

Effects of Electrostatic Anisotropy on the Properties of *de Novo* 4- α -Helix Bundles

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Dramatic progress has been made in the *de novo* design of 4- α -helix bundle proteins based upon hydrophobic stabilization. Bundles with the expected size, mass, aggregation, and helicity have been synthesized;¹ however, conformational lability is a common problem^{1c,d,h,j} resulting in proteins that resemble a “molten globule”.² A novel model of the bundle is proposed which provides insight into the source of the conformational lability and into a source of very low-frequency vibrations of proteins containing amphiphilic α -helices.

The amphiphilic α -helix is the building block of the bundle. One face is hydrophobic or nonpolar; the other is polar. The polar side chains, assumed to be randomly oriented,³ must give a resultant radial dipole moment. The axial components of the side-chain dipoles will cancel, but the radial components will not as they exist only on the polar face (Figure 1). The vector sum of the axial dipole moment μ_l (3.5 debye/residue or $1/2q$ at the end of each helix⁴) and the radial dipole moment μ_r gives a resultant μ that is off-axis (Figure 2). I have constructed a model of the 4- α -helix bundle based on this concept. Calculation of the distance between opposite ends of two helix dipoles is shown in Figure 2 (r = magnitude of the radial moment) for the synchronous rotational mode indicated. Distances between dipole ends are

$$y(z,r) = \{l^2 + d^2 + 4dr \cos(z-\pi) + 4r^2 \cos(z-\pi)^2\}^{1/2};$$

$$b(z,r) = \{r^2 + d^2 - 2dr \cos(2\pi-z)\}^{1/2}$$

$$x(z,r) = \left\{l^2 + d^2 + 4dr \cos\left(\frac{3}{2}\pi-z\right) + 4r^2 \cos\left(\frac{3}{2}\pi-z\right)^2\right\}^{1/2}$$

$$a(z,r) = \left\{r^2 + d^2 - 2dr \cos\left(z-\frac{\pi}{2}\right)\right\}^{1/2};$$

$$c(z,r) = 2\left\{\left(\frac{d}{2}\sqrt{2}\right)^2 + r - dr\sqrt{2} \cos\left(\frac{9}{4}\pi-z\right)\right\}^{1/2}$$

$$g(z,r) = \left\{l^2 + r^2 + 2d^2 - 2dr\sqrt{2} \cos\left(\frac{9}{4}\pi-z\right)\right\}^{1/2};$$

$$h = (l^2 + d^2)^{1/2}; e = d\sqrt{2}$$

The total electrostatic energy is then, with $\epsilon = 10\epsilon_0 = 8.9 \times 10^{-11} \text{ J}^{-1} \text{ C}^2 \text{ m}^{-1}$

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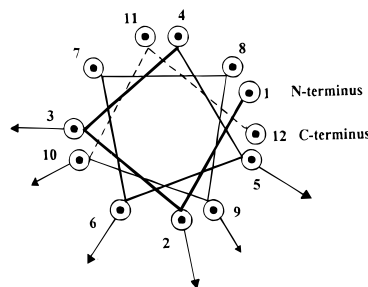


Figure 1. Helical wheel diagram for an α -helix looking down the long axis from the N-terminus to the C-terminus. Axial dipole moments (filled circles) are coming out of the paper; radial dipole moments are represented by arrows.

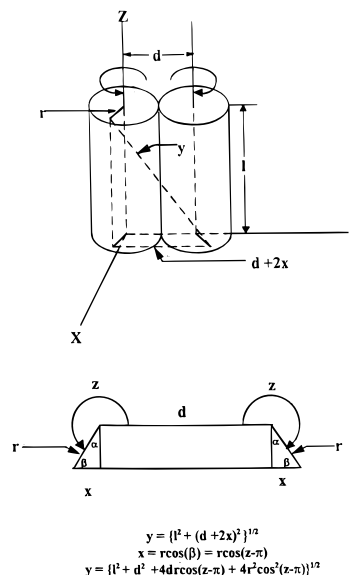


Figure 2. Geometry for and deviation of $y(z,r)$.

$$E(z,r) = \frac{(0.5q)^2 (6.02 \times 10^{23})}{4\pi\epsilon} \times \left[-\frac{4}{a} - \frac{4}{b} + \frac{2}{x} + \frac{2}{y} + \frac{2}{c} + \frac{2}{e} - \frac{4}{g} + \frac{4}{h} \right] \text{ J} \cdot \text{mol}^{-1}$$

A graph of $E(z,r)$ vs z and r is shown in Figure 3. The following parameters are standard: interhelical distance $d = 12 \times 10^{-10}$ m, helical length $l = 15 \times 10^{-10}$ m, charge $0.5q$, $-3\pi/4 \leq z \leq 5\pi/4$, $0 \leq r \leq 6.0 \times 10^{-10}$ m, and the dielectric constant⁵ is 10. The popular approach of using the gas phase dielectric constant ϵ_0 is unrealistic because it overestimates interaction energies by a factor of 10–40.⁵ The magnitude of r is chosen arbitrarily to be less than the helix radius. The interaction of the solvent is treated according to Chothia's analysis⁶ of the hydrophobic effect: both polar and nonpolar side chains exhibit an equal effect when normalized to equal surface area. The free energy for synchronous rotation of the helices about their long axes is then $\Delta G_{\text{rot}} = \Delta E(z,r)$. Conformational entropy effects for axial helical rotation, shown to be important for certain crystalline homopolymers,⁷ are modeled as negligible for bundles in water because of compensating effects⁸ as side chains move from the protein interior to solvent and vice versa. Connecting loops will stabilize the bundle against unfolding,⁹ but the fine structure of the bottom of the energy well is determined by $E(z,r)$.

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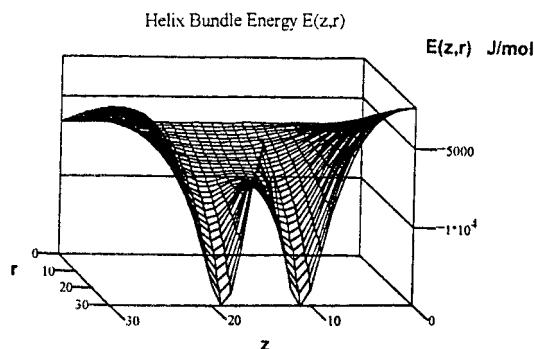


Figure 3. 3-D surface plot of $E(z,r)$; $i = 0...30$, $z_i = -3\pi/4 + \pi i/15$, $r_i = 0.2 \times 10^{-10}$.

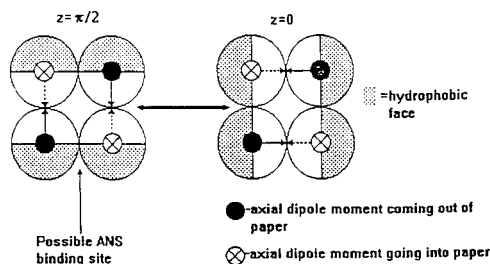


Figure 4. The most stable rotational conformers of a bundle with a significant dipole moment.

Though highly simplified, the model affords insight into the properties of *de novo* bundles. If the radial dipole moment of the helices is small ($r < 3$), the bundle will exist in wide range of rotational states of nearly equal energy (Figure 3) as expected of a "molten globule". Nonpolar side chains will be exposed to solvent and thus able to bind ANS¹⁰ (Figure 4). The bundle will be conformationally labile as reflected in proton magnetic resonance dispersions,^{1e,j} fluorescence emissions, and fluorescence decay constants.^{1e,j,11} Furthermore, one would not expect the bundle to crystallize; as yet, no crystalline species of *de novo* 4- α -helix bundles have been isolated. For $r > 4$ two isoenergetic conformers will exist ($z = 0$, $z = \pi/2$, Figure 4). I suggest that the *de novo* α_4 bundle described as existing in two nearly isoenergetic states, as binding ANS, and as having poor ¹H NMR dispersions^{1j} might contain helices with substantial radial dipole moments.

According to this model, a rigid helix should undergo harmonic vibrations about the long axis. The differential equation describing such vibrations as a function of torsional angle and potential energy is

$$I \frac{d^2 z}{dt^2} = -\frac{dE(z)}{dz} = -kz \quad (1)$$

The moment of inertia of the helix is I , z the torsional angle, $E(z)$ the potential energy, and k the force constant. The $1/g(z,r)$ potential energy term can be simplified as follows:

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(1) multiply and divide by $(l^2 + r^2 + 2d^2)$

$$g(z)^{-1} = \left\{ 1 - \frac{2dr\sqrt{2} \cos\left(\frac{9\pi}{4} - z\right)}{(l^2 + r^2 + 2d^2)} \right\}^{-1/2} (l^2 + r^2 + 2d^2)^{-1/2}$$

(2) simplify using $(1 + p)^{-1/2} \cong 1 - (p/2)$ for $p \leq 1$

$$g(z)^{-1} \cong \left\{ 1 + \frac{dr\sqrt{2} \cos\left(\frac{9\pi}{4} - z\right)}{(l^2 + r^2 + 2d^2)} \right\} (l^2 + r^2 + 2d^2)^{-1/2}$$

(3) simplify further by expanding $\cos((9\pi/4) - z)$ about $9\pi/4$

($z = 0$): $\cos q \cong (1 - (q^2/2))$

$$g(z)^{-1} \cong \left\{ 1 + \frac{dr\sqrt{2}}{(l^2 + r^2 + 2d^2)} \left(1 - \frac{z^2}{2}\right) \right\} (l^2 + r^2 + 2d^2)^{-1/2}$$

(4) the derivative with respect to z , the torsional angle, is

$$\frac{dg(z)^{-1}}{dz} = -\frac{\sqrt{2}drz}{(l^2 + r^2 + 2d^2)^{3/2}}$$

A similar treatment of the other potential energy terms ($r < 5.3 \times 10^{-10}$; drop z^4 terms¹²) yields

$$\frac{dE(z)}{dz} = \frac{(0.5q)^2}{4\pi\epsilon} \left\{ \frac{8dr}{(r^2 + d^2)^{3/2}} + \frac{8r(d + 2r)}{(l^2 + d^2)^{3/2}} - \frac{2dr\sqrt{2}}{(r^2 + \frac{d^2}{2})^{3/2}} + \frac{4dr\sqrt{2}}{(r^2 + l^2 + 2d^2)^{3/2}} \right\} z = kz$$

The differential equation (1) is now that of simple harmonic motion with frequency

$$\nu_0 = \frac{1}{2\pi} \sqrt{\frac{k}{I}} = 3.7 \times 10^{10} \text{ Hz (1.2 cm}^{-1}\text{)}$$

for $I = 8.9 \times 10^{-44} \text{ kg}\cdot\text{m}^2$ (glycine decapeptide). This frequency is in the far-infrared region of the spectrum and is an upper limit, as the moment of inertia will be larger for amphiphilic helices with side chains other than hydrogen. The model for torsional vibrations may account for some of the low-frequency absorption bands that have been observed for protein films and crystals.¹³

The assumption of an α -helix radial dipole moment leads to a straightforward explanation of the conformational properties of *de novo* α -helix bundles and yields an analytical expression for the force constant of a type of low-frequency vibrational mode. Further testing of the model will require an estimate of the radial dipole moment of the helices in a *de novo* bundle.

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(12) For the approximation in step 2 to be valid, p must be less than or equal to 1. For $x(z,r)$ and $y(z,r)$: $p = (4dr(1 - (z^2/2)) + 4r^2(1 - z^2))/(l^2 + d^2) \leq 1$ for $l = 15 \times 10^{-10} \text{ m}$, $d = 12 \times 10^{-10} \text{ m}$, $r \leq 5.3 \times 10^{-10} \text{ m}$, z negligible.

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