but since corresponding edges will not be involved one edge could have more of the hydrophobic and charge character of the α2 sequence than the other. Neither the structure of the molecule nor the arrangement of molecules in a fibril are sufficiently well defined for a full three-dimensional analysis to be possible (see reference 7). We therefore tested our proposal, as far as we were able, in one dimension.

Our method of testing the proposal was to show that the nett strengths of interaction for a +1D stagger were clearly not equal when calculated from the convolution of an α1 sequence function with an α2 sequence function and when the order of convolution was reversed. (The sequence

function represented the distribution of charged and hydrophobic residues and was defined in the same way as in reference 1.) Thus the two sequences are sufficiently different to resolve the ambiguity of direction. A difficulty arose in that no complete α2 sequence was available and we were obliged to use the α1 sequence (1) with those positions of amino acids which were known in the α2 sequence (8) substituted appropriately. Therefore the data were biased against the success of the test which nevertheless supported our proposal.

Conclusions The proposal has implications for the three-dimensional structure of the collagen fibril. If the α2 chain is necessary for specifying the exact relationship between molecules then those collagen molecules with three identical chains, like cartilage collagen, will not form ordered three-dimensional lattices and the axial relationship between molecules will merely be a statistical one. A regular lattice has only been observed for the packing of collagen molecules in the fibrils of tendon; these molecules contain an α2 chain. We predict that such lattices will not be observed for collagen species with three identical chains.

If our proposal is correct we have in collagen a structure where a non-identical protein chain is exploited as a source of further information with the consequence of greater specifity and possible enhancement of function. The need for this extra specificity does not arise in the formation of tropomyosin tactoids, the only other assembly of fibrous protein molecules to be investigated in such detail (9), because their structures do not involve an extended array of staggered molecules.

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