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Communication

Orientation of TOAC amino-acid spin labels in α-helices and β-strands

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Abstract

The orientation of α-helices or β-strands, e.g., in membranes, can be determined from EPR order parameters of (2,2,6,6-tetramethyl-piperidine-1-oxy-4-amino-4-carboxylic acid) TOAC amino-acid spin labels incorporated in the polypeptide backbone. This requires knowledge of the inclination of the nitroxide axes, relative to the α-helix or β-strand axis. Crystal structures of TOAC-containing peptides are used to derive the spin-label orientation relative to refined α-poly-L-alanine and β-poly-L-alanine structures. The spin-label *z*-axes of the two mirror-image TOAC twist-boat conformers are inclined at $13 \pm 2°$ and $65 \pm 3°$, respectively, to the α-helix axis, or at $25 \pm 3°$ and $32 \pm 3°$ to the β-strand axis.

Keywords: TOAC; Nitroxide axes; Order parameter; α-Helix; β-Sheet; EPR

1. Introduction

The 2,2,6,6-tetramethyl-piperidine-1-oxy-4-amino-4-carboxylic acid (TOAC; see Fig. 1) nitroxyl amino acid was introduced by Nakaie et al. [1–3] as a means for spin-labelling the backbone of peptides. Because the nitroxide is rigidly coupled to the peptide backbone, measurement of angular order parameters, S_{zz}, from the spin label EPR spectrum provides direct information on the orientation of the secondary structural elements, e.g., in membranes [4,5]. The experimental order parameter, however, provides the orientation of the spin-label group, which must then be related to that of the secondary structural elements.

For uniaxial motional averaging, the EPR order parameter of TOAC in a regular secondary structure is given by:

$$S_{zz} = \langle P_2(\cos\gamma)\rangle \cdot P_2(\cos\theta_z), \tag{1}$$

where γ is the angle that the helix or β-strand axis makes with the director (e.g., membrane normal), and θ_z is the inclination of the nitroxide *z*-axis to the helix or β-strand axis. $P_2(x) = \frac{1}{2}(3x^2 - 1)$ is a second-order Legendre polynomial, and angular brackets indicate a time average over the molecular motion. The EPR order parameters are determined directly from the motionally averaged hyperfine splittings, in the case of fast motion (e.g., [6]), or by simulation in the case of slow motion [7]. To determine the orientational order parameter, $\langle P_2(\cos\gamma)\rangle$, of the helix or β-strand axis (or, in general, the molecular diffusion axis) from the EPR measurements, however, it is necessary to know the angle, θ_z, that the spin-label *z*-axis makes with the helix or strand axis. In the absence of uniaxial averaging, the static tilt of the α-helix or β-strand axis can only be determined from measurements on aligned samples. This then requires knowledge of the direction cosines ($\cos\theta_x$, $\cos\theta_y$, and $\cos\theta_z$) of all three nitroxide axes, relative to the helix or strand axis (see e.g., [8]).

Several crystal structures of the TOAC amino acid in peptides have revealed that the preferred conformation of the spin-label ring is the twist-boat form, of which there are two possible conformers [9,10]. The purpose of the present communication is to determine the orientation of the TOAC nitroxide to the α-helix axis, when the various TOAC crystal structures are built into the refined coordinates of α-poly-L-alanine [11]. It is found that one TOAC conformer is oriented with the spin-label *z*-axis close to the helix axis, whereas, in the other conformer, the *z*-axis

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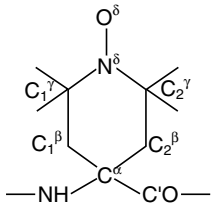

Fig. 1. TOAC amino acid spin label.

is tilted away from the axis of the helix. A similar determination is made also with the refined coordinates of β-poly-L-alanine [12] to determine the TOAC orientation in β-strands. In this case, it is found that the spin-label z-axis of both conformers is inclined at a considerable angle to the axis of the β-strands.

2. Conformation of TOAC in a peptide

Crystal structure studies on peptides have indicated a preference of the TOAC ring for the twist-boat conformation, although isolated occurrences of a near boat conformer and an approximate chair conformation are also found [9,10].

Of interest for the orientation of the TOAC spin label in peptides, is the orientation of the nitroxide p-π orbital (z-axis) relative to the NC$^{\alpha}$ C′ plane. The nitrogen p-π orbital is oriented perpendicular to the $C_1^{\gamma}(C_2^{\gamma})N^{\delta}O^{\delta}$ plane (cf. Fig. 1). The normal to the plane is defined by the vector

$$\mathbf{z} = \overline{C_1^{\gamma}N^{\delta}} \times \overline{N^{\delta}O^{\delta}}, \tag{2}$$

where C_1^{γ} is the (pro-L)C^{γ} atom. With appropriate permutation, the **z**-vector can alternatively be defined, in terms of the vector $C_2^{\gamma}N^{\delta}$, where C_2^{γ} is the (pro-D)C^{γ} atom. The nitroxide x-axis lies along the N-O bond and the y-axis lies closest to the C_1^{γ}-N^{δ} bond.

The normal to the NC$^{\alpha}$C′ plane is defined correspondingly by

$$\mathbf{n} = \overline{C^{\alpha}N^{\delta}} \times \overline{C^{\alpha}C'}, \tag{3}$$

where the direction of **n** lies closest to the C^{α}-C_1^{β} bond. The angle θ between the nitroxide z-axis and the normal to the NC$^{\alpha}$C′ plane is then given by

$$\cos\theta = \mathbf{z}\cdot\mathbf{n}/(|\mathbf{z}||\mathbf{n}|). \tag{4}$$

The inclination of the nitroxide z-axis to the NC$^{\alpha}$C′ plane is the complement of θ. The dihedral angle, ω, between the **z** and **n** vectors is given correspondingly by

$$\cos\omega = \frac{\left(\mathbf{z}\times\overline{C^{\alpha}N^{\delta}}\right)\cdot\left(\mathbf{n}\times\overline{C^{\alpha}N^{\delta}}\right)}{\left|\mathbf{z}\times\overline{C^{\alpha}N^{\delta}}\right|\left|\mathbf{n}\times\overline{C^{\alpha}N^{\delta}}\right|}, \tag{5}$$

where vertical bars indicate the lengths of the corresponding vectors.

Table 1 lists the values of the tilt angle θ and the dihedral angle ω between the nitroxide z-axis and the normal to the NC$^{\alpha}$C′ plane, for various TOAC peptide crystal structures. Differences between the values deduced taking the $C_1^{\gamma}N^{\delta}O^{\delta}$ normal or the $C_2^{\gamma}N^{\delta}O^{\delta}$ normal in the definition of the nitroxide z-axis indicate slight deviations from coplanarity, corresponding to a small pucker about the N^{δ} nitrogen. The near coincidence of the values for the inclination θ and the dihedral ω, arises because the **z** and **n** vectors are almost perpendicular to the $C^{\alpha}N^{\delta}$ vector. This somewhat simplifies visualisation of the orientation of the TOAC nitroxide in peptide helices.

Table 1
Angle, θ, between the nitroxide z-axis and the normal to the NC$^{\alpha}$C′ plane, and their dihedral angle, ω, for TOAC peptide derivatives

Peptide/residue[a]	θ(°)		ω(°)		Ref.
	$C_1^{\gamma}N^{\delta}O^{\delta}$[b]	$C_2^{\gamma}N^{\delta}O^{\delta}$[c]	$C_1^{\gamma}N^{\delta}O^{\delta}$[b]	$C_2^{\gamma}N^{\delta}O^{\delta}$[c]	
I/TOAC^{1}A	64.9	61.6	64.7	61.4	[8]
I/TOAC^{1}B	66.9	66.0	66.1	66.1	
II/TOAC^1	61.1	62.5	60.7	62.2	[8]
II/TOAC^4	62.8	67.0	62.9	67.1	
III/TOAC^1	64.8	66.2	64.6	66.0	[10]
IV/TOAC^{4}A	63.3	59.3	63.1	59.1	[9]
IV/TOAC^{4}B	62.1	64.0	62.2	64.1	
IV/TOAC^{8}A	63.9	66.3	63.8	66.3	
IV/TOAC^{8}B	63.8	68.0	62.8	67.2	
V/TOAC^1	−61.7	−57.1	−60.8	−56.2	[10]
VI/TOAC^1	−60.9	−62.2	−61.1	−62.3	[10]
VI/TOAC^2	−62.2	−67.2	−62.2	−67.2	

[a] Peptides and coordinates: I, Z-TOAC-(L-Ala)$_2$-NHtBu, CCDC 123753; II, pBrBz-TOAC-(L-Ala)$_2$-TOAC-L-Ala-NHtBu, CCDC 123754; III, Boc-TOAC-[L-(αMe)Val]$_4$-NHtBu, CCDC 257672; IV, trichogin GA IV nOct-[TOAC^{4,8},Leu-OMe^{11}], CCDC 120048; V, Ac-TOAC-(Aib)$_3$-L-Trp-Aib-OtBu, CCDC 257673; VI, Fmoc-(TOAC)$_2$-(Aib)$_4$-OtBu, CCDC 257674. A and B indicate two inequivalent molecules in the asymmetric unit.

[b] Nitroxide z-axis defined as normal to (pro-L)$C_1^{\gamma}N^{\delta}O^{\delta}$ plane.

[c] Nitroxide z-axis defined as normal to (pro-D)$C_2^{\gamma}N^{\delta}O^{\delta}$ plane.

The peptides divide themselves into two groups of twist-boat conformers. The larger group, with $\theta = +(64.1 \pm 2.3)°$ and $\omega = +(63.9 \pm 2.3)°$, corresponds to the conformer with $^6T_2$ ring puckering [10], where 6 refers to the (pro-L)C_1^{γ} and 2 to the (pro-D)C_2^{γ} atom. The smaller group, with $\theta = -(61.9 \pm 3.2)°$ and $\omega = -(61.5 \pm 3.5)°$, corresponds to the mirror-image conformer with $^2T_6$ ring puckering, except that peptide V is closer to the $^3T_1$ twist-boat disposition [10].

3. Orientation of TOAC in an α-helix

To determine the orientation of the TOAC nitroxide axes in an α-helix it is necessary to transform the TOAC local axis system (x, y, and z) to that of the α-helix (X, Y, Z)

$$\mathbf{X} = \mathbf{R}_z(\gamma)\mathbf{R}_x(\beta)\mathbf{R}_z(\alpha)(\mathbf{x} - \mathbf{x}_o), \tag{6}$$

where $\mathbf{x}_o$ is the position of the origin of the TOAC axes in the helix axis system. $\mathbf{R}_i$ are rotation matrices and α, β, and γ are the Euler angles relating the two systems of axes.