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Comparison Between Diverse Nitroxide Spin Labels in Synthetically Accessible Peptides

Barbara Biondi[1], Victoria N. Syriamina[2], Antonio Barbon[3], Fernando Formaggio[1,3], Claudio Toniolo[1,3], and Sergei A. Dzuba[2]

[1]CNR-Institute of Biomolecular Chemistry, Padova, 35131 Padova, Italy; [2]RAS-Institute of Chemical Kinetics and Combustion, 630090 Novosibirsk, Russian Federation; [3]Department of Chemical Sciences, University of Padova, 35131 Padova, Italy

Introduction

Site-directed spin labeling (SDSL) in combination with electron paramagnetic resonance (EPR) spectroscopy is a very effective biophysical method to analyze structure and dynamics of proteins under physiological conditions [1,2]. To evaluate the reliability in distance measurements of different spin labels, we designed and synthesized a 20-mer long model peptide and two analogs (Figure 1) with a well-defined Aib-generated, stable α-helical structure. We introduced, at fixed separations (14 residues, about 2.2 nm), two α-amino acid residues of the helicogenic 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) or two S-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl (MTSL) groups conjugated to cysteines (Cys) in the sequence, respectively (Figure 2). The latter nitroxyl labeling methodology is by far the most widely utilized in protein investigations.

Ac-20-NH_2	Ac-Aib-Lys-Aib-Ala-(Aib-Lys-Aib-Ala)$_3$-Aib-Ala-Aib-Lys-NH_2	**I**
Ac-[TOAC^{3,17}]-20-NH_2	Ac-Aib-Lys-**TOAC**-Ala-(Aib-Lys-Aib-Ala)$_3$-**TOAC**-Ala-Aib-Lys-NH_2	**II**
Ac-[Cys(MTSL)^{4,18}] -20-NH_2	Ac-Aib-Lys-Aib-Cys(**MSTL**)-(Aib-Lys-Aib-Ala)$_3$-Aib-Cys(**MSTL**)-Aib-Lys-NH_2	**III**

Fig. 1. Amino acid sequences of the 20-mer model peptide (I) and its two analogs (II and III).

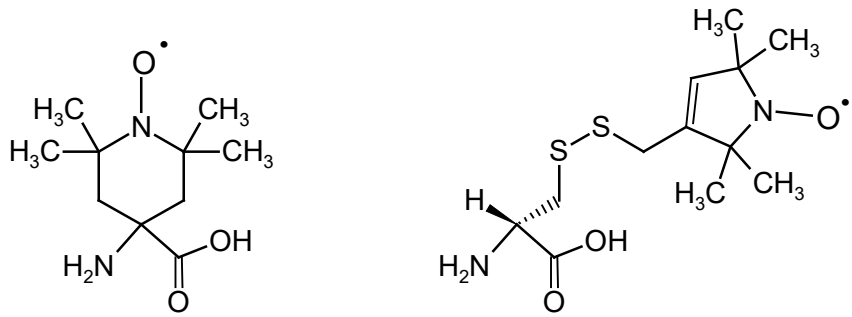

Fig. 2. Chemical structures of the TOAC and (S)-Cys(MTSL) spin labeled residues.

Results and Discussion

Through an in-depth conformational analysis (FT-IR absorption, CD and NMR), we confirmed a stable α-helical structure for all three peptides in different (water, methanol) environments. Our detailed EPR

analysis took advantage from both continuous-wave (CW) and double electron-electron resonance (DEER) experiments. The DEER data indicated a much narrower distance distribution for the TOAC-labeled peptide as compared with that of its MTSL-labeled counterpart (Figure 3). In the latter case, the experimental distance distribution exhibits two maxima which unambiguously point to the existence of two labeled conformers. We conclude that TOAC labels are much more rigid than MTSL labels, therefore providing more precise data on distance distributions in helical peptides.

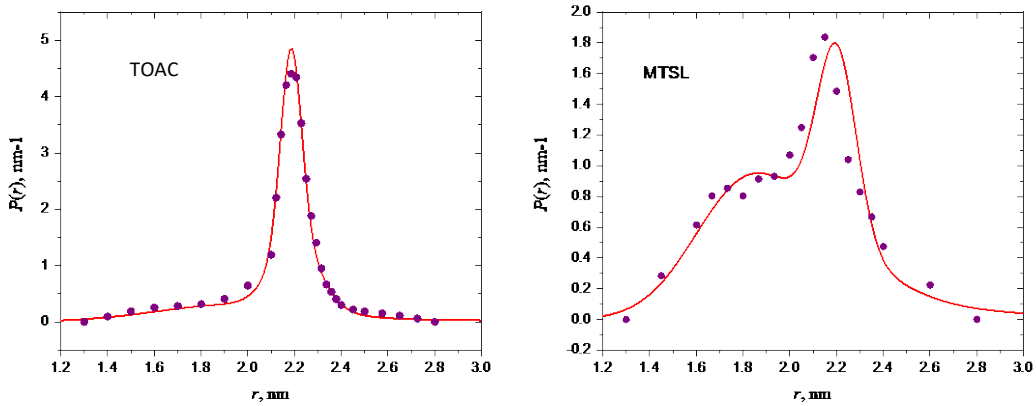

Fig. 3. These representations describe the obtained distance distributions employing the multi-Gaussian approximation (red curve) and the distance discretization (blue points).

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