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On collagen II fibrillogenesis

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Collagen fibrils exhibit noticeable differences in their molecular order in the direction perpendicular to fibril axis. For instance, three-dimensional crystallinity prevails for type I collagen (e.g. tendons) whereas only liquid-like order in the lateral direction prevails for type II collagen (e.g. cartilage). The latter situation has been likened to that of a smectic liquid crystal. It is suggested here that the fibrillogenesis of type II collagen is indeed directed by a possibly metastable liquid crystalline mesophase involving a supramolecular assembling process. The occurrence of decoration on the fibrillar surface enhances a liquid crystalline phase due to preferential growth assumed to continue along the axial direction, and an increasing persistence length with respect to triple-helical collagen molecules.

1. Introduction

Molecular order and lateral packing of collagen fibrils has been a subject of interest and debate for some time. Fibrillar structures are produced by extracellular self-assembly of triple-helical collagen molecules terminated by short non-collagenous telopeptide at either end [1]. The classical triple helix has a pitch of 10.4 nm, length $L \sim 300$ nm, diameter $d \sim 1.4$ nm, but the constituent chains do not necessarily have the same amino acid composition [2]. Type I and type II classes of fibrils correspond, respectively, to association of heterotrimeric ($\alpha_1(I)_2\alpha_2 I$) and homotrimeric ($\alpha_1(II)_3$) triple helices. The chemical/structural variations between various collagen types reflect different functional roles as extracellular matrix components [3]. For instance, type II fibrils, occurring in cartilage and vitreous, are thinner (diameter up to 40 nm), less crystalline and show a more arquated geometry than type I fibrils (diameter up to 500 nm) occurring in tendons [1, 4]. It is also recognized that the surface of fibrils contains sites for the interaction with other collagen types and decoration by proteoglycan [5]. The information encoded in the triple helix and in the fibrillar surface may indeed be the strategy used by Nature to monitor fibril diameter for the intended mechanical properties [5, 6].

The decoration prevents fusion into larger diameter fibrils, but decorated fibrils may nevertheless form suprafibrillar assemblies through interconnected *bundles*, as documented by electron microscope (EM) studies on type II collagen [7]. Diffuse equatorial X-ray scattering usually evidence reduced, liquid-like order in the direction perpendicular to fibre axis for type II tissues. For this reason, an analogy to smectic A liquid crystals was suggested [8]. On the other hand, even type I tissues with larger diameter and crystallinity may be interconnected by less organized collagenous and non-collagenous material. For instance, early X-ray diffraction data by Puett *et al.* [9] for rat-tail tendons showed that the equatorial reflection of the spacing between adjacent triple-helices was unaltered in spite of a 10-fold increase of the cross-sectional degree of osmotic swelling at pH=2. Therefore, basic fibrillar units resist swelling and are interconnected by swellable and less organized material. Hulmes *et al.* [8] elaborated a model in which radial growth unrestricted by decoration occurs concentrically about the fibrillar core.

2. Proposed Mechanism

The foregoing presentation highlights the contentious aspect of molecular order in the lateral packing of collagen fibrils. Some aspect of the controversy could be solved by the following new analysis of the role of restrained radial growth on the general mechanism of supramolecular polymerization and supramolecular liquid crystallinity [10, 11]. To this end, we consider the familiar schematization in figure 1a of the long range order of a four strands assembly of triple-helical collagen molecules. A stagger of 67 nm (nearly ¼ the molecular length) for molecules in neighbouring strands generates the well known alternation of high and low electron density in TEM and AFM images [12] and meridional X-ray reflections, all supporting a definite and less contentious molecular order along the axial direction. The corresponding length of fibrils has been

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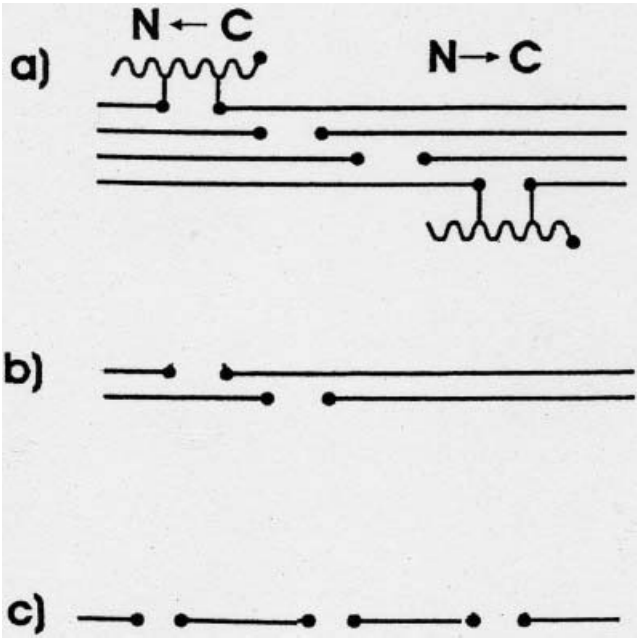

Figure 1. Schematic representation of: (a) staggered growth of a type II collagen fibril showing the antiparallel decoration by collagen type IX molecules [13]; (b) staggered growth of a linear supramolecular polymer; (c) head-to-tail growth of a linear supramolecular polymer. Black dots indicate the N and C terminals of individual triple helices.

difficult to measure but could attain the mm range [1]. In a real fibril there will be well over 1000 axially aligned triple-helices in each strand, and near 100 strands assuming a diameter in the order of 10 nm. The external surface of the strands at the boundary of the fibril will be coated by a decoration. Figure 1a includes the decoration of type II collagen fibrils by collagen type IX molecules [13]. An antiparallel arrangement allows both α_1(II)N-telopeptide and α_1(II)C-telopeptide to bind a single type IX molecule. The decoration prevents further lateral growth but since there are uncompensated N and C sites at the ends of the fibril there is possibility of further staggered growth along the axial direction.

We focus on the thermodynamics of the process by considering the assembly of just two strands of triple helices. Figure 1b represents a one-dimensional staggered analogue growing only linearly through supramolecular polymerization in the absence of cooperative effects. The interaction sites allowing growth are localized at the C and N terminal of each molecule and on the side groups distributed along the length of any two staggered molecules. The direct interaction of the C and N terminals of triple helices is modulated by intervening telopeptides. Ortolani *et al.* [14] have suggested an S-folded conformation for the telopeptides of collagen II, allowing their packing outside the helical region and adding a possible intra-telopeptide hydrophobic interaction. The telopeptides are also the sites for subsequent cross-linking formation. Even three H-bonds formed by head-to-tail arrangement of two triple-helices would not form a supramolecular bond strong enough for large growth to occur. In fact, a simple head-to-tail arrangement (schematized in figure 1c) is known to allow large linear growth (degree of polymerization reaching 1000 even for isodesmic, non-nucleated polymerization) provided the head-to-tail linkage involves four or more H-bonds [11, 15]. The driving force for an extensive growth of the assembly in figure 1b should therefore arise from the staggered interactions, which could be evaluated from known or simulated details of the molecular path of staggered helices [1, 14]. The staggered arrangement in figure 1b was also proposed for the linear growth of supramolecular polymers based on partly complementary sequences of nucleotide pairs [16]. In the case of actin [11, 17], strong head-to-tail (N-S) and staggered (N-E and S-E) interactions are known to stabilize the double-helical supramolecular assembly when a cooperative nucleation effect is known to be operative.

It is known that the assembly of an organic crystal may be described using the basic concepts of supramolecular polymerization. The main differences with respect to linear assembly is the need to consider multidimensional binding, and the all-or-none feature of the crystallization process as opposed to the broad size distribution expected for nucleated linear growth [18]. The need to consider different binding constants along the longitudinal and lateral direction was discussed by the author in early work on the assembly of rigid polyelectrotytes and complementarily charged surfactants [19]. In the case of collagen, the staggered feature of the assembly, coupled with weak head-to-tail interaction, might not require different binding constants for axial and lateral growth. Moreover, the inhibition to unlimited growth along the lateral direction for type II collagen (due to decoration) allows the focus to be on further growth *restricted* to the axial direction. Most interesting features may than be predicted for the liquid crystallinity of the system.

It is useful to analyze the role of liquid crystallinity on the formation of ordered fibrils expected for a solution of molecularly dispersed triple-helices. The critical volume fraction v^* at which nematic alignment begins is predicted to be

$$v^* \sim \text{const}/X, \tag{1}$$

where the constant is between 3 and 6 depending upon the particular theory (virial or lattice; see Ciferri [20] for

a general discussion of these treatments and their experimental verification) and X is the axial ratio expressed by the length/diameter ratio ($X=L/d$) for rigid rods, or by the persistence length/diameter ratio ($X=2q/d$) for worm-like chains (note that the above theories are based on excluded volume calculation which are valid irrespective of the size or dilution of the dispersed entity). Recent evaluation of the persistent length of both type II and type I collagen using optical tweezers under physiological conditions [21] indicate a value of $q=11.7$ nm, considerably smaller than the actual length (300 nm) of the triple helix. The expected critical volume fraction from equation (1) turns out to be greater than 0.2, a value definitively above the known collagen concentration for type I fibril crystallization under isoelectric and physiological conditions [22, 23]. The rigidity of the triple helix is simply too small to promote liquid crystalline orientation prior to crystallization [24].

Development of liquid crystallinity at considerably smaller v^* values could nevertheless be expected since the growing, *supramolecular* assembly, rather than the monomeric triple-helix, is actually the most rigid entity suspended in the extracellular fluid. The incorporation of additional triple helices to a crystallizing fibril occurs primarily by virtue of the staggered interaction causing an increase of both fibrillar length and diameter. However, when the limitation on the growth of the diameter due to decoration occurs, growth should continue only along the axial direction. Under these conditions, the geometrical asymmetry of the particle increases and development of liquid crystalline orientation eventually occurs, as detailed in recent work by the author [25] based on the theory of supramolecular liquid crystallinity [26–28]. A large persistence length of the supramolecular assembly and a sufficiently large binding constant for the staggered interaction become the relevant parameters controlling the critical concentration [25–28]. The system may thus acquire order and orientation even before a minor extent of interfibrillar association or cross-linking complete the stabilization of the structure.

The persistence length of the growing fibril is expected to considerably exceed that of the triple-helix as a result of the lateral association of just a few strands. Even though there are no *ad hoc* data for the increase of persistence length with the number of associating collagen strands, extremely large values of q reported in early studies were attributed to the readiness of aggregation shown by collagen [21]. In several other systems, particularly aggregan, the persistence length of individual components was greatly increased by lateral aggregation [29]. Measured persistence lengths for a number of multistrand supramolecular polymers have been shown to attain values in the μm range [11]. Both theory and experiments support a rapid increase of the length of several assemblies at a low volume fraction in the absence of crystallization [25]. For large values of the binding constant (i.e. $>10^7$/M), growth was shown to be hierarchically *followed* by liquid crystalline orientation. For intermediate values of the binding constant (10^4–10^6) growth of individual assemblies occurs *simultaneously* with their mutual alignment (a process described as growth-coupled-to-supramolecular liquid crystalline orientation [25–28]).

3. Concluding remarks

Decoration, regarded as a termination step for the lateral growth of a collagen fibril, prevents the formation of fibrils having diameter larger than $\sim$40 nm, producing a situation of frustrated crystallization and enhanced compatibility with the solvent. Axial growth of individual decorated fibril was assumed to continue and to conform to the general rules of supramolecular polymerization according to which growth is controlled by the strength of staggered interaction of triple helices, and by the geometrical asymmetry of the assembly. Liquid crystalline alignment of growing fibrils can thus occur even in the absence of crystallization.

The lack of crystalline order within each growing fibril may be attributed to their small (10–40 nm) diameter or to an imperfect core structure [8], probably associated to biochemical regulatory processes occurring at the triple-helix level. Since age-induced aggregation of collagen II fibrils is known to occur [1, 5, 6], non-equilibrium processes may be superimposed on crystalline and liquid crystalline thermodynamic equilibria. A time evolution toward an equilibrium crystalline phase would be consistent with both the ageing process and the observation that crystallinity is occasionally exhibited by collagen II (i.e. in samples from lamprey motochord [1]).

In the case of collagen I, decoration appears to occur when fibrils attain diameter much larger than for collagen II (see introduction), allowing three-dimensional order to extensively develop. As discussed elsewhere, even in this case crystallization ought to be directed by a nematic precursor [24]. However, a large gap between the crystallization and the mesophase boundary will disfavour the observation of a liquid crystalline phase even under metastable conditions [24].

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