Self-assembly in the major ampullate gland of Nephila clavipes

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Abstract

We present a tentative interpretation of the origin of nematic liquid crystalline order exhibited by dragline silk fibroin solutions collected from the spider Nephila clavipes. Liquid crystallinity is thought to confer certain rheological properties on the fibroin solution which are exploited during the dragline spinning process. We show that the feasibility of liquid crystallinity under physiological conditions depends critically on parameters characterising the amino-acid sequence of the fibroin molecules.

“Dragline” silk secreted by the major ampullate gland of the orb-weaver spider Nephila clavipes is one of the best-characterised natural silks, notable in respect of its singular mechanical properties [1]. The spinning process itself, however, i.e., the structural transition undergone by fibroins initially dispersed in the aqueous environment of the gland, culminating with the extrusion of water-insoluble fiber, remains relatively poorly understood [2]. Recent in vitro experiments [3] have suggested that the solution in the gland enters a nematic (or twisted nematic) liquid crystalline state, which might play a rheological role in the spinning process and hence affect the mechanical quality of the extruded fiber [4]. Interestingly, Gatesy et al. [5] have argued that, in the evolutionary context, mechanical performance of natural silks exerts a particularly acute selection pressure on the amino-acid sequence of the constituent fibroin molecules. A stabilizing source of selection pressure appears necessary to explain the remarkably long evolutionary timescale (∼125 million years) over which the characteristic repeated-motif sequences of orb-weaving spiders have remained essentially unchanged. In this regard, correspondence between sequence and the ability to form a liquid crystalline phase in the gland, affecting spinning conditions, and hence dragline quality, would present a relevant perspective.

Our objective here is to tentatively establish such a connection, addressing at the molecular level how nematic order emerges in the gland, and is controlled by sequence-related parameters. We develop from the premise that the dragline fibroin solution belongs to a general class of fibrillizing globular protein systems of which hemoglobin S is a well-known example [6]. The globules of these systems assemble reversibly into supramolecular rod-like structures, which, beyond a critical axial ratio, undergo a nematic ordering transition.

The details of the assembly process are as follows. We identify three generic sources of protein-protein interaction contributing to an effective binding potential between fibroins. The principal contribution derives from the free energy of transfer $\Delta F$ of nonpolar amino acid from a hydrophobic environment to water - the so-called hydrophobic effect. According to data collated and interpreted by Dill et al. [7], $\Delta F$ has a magnitude of approximately 2 kcal/mol, increasing with temperature up to a maximum at 60-80°C:
\[ \Delta F(T) = \Delta H^0 + \Delta C_p (T - T_0) - T[\Delta S^0 + \Delta C_p \ln(T/T_0)], \]

where \( \Delta H^0 = 0 \) and \( \Delta S^0 = -6.7 \text{ cal/K/mol} \) are the enthalpy and entropy of transfer at room temperature \( T_0 = 298K \), and \( \Delta C_p = 55 \text{ cal/K/mol} \) is the heat capacity.

If we assume that each fibroin monomer is in a dense-packed roughly spherical globular state (radius \( r \)), the hydrophobic effect generates an effective fibroin-fibroin binding potential

\[ \gamma_h \approx n_h \Delta F(T) \sim 10 \text{ cal/mol/Å}^2, \quad (1) \]

per unit area of surface buried at the binding contact, where, with \( a \sim 1\text{nm} \) a characteristic residue lengthscale, \( n_h \sim a^{-2} \) is the area density of hydrophobic residues exposed at the globule surface.

Dispersion forces likewise favour association, but contribute a much smaller term [8]

\[ \gamma_d = \frac{A}{24 \pi a^2} < 1 \text{cal/mol/Å}^2, \quad (2) \]

where the Hamaker constant \( A \) gives the strength of water-mediated dispersion interaction between amino-acids residues. This estimate assumes \( A \simeq 500 \text{ cal/mol} \), as for typical alkane chains in water [8].

Thirdly, the silk fibroin sequence [9] features a number \( N_{\text{Arg}} \sim 20 \) of arginine residues, which carry a positive charge at normal pH [10]. These residues generate an electrostatic double layer effect which we can examine, to a first approximation, by treating fibroin globules as conducting spheres each carrying a charge \( \alpha N_{\text{Arg}} \), where the ‘degree of ionization’ \( \alpha \) reflects the extent to which the solvent is able to penetrate into the globule interior.

The free energy of formation of the double layer is [11]

\[ -\frac{\alpha^2 N_{\text{Arg}}^2 e^2}{8 \pi \epsilon r^3} \left( \frac{\xi}{r} \right) \quad (r >> \xi), \]

where \( \xi \) is the Debye screening length, and \( \epsilon \simeq 7 \times 10^{-11} \text{ F/m} \) is the dielectric constant of water. The limit \( r >> \xi \) is appropriate at salt concentrations beyond the order of \( 10^{-2}M \), for which the Debye length is comparable with the amino-acid residue dimension, \( \xi \sim a \).

A negative contribution to the binding potential per unit area of fibroin-fibroin interface follows from the unfavourable cost of breaking up this double layer,

\[ \gamma_e \simeq -\frac{\alpha^2 N_{\text{Arg}}^2 e^2}{32 \pi \epsilon r^3} \left( \frac{\xi}{r} \right) \quad (3) \]

We observe that binding is only feasible if the degree of ionization is very low, since otherwise only three or four arginine residues would be sufficient to generate magnitudes \( \gamma_e \sim -10 \text{ cal/mol/Å}^2 \) comparable with the hydrophobic term, hence preventing association.

The buried surface area of a fibroin embedded within a supramolecular mesogen scales with the fibroin size as \( r^2 \), yielding for the net binding free energy due to the respective contributions

\[ E_b = \text{const} \times r^2 (\gamma_h + \gamma_d + \gamma_e), \quad (4) \]
where the constant is a geometrical factor of order $\sim 4\pi$ which depends on the morphology of the supramolecular structure. In a ‘close-packed’ estimate for $r$, we have $r/a = (3N/4\pi)^{1/3} \approx 6$, where $N \sim 700$ is the total number of residues per globule according to the sequence data [9].

The binding potential is attractive when the hydrophobic term dominates over the electrostatic repulsion, yielding magnitudes $E_b \sim 10RT$ cal/mol. In order to relate $E_b$ to mesogen assembly, we adopt a well-known approximate result of generalized Flory-Huggins lattice statistics [12], giving for the mean axial ratio of rods reversibly assembled from associating monomers at volume fraction $\Phi$ [13],

$$x(\Phi, T) = s^{-3}\Phi^{1/2} \exp(E_b/2RT). \quad (5)$$

Here $s$ specifies the number of monomer ‘strands’ in the rod cross-section. In the example of hemoglobin S fibrils, $s = 14$ entwined strands have been identified from crystallographic data [6]. Unfortunately, in the absence of similar data for silk fibrils in the major ampullate gland, $s$ is an unknown parameter in the present context [14].

Nematic-isotropic coexistence is naively calculated in Fig.1 (for a hypothetical choice of $s$), by solving Onsager-like criteria [15] defining respectively the isotropic (I) and nematic (N) nodal lines bounding the biphasic region,

$$x(\Phi, T)|_I = 5/\Phi \quad x(\Phi, T)|_N = 8/\Phi. \quad (6)$$

In this type of calculation, we neglect thermodynamic coupling between nematic ordering and mesogen assembly [16], and related coupling to polydispersity [17] and flexibility [18] of the mesogens. Anisotropic electrostatic [19] and dispersion force [20] contributions to the effective mesogen-mesogen interaction, which is purely steric in the Onsager approach, are similarly neglected.

In summary, we have shown that it is feasible for silk solutions to exhibit nematic liquid crystalline order along broadly similar lines to the hemoglobin S mechanism. The hydrophobic effect drives thermodynamically reversible assembly of supramolecular mesogens, which are capable of ordering according to an Onsager-like criterion.

This interpretation remains only tentative, insofar as we do not have crystallographic data to hand against which to elucidate structural features of fibroin monomers and fibrils which are implicit to the approach. In particular, values for $s$, characterising mesogen morphology, and $n_h$, specifying the hydrophobic surface topology of fibroin monomers, are unknown.

The strong sensitivity of the model to these parameters, along with unspecified geometrical constants, renders the semi-quantitative phase diagram calculated in the figure somewhat academic. However, the reentrant nature of the phase diagram, reflecting the temperature dependence of the hydrophobic effect, presents a strong qualitative signature which might be looked for experimentally.

There is some scope for comment on the suggestion that spiders regulate their silk gland in vivo by pumping in additional protons and salts (see [21] and references therein). According to our Eqns (3-4), there is a strengthening of the net fibroin-fibroin binding potential in response to added salt, due to increased screening of the electrostatic double layer repulsion, which should favour mesogen assembly and the onset of a nematic phase. This is in line with very recent observations of Chen et al. [21], who report that nanofibril formation
can be induced in *Nephila senegalensis* major ampullate fibroin solutions by the addition of KCl. These authors also observe a marked transition in the rheological character of the solution occurring between pH 6.4 and 6.8, which provides us with some justification for the significance attributed in the model to histidine residues (pK value 6.5).

Finally, the model affords some insight into how sequence mutations might be expected to interfere with liquid crystallinity of the fibroin solution. The strong shift of the biphasic region calculated in the figure, for example, roughly reflects a single site hydrophobic→polar mutation in the surface of the interacting globules. We infer that mutations are in general easily capable of disrupting the dragline spinning process by rendering the nematic phase physiologically inaccessible. Arguably, this has attendant consequences for the evolution of the fibroins. Sequence mutations which interfere with liquid crystallinity are discouraged by selection pressure, since by disrupting the spinning process they would also degrade mechanical performance of the extruded dragline.
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FIGURES

FIG. 1. Nematic-isotropic biphasic region of the major ampullate gland phase diagram, from Eqns (5-6) with $E_b = n_h \Delta F(T)$. The dispersion force and electrostatic contributions to $E_b$ as presented in the text are relatively weak and have been neglected. Substituted parameters are $s = 8$, $r/a = 5$, and the thermodynamic parameters for $\Delta F(T)$ quoted in the text. We contrast the biphasic region for $n_h = .25a^{-2}$ (solid lines) with $n_h = .23a^{-2}$ (dashed lines). This parameter shift is chosen to roughly reflect a single site hydrophobic→polar mutation in the surface of the interacting globules.
A graph depicting the relationship between fibroin vol fraction and temp (deg C) with increasing nh. The graph shows different regions labeled iso and nem.