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Geometry of kinked protein helices from NMR data

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ABSTRACT

Mathematical questions related to determining the structure of a protein from NMR orientational restraints are discussed. The protein segment is a kinked alpha helix modeled as a regular alpha helix in which two adjacent torsion angles have been varied from their ideal values. Varying these torsion angles breaks the helix into two regular helical segments joined at a kink. The problem is to find the torsion angles at the kink from the relationship of the helical segments to the direction of the magnetic field.

1. Introduction

1.1. Membrane proteins

Fine structural detail is important for understanding the functional mechanisms of membrane proteins. For channels conducting ions the mechanism requires a precise alignment of the atoms near the active site. In the M2 protein from influenza A, for example, it is known [1] that a glycine is one of the most conserved amino acids in the evolution of the protein. A detailed knowledge of the structure near that residue will help in understanding why it is conserved, and why it is important in conduction of protons [2]. One possibility is that the helix forms a kink there. Either the kink at Gly helps to align the other residues in the helix properly, or else the kink exposes a carbonyl oxygen to function in conduction of the ions. This paper explores a model in which NMR orientation restraints can provide information on the structure at a kink.

1.2. NMR orientational restraints

Orientational restraints are obtained from NMR spectra of samples that are uniformly aligned with respect to the magnetic field. These orientational restraints are obtained from a class of separated local field experiments that represents refinements [3,4] on the PISEMA experiment [5]. Utilizing these restraints has been described in detail [6–8] [9]. If proteins are put in a fixed orientation in a magnetic field, the signal from the nuclear spins in the molecule is dependent on the orientation of the sample, and one can find the coordinates of the unit magnetic field direction in molecular frames rigidly attached to the molecule. This approach has been used to characterize the structure of quite a few membrane protein structures [10,1,11–20].

Orientation dependent structural restraints can also be obtained in solution NMR experiments through residual dipolar coupling (RDC) measurements of proteins in partially oriented

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media. The incorporation of RDC measurements has allowed structural characterization of large soluble proteins [22,23] as well as large membrane proteins [24]. RDC values obtained from membrane proteins solubilized in detergent micelles provide an opportunity to characterize protein secondary structure topology. Secondary structure dependent patterns are expected for RDC spectra [25] which allows the direct comparison of secondary structure topologies determined from both solution and solid state NMR. These techniques are becoming common for membrane proteins [26–29,14].

1.3. Kinked alpha helix

In NMR experiments such as PISEMA an alpha helix shows a resonance pattern called a PISA wheel, and the angle between the helix axis and the magnetic field direction can be found from the pattern [32,9,8,16]. This angle is called the *tilt angle* and is denoted τ. When this helix is a segment of a membrane protein in a lipid bilayer, and the bilayer normal is parallel to the magnetic field direction, τ also gives the tilt of the helix in the membrane. When PISEMA shows two distinct resonance patterns for a single helix, this indicates a kinked alpha helix with two straight alpha helical segments, and the tilt angles τ and τ' for each segment can be found.

Of more importance in understanding the protein structure is the *kink angle* κ, the angle between axes of the two helical segments. This cannot be determined from τ and τ' alone. From elementary considerations, κ is between $|\tau'-\tau|$ and $\tau' + \tau$ (For proteins crossing the membrane, the tilt angle can be assumed to be between 0° and 90°.).

To find the kink angle, more information than the two tilt angles is needed (see Fig. 1). For each alpha helical segment, the PISEMA experiment is capable of finding a rotation angle ρ in addition to the tilt angle τ. The angles ρ, τ are the spherical coordinates of the magnetic field direction in a frame rigidly attached to the straight alpha helix segment. This frame is referred to as a *helix axis frame*.

Given a simplified model of a kinked alpha helix, the kink angle κ can be found from the angles ρ, τ and ρ', τ'. This paper explains the model and the method of solving for the kink angle. In the model, a kinked alpha helix is a pair of straight alpha helix segments with uniform ϕ_0, ψ_0 torsion angles except for two torsion angles varied from ϕ_0, ψ_0 at a single alpha carbon between them where the kink is formed. Now the angles ϕ, ψ can be solved given ρ, τ and ρ', τ', and from ϕ, ψ the kink angle κ can be computed. The calculation is carried out in a very general fashion so that the solution is in terms of ϕ_0, ψ_0 and the various bond angles along the backbone, and these can be chosen appropriate to the protein environment.

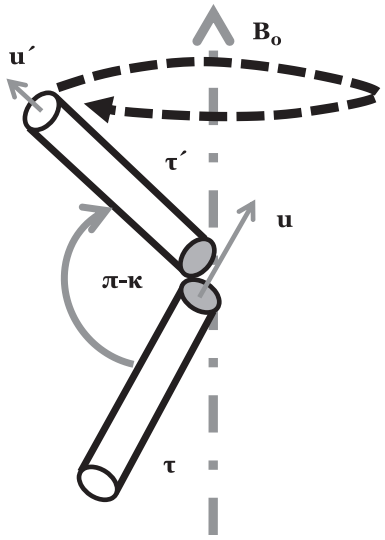

Fig. 1. A kinked helix with segments having tilt angles τ and τ'. B_0 is the unit direction of the magnetic field and also the normal to the membrane bilayer surface. The nitrogen atoms of the straight helical segments lie on the two cylinders. Rotating helix 2 about B_0 does not change the tilts and gives values of the kink angle κ in the range $|\tau' - \tau| \leqslant \kappa \leqslant \tau' + \tau$. The kink angle cannot be found from the two tilt angles alone.

1.4. Mathematical tools

A string of bonded atoms such as the backbone of a protein can be thought of as a sequence of points in space, a discrete curve. Finding the shape of this curve is one important step in determining structure. In the differential geometry of smooth curves the Frenet frame is used to describe the shape of the curve in terms of curvature and torsion. A modification of the Frenet frame is used here to explicitly write the transformation between frames in terms of torsion angles and bond angles. The transformations can be written using rotation matrices or quaternions. Similar computations are found in earlier papers [30,31]. The concept of a Frenet Frame unifies these ideas in terms of differential geometry of discrete curves.

Given a frame at a nitrogen on the helix, the frame at the subsequent nitrogen is given by rotating this frame about the helix axis. (Since we are concerned only with orientations, the translation is ignored.) For an alpha helix the rotation angle is approximately 100°, giving 3.6 nitrogen atoms on the backbone per turn of the helix. This rotation angle and axis can be computed in terms of torsion angles and bond angles using Frenet frame calculations.

A regular alpha helix is one whose torsion angles are the same at each alpha carbon. The torsion angles are fixed at ideal values denoted ϕ_0 and ψ_0 in the region of the Ramachandran plane for alpha helices. A kinked helix is formed by taking a single regular alpha helix and varying from the ideal values two torsion angles ϕ and ψ at an alpha carbon near the middle of the helix. These torsion angles parameterize the kink. The resulting kinked helix is formed by two regular alpha helical segments. The angle between the axes of the helical segments is called the kink angle κ.

The orientation of the segments is given by frames H_1 and H_2 for the first and second segments respectively. The third vector in the frame H_1 is in the direction of the axis of the first helical segment and similarly for H_2. For this reason they are referred to as helix axis frames. The frames are chosen to be fixed in one of the molecular frames along the helix segment. The orientation of the helix segments with respect to the unit magnetic field B_0 can be found if the coordinates of B_0 in each of the two frames is known. If the coordinates of B_0 in the frames H_1 and H_2 are given by vectors X_1 and X_2 respectively, then $H_1X_1 = H_2X_2$. This gives an equation of the form $X_2 = H_2^* H_1 X_1$, and since X_1 and X_2 are unit vectors, this vector equation gives essentially two equations for ϕ and ψ. If X_1 and X_2 are found from the experiment (usually in terms of spherical coordinates ρ, τ and ρ', τ', the equations can be solved for the torsion angles ϕ and ψ at the kink, giving the structure of the kinked helix. The kink angle κ can then be computed in terms of the torsion angles ϕ and ψ, Eq. (15).

2. Frenet frames

Here we discuss the Frenet frames for a discrete curve. If $\mathbf{p}_j$ is a sequence of points in 3D space then unit tangent vectors are defined by

$$\mathbf{t}_j = \frac{\mathbf{p}_{j+1} - \mathbf{p}_j}{|\mathbf{p}_{j+1} - \mathbf{p}_j|},$$