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Rigid-body oscillations of α-helices: implications for protein thermal stability

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Abstract

A quasi-continuity model protein consisting of two α-helices undergoing rigid-body torsional oscillations demonstrates that factors stabilizing the model protein, such as increased helix rigidity and hydrophobicity, are the same factors that stabilize thermophilic proteins relative to their mesophilic analogs. The model predicts oscillatory motions with frequencies in the microwave (10^{10} Hz) range. These oscillations decrease in frequency with increasing helix rigidity because of compensating increases in the force constant and moment of inertia, thus explaining the retention of activity in the more rigid thermophilic enzymes. Implications for protein design, based on the predictions of the model, are discussed.

Keywords: Thermophilic protein; Quasi-continuity model; Rigid-body oscillation; α-helix; Microwave absorption

1. Introduction

Investigators studying protein stability, protein evolution and protein engineering for biotechnology have been drawn to thermophilic proteins as model systems. Early studies directed towards ascertaining the physical differences between related mesophilic and thermophilic proteins have shown that the differences are subtle [1–3]. As more of the sequences and 3-D structures of thermophilic proteins and their mesophilic analogs have been solved, more detailed analyses of the differences have been possible.

Menendez-Arias and Argos [4], in a comprehensive statistical study of six protein families, 70 sequences, and 3-D structures found that residue substitutions from mesophilic to thermophilic proteins that decrease protein flexibility and increase hydrophobicity were the main determinants in achieving thermostability. Furthermore, they found that only the α-helical regions were consistently the foci of stabilizing substitutions. Paradoxically, their findings suggest strongly that these more hydrophobic substitutions should be on the solvent-exposed surface of the helices.

The following analysis, based on a quasi-continuity [5] model protein consisting of two α-helices undergoing rigid-body oscillations (Fig. 1),

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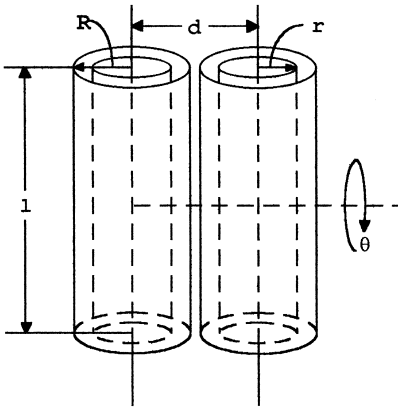

Fig. 1. The protein model consisting of 2 anti-parallel α-helices.

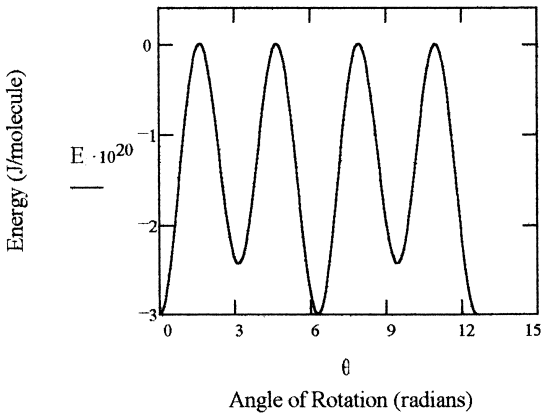

Fig. 2. Model protein energy as a function of rotational angle θ.

focuses on the analysis of Menendez-Arias and Argos. The model demonstrates that the factors stabilizing the model protein are those factors that stabilize thermophilic proteins and explains the physical basis for this stability. Furthermore, the model explains how, because of compensating increases in the force constant and moment of inertia as a function of helix length, enzyme activity is not compromised by increased rigidity,

2. Experimental

Consider two anti-parallel α-helices (Fig. 1). The electrostatic properties of this system have been analyzed by Hol et al. [6] and Rogers and Sternberg [7]. Basic geometric arguments lead to the following expression for the electrostatic energy of the protein as a function of rotational angle θ:

$$E_e = -\frac{(0.5q)^2}{2\pi\epsilon}\left\{\frac{1}{\left[\frac{l^2}{2}(1-\cos(\theta))+d^2\right]^{1/2}} - \frac{1}{\left[\frac{l^2}{2}(1+\cos(\theta))+d^2\right]^{1/2}}\right\} \quad (1)$$

For this system, unless stated otherwise, the standard conditions are $l = 12\times10^{-10}$ m, $d = 12\times10^{-10}$ m, $r = 3\times10^{-10}$ m, $R = 6\times10^{-10}$ m, the charges at the helical ends [8] $q = 1/2\ e$, and the permittivity $\epsilon = 8.85\times10^{-11}\ \mathrm{J^{-1}C^2m^{-1}}$ (dielectric constant of 10.0). The free energy gained by burying exposed surface area f is treated simply as a function of the angle of rotation θ:

$$E_h = -2\pi Rl\phi_o f(1+\cos(2\theta)) \quad (2)$$

The surface energy [9,10] ϕ_o is 22 cal/Å^2-mole and f is the fraction of the helix surface buried (at $\theta = 0$). Using a spherical water probe of radius 1.4×10^{-10} m and assuming that the helices are smooth cylinders, the buried surface is estimated to be 20%. The energy[1] of this model protein is then:

$$E = E_e + E_h \quad (3)$$

It is assumed that the lowest energy state is the one of greatest statistical weight [11] and that the entropy as a function of rotational angle is constant (i.e. the energy surface is parabolic, Fig. 2). However, a vibrational entropy term will be included when comparing states with different vibrational force constants (vide infra).

The differential equation describing torsional

[1]At $\theta = \pi/2$ there is residual buried surface $\delta(1\text{-}\cos(2\theta))$ the relative importance of which decreases with l. It will be ignored as it is small (helical contact is a point), though in more refined calculations it may have to be included.

oscillations of rigid cylindrical helices [12] as a function of torsional angle θ, potential energy E, moment of inertia l and force constant k is

$$I\frac{d^2\theta}{dt^2} = \frac{dE}{d\theta} = -k\theta \tag{4}$$

Differentiation of the energy term and expansion about $\theta = 0$ yields the following expression for the force constant for simple harmonic motion [12]:

$$k = \frac{(0.5q)^2}{4\pi\epsilon}\left[\frac{l^2}{\left(d^2 + \frac{l^2}{2}\right)^{3/2}}\right] + 8\pi Rl\phi_o f \;\; \frac{N\cdot m}{rad} \tag{5}$$

with frequency

$$\upsilon = \frac{1}{2\pi}\sqrt{\frac{\frac{(0.5q)^2}{4\pi\epsilon}\left[\frac{l^2}{\left(d^2 + \frac{l^2}{2}\right)^{3/2}}\right] + 8\pi Rl\phi_o f}{I}}\; s^{-1} \tag{6}$$

The reduced moment of inertia [13] I is

$$I = 6.3\times 10^{-16}\left\{l\frac{(R^2 + r^2)}{4} + \frac{l^3}{12}\right\} kg\cdot m^2 \tag{7}$$

The helix atoms are treated as point masses, forming a hollow cylindrical shell of inner radius 3×10^{-10} m and a uniform mass density shell of thickness of 3×10^{-10} m composed of flexible leucine side chains. For the parameters listed above and $f = 20\%$, a vibrational frequency of 9.1×10^{10} s^{-1} (3.0 cm^{-1}) is predicted. This type of vibration provides a mechanism for the non-thermal effects of microwave energy of 10.4 GHz on 2 thermophilic proteins [14] and some of the very low frequency vibrational bands that have been observed for protein films and crystals by Raman spectroscopy [5].

When the vibrational amplitude is large ($\theta \simeq \pi/2$), the vibrational motion becomes rotational [15]. At this angle, $E = 0$ and the protein unfolds. The *transition* from vibration to rotation occurs [15] when

$$(n + 1/2)h\upsilon > E(\pi/2) - E(0) \text{or } n_{\text{tran}} \simeq \frac{\Delta E}{h\upsilon} = \frac{\frac{(0.5q)^2}{2\pi\epsilon}\left\{\frac{1}{d} - \frac{1}{(l^2 + d^2)^{1/2}}\right\} + 4\pi Rl\phi_o f}{h\upsilon} \tag{8}$$

(drop the 1/2 for large n) with n_{tran} the vibrational quantum number above which vibration changes to rotation.

These low frequency vibrational states are highly populated at room temperature. From the partition function the number of populated levels at a given temperature can be approximated as

$$n_{\text{pop}} \simeq \frac{k_B T}{h\upsilon} \tag{9}$$

k_{B} is the Boltzmann constant. When $n_{\text{tran}} < n_{\text{pop}}$ the protein motion spills over from vibration to rotation, the electrostatic and hydrophobic energies become zero and the protein falls apart or 'denatures'; when $n_{\text{tran}} > n_{\text{pop}}$ the protein is stable. The ratio

$$n_{\text{tran}}/n_{\text{pop}} = \frac{\Delta E}{h\upsilon} \div \frac{k_B T}{h\upsilon} = \Delta E/k_B T \tag{10}$$

is then a definition of protein stability, the protein being stable when $\Delta E/k_{\text{B}}T > 1$. In the following discussion, stabilizing effects will be measured in terms of a surface of constant stability, $\Delta E/k_{\text{B}}T$ (Fig. 3).

1. Discussion

What justification exists for proposing that two rigid helices can serve as a model for a protein? First, a majority of proteins have at least one or more α-helices. These helices act as structural and functional modules. Those factors that strengthen helix–helix interactions will affect protein energetics. It will be demonstrated that there

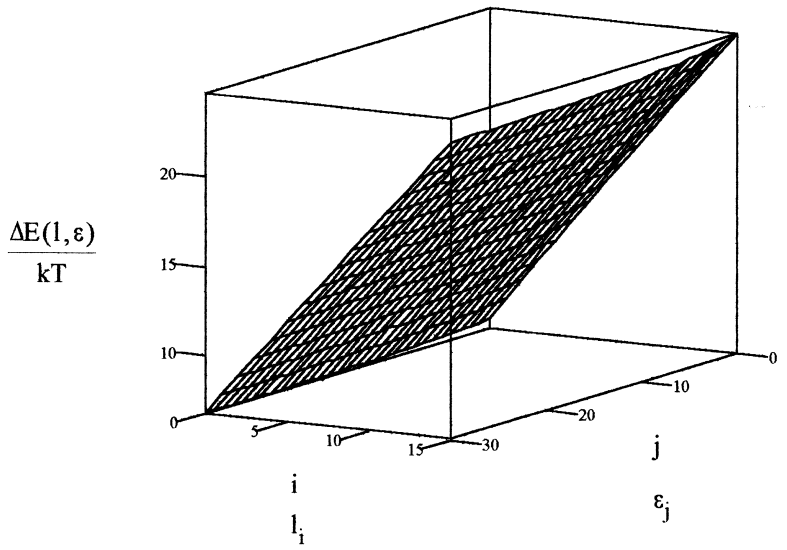

Fig. 3. Model protein stability as a function of helix length and dielectric constant. $l = 12 \times 10^{-10} + 2.0 \times 10^{-10}\ i$, $i = 0 \ldots 15$; $\epsilon = 8.85 \times 10^{-11} + 1.00 \times 10^{-11}\ j$, $j = 0 \ldots 30$; $T = 298$ K.

is a correspondence between the properties of real proteins and the model of two helices oscillating in a potential well consisting of electrostatic and hydrophobic terms.

The model predicts that the protein is stabilized by increasing the helix length (Fig. 3), and that the stability is very sensitive to this parameter. For example, increasing the helix length by one residue (1.5×10^{-10} m/residue) maintains constant protein stability through a temperature increase of 38 K (Table 1).

The model predicts that protein stability decreases with increasing dielectric constant (increasing denaturant or electrolyte concentration). If one is increased by 1.0×10^{-10} m, then a constant protein stability is maintained, though the dielectric constant increases from 10 to 57.

Table 1
Factors affecting model protein stability

T (K)	l (m)	f (%)	ϵ	$\Delta E/kT$
298	12.0×10^{-10}	20	10	7.28
336	13.5×10^{-10}	20	10	7.28
298	$13.0 - 10^{-10}$	20	57	7.28
298	12.0×10^{-10}	2	10	1.34
338	13.5×10^{-10}	2	10	1.34
298	13.0×10^{-10}	2	12	1.34

The same feature that increases thermostability also increases the model protein's stability to increased salt concentration, as has been observed for a number of thermophilic proteins [16–18]. The effect of increasing length is less pronounced for the dielectric constant at lower surface coverages, but is approximately the same for the thermostability (Table 1).

The helix length is a critical factor in determining the model protein stability and is an important determinant of the flexibility of the protein. The measure of flexibility used by Menendez-Arias and Argos is the temperature factor of the Cα atoms taken from X-ray crystallographic data [19]. The temperature factor is

$$B = 8\pi^2 U^2 \tag{11}$$

U being the amplitude of an alpha carbon atom. For a quantum oscillator [20], the vibrational amplitude is

$$U_n = \left\{ h(n + \tfrac{1}{2}) / \pi (kI)^{1/2} \right\}^{1/2} \tag{12}$$

The helix length l is the critical parameter for increasing rigidity, because increased length leads to an increase in the force constant k (Fig. 4) and in the moment of inertia I, which act multiplica-

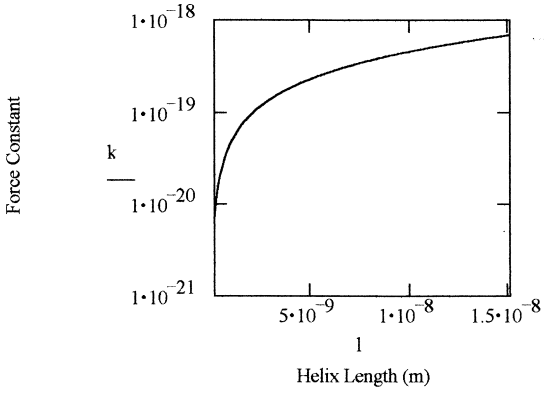

Fig. 4. The effect of increasing helix length on vibrational force constant.

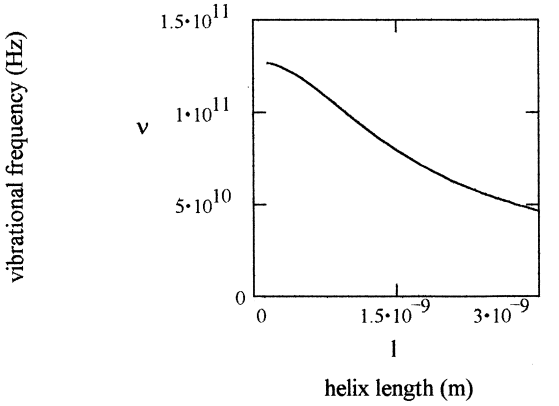

Fig. 5. The effect of increasing helix length on the vibrational frequency.

tively or cooperatively (Eq. (12)) to decrease the amplitude. Small increases in l increase both stability to heat and salt by increasing the rigidity of the protein, thus decreasing the energy E because: (1) oppositely charged ends of the helix macrodipoles move closer together on average; (2) like-charged ends move further apart; and (3) buried surface area increases.

The entropic loss expected from increased rigidity is compensated for by the unique character of the oscillations. As the rigidity increases (as measured by the helix length) the vibrational frequency actually decreases (Fig. 5) leading to an increase in entropy [5]

$$S = k_B\left\{\frac{h\upsilon/k_BT}{e^{h\upsilon/k_BT}-1} - \ln(1-e^{-h\upsilon/k_BT})\right\} \qquad (13)$$

which reduces to

$$\Delta S \simeq k_B\{\ln(\upsilon_2/\upsilon_1)\} \qquad (14)$$

in the low frequency approximation. For example, an increase in helix length of one residue results in an increase in the entropy of 0.54 $JK^{-1}mol^{-1}$.

The helix length can be increased directly by substituting residues with greater helix-forming propensity at the ends of the helix, or indirectly by substitutions along the length of the helix with residues of increased helix-forming propensity. The direct mechanism should lead to an increase in the percentage of α-helices in a thermophilic protein compared to its mesophilic analog as has been observed [20,21]. Substitutions along the helix length should increase the 'effective' length

$$l_{eff} = A/2\pi Rf \qquad (15)$$

defined as the length of a rigid helix that would bury the same surface area (A) as the more flexible helix (Fig. 6). An analogy with a horizontal load-supporting beam can be made: the stiffer the beam, the greater the distance it will span. For the helix, the stiffer it is the more surface it will bury. Such substitutions are harder to detect from crystallographic data, but should result in increased buried surface which can be calculated [9].

At a given temperature, a rigid protein should be less active than its more flexible analog, because the active site is not as accessible to the substrate. Should the active site be bounded by two helices, the nature of their oscillations would partially resolve this enigma. The vibrational frequency decreases with increasing length (Fig. 5), while the force constant increases (Fig. 4) with increasing length. The consequence is that the active site remains open longer as the amplitude

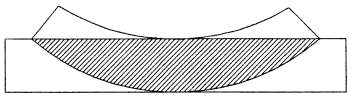

Fig. 6. Effective helical length and buried surface area.

decreases. The substrate has more opportunities to dock, though the slip is tighter. With increasing temperature, the amplitude would increase at constant frequency and the slip would get wider. Nature has balanced amplitude and frequency for the thermophilic proteins to maintain rigidity and activity.

That stabilizing substitutions are more hydrophobic and tend to be on the solvent-exposed surfaces of the helices are readily explained by the model. Such substitutions do not increase the distance d between the helices (which would destabilize the protein) and do not disrupt the internal side-chain packing. It would seem that putting these on the surface should destabilize the protein by increasing exposed surface area. But notice that these same substitutions also increase helix-forming propensity (Table 2), so they tend to increase the effective length and the rigidity of the helices, thus compensating for the increased surface area. Residues of increased hydrophobicity tend to have larger side-chains and thus increase the mass of the helices and the radius R. This increases the moment of inertia of the helices, which also compensates for the increased surface area by decreasing the vibrational amplitude.

The statistical findings of Menendez-Arias and Argos, rationalized by the physical model of oscillations of rigid helices, provide rough guidelines for protein engineering and for the design of more stable proteins. Helices involved in the early stages of unfolding would be the most likely targets for modification as they would most affect the stability of the native state. Molecular dynamics simulations of protein unfolding/folding can extend the model by identifying specific helices and residues amenable to stabilizing modifications. The tools of molecular biology, specifically site-directed mutagenesis, can provide the decisive test of the predictions.

Table 2
Hydrophobicity-helix propensity compensation

Substitution	ΔH_f[a]	Δs[b,c]
Lys to Arg	3.02	0.08
Ser to Ala	2.01	0.29
Gly to Ala	0.50	0.46
Ile to Val	−0.50	−0.16
Lys to Ala	5.53	0.12
Glu to Arg	0.50	0.06
Asp to Arg	0.50	0.36

[a] Hydrophobicity [22] difference.
[b] Zimm and Bragg's factor [23].
[c] Data from Scheraga [24,25].

1.1. Caveats

The findings of Menendez-Arias and Argos relate to mesophilic-to-thermophilic substitution patterns in proteins. One must proceed with caution in trying to extend their findings to the hyperthermophiles [26], especially in the case of small sample sizes.

The vibrational frequencies calculated for this system are probably damped out in water, but the trajectories are the same as for the undamped case [27].

Finally, the total energy of the model protein is sensitive to the choice of dielectric constant and to the value of buried surface. The value of the dielectric constant for proteins is a matter of some controversy [28], and the value of 10 chosen for this work is a compromise.

2. Conclusions

The model rationalizes the findings of Menendez-Arias and Argos implicating α-helices as the foci for amino acid substitutions that lead to increased thermal stability. The model protein, composed of two rigid α-helices undergoing torsional oscillations in an energy well consisting of electrostatic and hydrophobic energy terms, is stabilized by the same factors that stabilize proteins against thermal denaturation. This correspondence suggests that proteins containing at least two α-helices may have used helical pairs as scaffolding for the construction, along the evolutionary pathway, of their present structure.

The mesophilic-to-thermophilic amino acid substitutions that increase thermostability increase the rigidity of the helical pairs in the thermophilic proteins. The increased rigidity stabilizes the protein through the electrostatic and hydrophobic terms of the energy function.

The unique character of the vibrations (i.e. the frequency decreases as the force constant increases) explains why the thermostabile enzymes are able to remain active in spite of increased rigidity. The increased stiffness and decreased amplitude of vibrations is compensated for by a decrease in the frequency. In addition to explaining the experimental findings, the model predicts rigid-body oscillations in the 10^{10} Hz range.

The model is amenable to testing, as the stability of the protein can be tied to parameters of helical length, radius, moment of inertia, hydrophobicity, buried surface area, interhelical distance, dielectric constant, helix-forming propensity and temperature. The variables demonstrate intramolecular cooperative and compensating behavior. The model should serve as a departure point for the experimentalist coupling the powerful tools of molecular biology and high-speed computers to modify existing proteins or to design and link together modules built around finely tuned helical pairs.

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