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Closest Packing of Multistranded Coiled-Coils – a Possible Application to Collagen

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A general treatment of the closest packing of multistranded coiled-coils is given with a view to understanding the structure of assemblies of fibrous protein molecules. Expressions for the closest distance of approach of two coiled-coils are derived both for the case of adjacent coils having the same hand, and having opposing hands. An investigation of the lattice on which such coiled-coils would be closest packed is presented. The two-stranded coiled-coil is particularly interesting because when adjacent supercoil hands are opposing, the closest packed lattice is tetragonal. The possibility of this being the way that the molecules pack in the collagen fibril is discussed briefly from the point of view of the systematic absences predicted by such a packing.

1. Introduction

Some evidence is available for the view that fibrous proteins can exist as multistranded coiled-coils. The idea is an appealing one and has given rise to many specific structural hypotheses. An instance is the two-stranded coiled-coil of tropomyosin and a number of other α-proteins, and for some time a 9+2 strand structure was in vogue for α-keratin. Collagen has provided very fertile ground for coiled-coil models. Units of 2, 4, 5 and 7 strands have been proposed at different times and evidence for them found!

Some fibrous proteins play structural roles in animals and exist as rather large paracrystalline assemblies, for example, the fibril of collagen. It is of some interest to understand how coiled-coils might be arranged in structures such as the collagen fibril, in the crystallites of the praying mantis egg-case or in the thick filament of muscle. It is worth remarking that only one structure comprising coiled-coils has really been worked out in detail and established, that of the filamentous bacterial viruses (for a summary see Marvin & Wachtel, 1975).

Since close packing of coiled-coil units might be correlated with a minimization of the free energy of the whole assembly, one way of gaining some insight into possible structures is to examine geometrically the closest packing of the coiled-coils. In this paper the question is examined first by establishing the closest distance of approach of two coiled-coils and then by investigating the lattice which would allow closest packing. A consideration of the number of possible (geometrically idealized) contacts between one coil and its neighbours shows that the two-stranded coiled-coil is of particular interest since a packing of such coils with alternate supercoil hands allows more inter-coil contacts than the arrangement with hands of the same sign and, moreover, this is achieved when the arrangement is tetragonal. More generally, coiled-coils with odd numbers of strands show a greater number of contacts than those with even numbers of strands, and, although supercoiling with adjacent coils having opposing hands leads to a closer packing than if the coils all have the same hand, the number of possible contacts is the same for both. The packing will be characterized throughout the discussion by the packing fraction η defined to be the fraction of the total space occupied by the strands of the coiled-coils thought of as regular cylinders.

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2. Hexagonal close packing

Closest packing of parallel cylinders, as is well known, is achieved with a hexagonal array, and the packing fraction is 0·9069. The simplest way to consider the packing of coiled-coils is hexagonal packing of their circum-cylinders. This is the *closest* packing of coiled-coils having even numbers of strands, the same supercoil hand and identical azimuthal phases (see below). The circum-cylinders of regular coiled-coils of n strands, each of radius R, have a diameter δ where

$$\delta=2R\left[1+\operatorname{cosec}\left(\frac{\pi}{n}\right)\right]. \qquad (2.1)$$

Values of δ and corresponding packing fractions η are found in Table 1.

3. Closest approach of two coiled-coils of the same hand

Coils can approach more closely than δ and the closeness depends on whether adjacent coils have the same or opposing hands. There is a difference between coiled-coils having odd or even numbers of strands (see Fig. 1). If coiled-coils having even numbers of strands are arranged with the same azimuthal phase then their closest approach is given by δ; similarly if coils with odd numbers of strands are arranged with a phase differing by π/n. Conversely it seems that the closest distance of approach will be achieved for coils with odd numbers of strands if these have identical phases and for even numbers of strands if the phase of the coils differs by π/n.

The foregoing assertion has, of course, not been proved. Consider the general problem of the closest allowable distance between two coiled-coils with odd numbers of strands having a relative phase of α (taking values between 0 and π/n because of the rotational symmetry). Such a distance must allow any two strands, one on each coiled-coil, to approach each other to $2R$ but no closer (see Fig. 2). Given that constraint, the distance between the supercoil centres, y, is given by

$$y=R\operatorname{cosec}\frac{\pi}{n}\,.\cos\theta+R\operatorname{cosec}\frac{\pi}{n}\,.\cos\left(\frac{\pi}{n}-\alpha-\theta\right)+\left[4R^2-\left\{R\operatorname{cosec}\frac{\pi}{n}\,.\sin\left(\frac{\pi}{n}-\alpha-\theta\right)-R\operatorname{cosec}\frac{\pi}{n}\sin\theta\right\}^2\right]^{1/2} \quad (3.1)$$

where θ represents the azimuthal angle that the supercoils have turned from their initial positions.

Differentiation of (3.1) w.r.t. θ shows that the minimum value of y is achieved when $2\theta=\pi/n-\alpha$ so that given a value of α, the smallest possible y is

$$y_{\min}=2R\left\{1+\operatorname{cosec}\frac{\pi}{n}\,.\cos\left(\frac{\alpha-\frac{\pi}{n}}{2}\right)\right\}. \quad (3.2)$$

If $\alpha=\pi/n$ we recover the expression (2.1) since now the coils with odd numbers of strands are out of phase by π/n as discussed at the beginning of the section. Since α is constrained to be between 0 and π/n the smallest possible value of $y_{\min}$ is achieved with $\alpha=0$ in which case the smallest distance of approach is

$$\xi_s=2R\left\{1+\operatorname{cosec}\frac{\pi}{n}\,.\cos\frac{\pi}{2n}\right\} \quad (3.3)$$

$$=2R\left\{1+\tfrac{1}{2}\operatorname{cosec}\frac{\pi}{2n}\right\}. \quad (3.4)$$

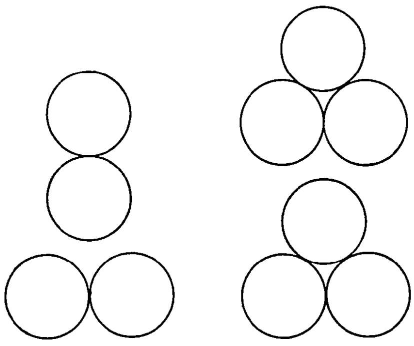
(I) (II)

Fig. 1. Diagram showing that on a large-scale knobs-into-holes basis, close packing of coiled-coils is likely to be achieved for (I) even numbers of strands with the initial phase difference π/n, (II) odd numbers of strands with the initial phase difference zero.

Table 1. *Values of closest distance of approach, close packed lattice and packing fractions for coiled-coils having different numbers of strands*

Number of strands	Circum diameter $\delta/2R$ of cylinder	Packing fraction η for hexagonal packing of circum-cylinders	Closest distance of approach $\xi_s/2R$ supercoils of same hand	Angle γ	Packing fraction of closest packing of coiled coils η_s
1	1·0000	0·9069	1·0000	60°	0·9069
2	2·0000	0·4534	1·7071	71°42′	0·5678
3	2·1547	0·5860	2·0000	60°	0·6801
4	2·4142	0·6224	2·3066	63°6′	0·6621
5	2·7013	0·6214	2·6180	60°	0·6616
6	3·0000	0·6046	2·9319	61°32′	0·6736
7	3·4046	0·5634	3·2453	60°	0·6028
8	3·6131	0·5558	3·5629	60°46′	0·5673
9	3·9238	0·5301	3·8794	60°	0·5424

Number of strands	Closest distance of approach $\xi_o/2R$ super coils of opposite hand	Angle γ	Packing fraction of closest packing of coiled coils η_o
1	1·0000	60°	0·9069
2	1·4142	90°	0·7854
3	1·8165	66°48′	0·7770
4	2·1473	68°24′	0·7328
5	2·4686	64°4′	0·7166
6	2·7761	65°24′	0·6725
7	3·1055	63°0′	0·6398
8	3·4411	63°20′	0·5984
9	3·7409	62°28′	0·5696

(The suffix s in ξ_s denotes coils of the same hand.)

An exactly similar analysis shows that the relationship (3.3) holds for coiled-coils with even numbers of strands, but here, as conjectured earlier, the coils must differ in phase by π/n. Values of ξ_s are shown in Table 1.

Notice that when the distance between two coiled-coils is a minimum, each strand of the coiled-coil makes two contacts with strands on the other in one pitch of the supercoil. If the distance is not a minimum ($\alpha \neq 0$) then each strand makes only one contact.

4. Adjacent supercoils having opposing hands

With slight modifications the analysis of § 3 can be extended to cover the case of opposing hands yielding a value of closest approach of

$$\xi_o = 2R\left[\operatorname{cosec}\frac{\pi}{n} \cdot \cos\frac{\pi}{2n} + \sqrt{1 - \left(\operatorname{cosec}\frac{\pi}{n} \cdot \sin\frac{\pi}{2n}\right)^2}\,\right]. \tag{4.1}$$

(The suffix o in ξ_o denotes coils of opposing hand.)

The values of ξ_o are given in Table 1 and it can be seen that having neighbouring supercoils with opposing hands favours closer packing.

5. Closest packing of coiled-coils

The simplest problem is that of coiled-coils with odd numbers of strands, adjacent coils having the same hand. Since all the coiled-coils are identical in all respects, closest packing is achieved with a cell of side ξ_s and internal angle 60°, *i.e.* hexagonal packing.

The problem of packing coils with even numbers of strands is slightly more complicated. The phases of neighbouring coils differ by π/n for closest distance of approach so that the arrangement is an alternating one and cannot be hexagonal. Consider a unit cell of side ξ_s and internal angle γ. The length of the shortest diagonal of the cell, d, is given by

$$d = (2\xi_s^2 - 2\xi_s^2 \cos\gamma)^{1/2}. \tag{5.1}$$

Since coiled-coils at the ends of the diagonal have the same phase, the length of the diagonal must be at least δ [as defined in (2.1)] so that

$$\sin\frac{\gamma}{2} = \frac{\delta}{2\xi_s} \tag{5.2}$$

yields the closest packing.

Similar considerations may be applied to adjacent coils having different hands, and here all lattices are alternating. For odd numbers of strands the alternating property is merely supercoil hand while for even numbers it is both hand and phase. It follows, therefore, that for even numbers where the closest distance of approach is ξ_o

$$\sin\frac{\gamma}{2} = \frac{\delta}{2\xi_o} \tag{5.3}$$

and for odd numbers

$$\sin\frac{\gamma}{2} = \frac{\xi_s}{2\xi_o}. \tag{5.4}$$

The calculated values of γ are given in Table 1 together with the packing fraction calculated as

$$\eta = \frac{n\pi R^2}{\xi^2 \sin\gamma}. \tag{5.5}$$

6. Numbers of intermolecular contacts made in the supercoil pitch

No restriction on the pitch of the coiled-coil is made by the packing schemes suggested in the last section. In practice, short pitches would not be favourable for isolated coiled-coils because the strain energy associated with it would be large and pitches (other things being equal) would tend to be larger, the larger the number of strands. On the other hand, the shorter the pitch, the more intercoil contacts are made per unit length, and the greater the number of strands, the more contacts are made per pitch. Thus there is no reason to suppose that pitches in isolated coiled-coils need necessarily be the same as in an assembly of coils.

It is quite simple to calculate the number of contacts per pitch for the various structures described. First consider coiled-coils with odd numbers of strands, all the coils having the same hand. Each coiled-coil makes $2n$ contacts with each of six near neighbours so the number per pitch is $12n$. For coils with even numbers of strands, each makes $2n$ contacts with its four nearest neighbours and n contacts with its two next nearest neighbours so the total is $10n$. The same is true for supercoils with opposite hands. So, although the effect of supercoiling adjacent coils with opposing hands leads to closer packing, it does not lead, in general, to an increase in the number of contacts, ex-

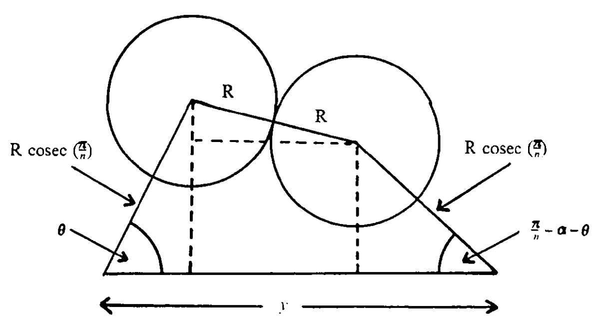

Fig. 2. Diagram to show the origin of expression (3.1). The analysis is based on the idea that a coiled-coil can be thought of as a transverse section rotating with constant angular velocity.

cept in the case of the two-stranded coiled-coil. Here, when the supercoils have opposing hands, the packing is tetragonal, so each coil has four nearest neighbours and four next nearest, rather than two as is otherwise the case. Thus the number of contacts increases from 20 to 24 per pitch length.

7. Discussion

The analysis of §5 does not, of course, contain an explicit proof that the derived lattice represent situations of closest packing. Such a proof, although troublesome, is not in fact difficult, and its inclusion in the paper would have added very little. It seems worth pointing out that the particular lattices tabulated are not the only ones which do, in fact, possess the property of closest packing. They are, however, crystallographically the simplest and the alternatives differ only trivially from them.

A pertinent question is – does the analysis outlined in the paper give any useful insights into real structures? The answer is – not in general, because other important factors are involved. Closest packing seems to be a feature of the arrangement of amino acids in the interior of globular proteins but there the steric considerations are at a much finer level than is implied in an analysis of the packing of smooth cylinders. The analysis may, however, be helpful in some particular situations. For example, a four-stranded coiled-coil has been suggested for the α-protein of the silk of *Apis Mellifera* by Atkins (1967). It will be interesting to see whether that view can be sustained and, if so, how the coiled-coils pack together. The treatment here gives grounds for a possibility.

A less speculative problem is the packing of the two-stranded coiled-coil structures more customarily attributed to α-proteins. The problem seems first to have been considered by Rudall (1955–56). It is possible that he knew the closest distance of approach of two coiled-coils, but he suggested hexagonal close packing. If he did know the approach distance, then he should have known that they could not have been packed hexagonally, however, even without a detailed analysis since an alternating lattice cannot also be hexagonal. Later the suggestion was made by Frazer (referred to in Elliott & Lowy, 1970) that two-stranded coiled-coils would tend to pack on a body-centred square lattice, but no reason for it was advanced beyond it appearing convenient. More recently, Longley (1975) has discussed this problem in more detail and shown the closest distance of approach for two-stranded coiled-coils of the same hand.

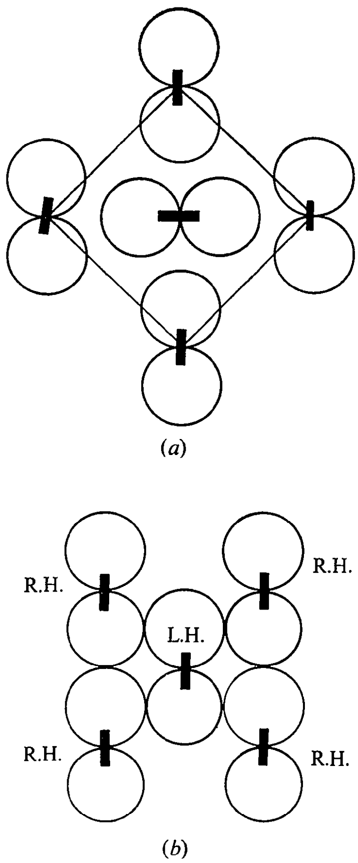

Fig. 3. Two body-centred tetragonal lattices involving (*a*) a rotation by $\pi/2$ between neighbouring coiled-coils of the same hand, (*b*) alternation of hand between neighbours.

8. A possible application to collagen

The most interesting result of this paper is that of the tetragonal packing of two-stranded coiled-coils when the hand of the coil alternates. The idea of coiled-coils existing with each of two hands is not one allowed within the present established conventions of molecular biology. Recently, bacterial flagella have been found existing with helices of each hand (Shamada, Kamiya & Asakura, 1975) but this situation is too remote to offer much hope as circumstantial evidence for the possibility of the same phenomenon at a molecular level. Evidence has not been found for the existence of α-helix in globular proteins having each of two hands although it was believed possible in some early studies.

However, a model involving the features of alternating hand and tetragonal packing has been suggested for collagen on the basis of numerological and crystallographic features of the near equatorial, low-angle X-ray diffraction pattern of rat-tail tendon (Woodhead-Galloway, Hukins & Wray, 1975). It seems worthwhile discussing that model from a crystallographic point of view.

A rather unsystematic indexing scheme was given by Miller & Parry (1973) for the row lines of the near equatorial diffraction pattern of rat-tail tendon, and the idea that this represented a body-centred square lattice of side 55 Å put forward. The reflexions were reindexed by Woodhead-Galloway *et al.* (1975) in a more systematic way but the conclusion of body centring was adhered to. The most notable feature of both indexing schemes is the absence of both the 1,0 and 3,0 row lines. The nature of the body centring was suggested by Miller & Parry to be a rotation of the coiled-coil (in their case the coiled-coil was a five-stranded one) and by Woodhead-Galloway *et al.* to be an alternation of coil hand. The absence of the 1,0 (and 3,0) row lines is the feature which must be most immediately explained by a model beyond those already discussed in Woodhead-Galloway *et al.* A full discussion of the model and detailed intensity calculations must be left to a later paper, but the point can be discussed in a much simplified way. This will be done by considering the systematic absences of the two models shown schematically in Fig. 3. The first represents a tetragonal model of the sort suggested in Elliott & Lowy, the second shows the packing in the collagen model referred to. In both, the distance between neighbouring coiled-coils is presumed to be the smallest possible, but this is not in any way critical to the crystallographic argument.

For the model in Fig. 3(*a*) the systematic absences for reflexions *hkl* are: (i) l odd, all reflexions, (ii) $h + k$ odd, layer lines such that $\sin(l\pi/4)=0$, (iii) $h + k$ even, layer lines such that $\cos(l\pi/4)=0$. Reflexions therefore appear on all row lines. Clearly the same is true for a five-stranded coiled-coil model with rotations of π (rather than $\pi/2$ as is involved here).

Contrast the situation shown in Fig. 3(*b*). The Fourier transforms of coiled-coils of opposing hand are related as complex conjugates so that the systematic absences are: (i) l odd, (ii) $h + k$ odd $\sin(l\psi)=0$, (iii) $h + k$ even $\cos(l\psi)=0$, where $\psi=\tan^{-1} h/k$, the most immediate consequence of which is that the row lines 1,0; 3,0; 5,0 *etc.* are identically absent in agreement with observation. Crystallographic situations analogous to this are found in ($\pm$) racemates. It is hoped that a fuller discussion of systematic absences produced by coiled-coils of alternating hand will be produced later.

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