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Conformational dynamics and distribution of nitroxide spin labels

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ABSTRACT

Long-range distance measurements based on paramagnetic relaxation enhancement (PRE) in NMR, quantification of surface water dynamics near biomacromolecules by Overhauser dynamic nuclear polarization (DNP) and sensitivity enhancement by solid-state DNP all depend on introducing paramagnetic species into an otherwise diamagnetic NMR sample. The species can be introduced by site-directed spin labeling, which offers precise control for positioning the label in the sequence of a biopolymer. However, internal flexibility of the spin label gives rise to dynamic processes that potentially influence PRE and DNP behavior and leads to a spatial distribution of the electron spin even in solid samples. Internal dynamics of spin labels and their static conformational distributions have been studied mainly by electron paramagnetic resonance spectroscopy and molecular dynamics simulations, with a large body of results for the most widely applied methanethiosulfonate spin label MTSL. These results are critically discussed in a unifying picture based on rotameric states of the group that carries the spin label. Deficiencies in our current understanding of dynamics and conformations of spin labeled groups and of their influence on NMR observables are highlighted and directions for further research suggested.

Contents

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1. Introduction

Nitroxide free radicals [1] can be attached as spin labels [2] to proteins, nucleic acids, peptides, or synthetic macromolecules. Such labeling schemes allow the targeted introduction of an electron spin into otherwise diamagnetic systems. This electron spin can be observed either directly by electron paramagnetic resonance (EPR) or indirectly by NMR spectroscopy via its hyperfine interactions with nuclear spins. Since the magnetic moment of an electron spin is 658 times larger than the one of a proton, such experiments can provide access to longer distances than NMR experiments on systems that only contain nuclear spins, for instance by measuring the paramagnetic relaxation enhancement (PRE) for soluble proteins in liquid phase [3–5], including detergent-solubilized membrane proteins [6], and intrinsically disordered proteins [7]. PRE measurements have also been performed in the solid state [8]. Furthermore, the large magnetic moment leads to a proportionally large Boltzmann polarization of electron spin transitions compared to nuclear spin transitions. Polarization transfer induced by microwave irradiation and mediated by the hyperfine coupling, called dynamic nuclear polarization (DNP) [9], can be used to enhance sensitivity of NMR experiments [10,11]. In addition, the larger magnetic moment of the electron spin leads to higher frequencies at comparable magnetic fields and to larger anisotropies of the interaction of the electron spin with the magnetic field or with other spins than can be found in spin systems containing only nuclei. This in turn causes a strong sensitivity of paramagnetic systems to motions on time scales where NMR of diamagnetic systems is less sensitive. For instance, liquid-state DNP induced by the Overhauser effect is sensitive on the time scale of translational diffusion of water [12].

Experiments that depend on the introduction of electron spins into otherwise diamagnetic systems can strongly benefit from site-directed spin labeling (SDSL), where the nitroxide spin label is introduced at a specified position in a protein [13,14] or DNA molecule [15]. Early EPR [16] and NMR [17] studies have indicated that perturbations of structure due to the label are usually small and rather local, a finding that has been largely confirmed by later work. Such SDSL techniques are widely applied in PRE studies. They have also been used in recent liquid-state DNP work [18,19], and may become attractive for solid-state DNP [20]. Once a protein or nucleic acid is spin labeled, further information can be obtained from CW EPR experiments [14] or distance measurements between spin labels by pulsed EPR techniques [21]. Constraints obtained from NMR and EPR experiments may complement each other in cases where constraints from NMR alone are insufficient for a full determination of the structure of a protein [22] or protein complex [23]. The spectroscopic signature in SDSL experiments is determined not only by inherent properties of the system under investigation, but also by specific properties of the spin label itself. In particular, the average site of the electron spin, which is approximately located at the center of the nitroxide N–O bond, is connected to the macromolecule by a linker that is generally flexible. Differences between the free energies of different linker conformations are usually smaller than the thermal energy. Hence, the site of the electron spin is distributed over space to an extent that depends on the type and conformational distribution of the linker. This distribution must be taken into account in the interpretation of distance measurements between spin labels in the solid state [24] and for a quantitative analysis of solid-state PRE measurements [25]. Furthermore, liquid-state PRE and DNP efficiency depend on the spectral density functions of dynamic processes that modulate the hyperfine couplings between the electron and nuclear spins. Conformational dynamics of the label contributes to this spectral density function and thus cannot be neglected.

Current knowledge of the conformational dynamics of spin labels originates mainly from continuous-wave (CW) EPR studies and molecular dynamics (MD) simulations that were aimed at explaining the observed spectral lineshapes. This article is devoted to a critical review of the results of such studies and an assessment of their importance for the interpretation of NMR experiments. Conformational dynamics of labels in liquid phase is linked to their static distribution in solid phase. Direct information on preferred conformations can be obtained from diffraction of crystallized spin-labeled proteins. Indirect information can be derived from label-to-label distance distributions measured in proteins with known structures. Since such measurements are less demanding than protein crystallization, they have become very popular in the past decade, so that a large number of distances are known experimentally. The combined analysis of conformations of spin labels in protein crystals and label-to-label distances is the second main topic of this article. The majority of experimental results discussed in this review stems from EPR experiments. Care is taken to highlight the importance of these results for the interpretation and quantitative analysis of NMR experiments.

This review is structured as follows. Section 2 discusses how the distribution and dynamics of conformations can be influenced by the choice of the spin label and the labeling site. Section 3 is devoted to theoretical, computational, and experimental approaches to the dynamics of labels. First, motional processes and interactions between the macromolecule and the label are considered since they influence the spatial dynamics of the electron spin and determine the spectral response. Second, findings from MD simulations and limitations of this approach are discussed. Third, we examine how dynamics of the macromolecule and the label influence CW EPR lineshapes. Fourth, the relation between spatial dynamics of the electron spin and the relaxation times of the electron spin itself and of nearby nuclear spins is clarified and the implications for the interpretation of PRE and Overhauser DNP data are reviewed. Section 4 focuses on the distribution of conformations of spin labels in solid samples. The "rotamer library approach" is presented as a computationally inexpensive way of predicting the distribution of conformations of spin labels from a structural model of the unlabeled macromolecule, and alternative approaches are mentioned. The crystal structures of spin-labeled proteins tell us about preferences of the label for certain rotameric states and label-to-label distances in proteins with known structures can discriminate between different models of the distribution of rotameric states and different approaches for modeling conformational distributions. The review ends with a summary of the most important findings and open questions.

2. Choice of spin labels and labeling sites

2.1. Labeling strategy and common spin labels

Spin labels can be incorporated at specific sites into macromolecules either during step-by-step synthesis of the macromolecule or by post-synthetic modification of specific residues. The former strategy is usually applied in solid-state [26] or solution-state [27] synthesis of peptides and for oligonucleotides [15] and allows a broad variation of labeling chemistry [28]. In this scenario, one often aims for a rigid coupling of the label to the backbone of the macromolecule and for the least possible conformational ambiguity of the label. For peptides, this can be achieved by incorporating the unnatural amino acid 2,2,6,6-tetramethylpiperidine-N-oxyl-4-amino-4-carboxylic acid (TOAC, Fig. 1) [26,27]. While TOAC is a good substitute for the rare amino acid α-aminoisobutyric acid that features in antibiotic peptaibols [29], it is an achiral amino acid with a tetrasubstituted C^{α} atom and unusual preferences for a limited range of backbone dihedral angles. Thus, TOAC is liable