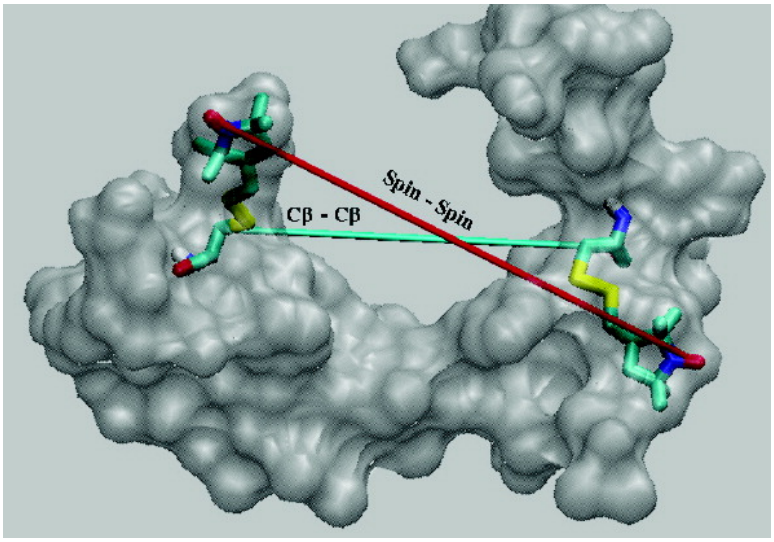
Communication

Explicit Treatment of Spin Labels in Modeling of Distance Constraints from Dipolar EPR and DEER

Ken Sale, Likai Song, Yi-Shiuan Liu, Eduardo Perozo, and Piotr Fajer

J. Am. Chem. Soc., **2005**, 127 (26), 9334-9335• DOI: 10.1021/ja051652w • Publication Date (Web): 09 June 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 9 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 06/09/2005

Explicit Treatment of Spin Labels in Modeling of Distance Constraints from Dipolar EPR and DEER

Ken Sale,*,§ Likai Song,† Yi-Shiuan Liu,‡ Eduardo Perozo,‡ and Piotr Fajer*,†

Biosystems Research Department, Sandia National Laboratories, Livermore, California, Institute of Molecular Biophysics, The National High Magnetic Field Laboratory, Department of Biological Sciences, Florida State University, Tallahassee, Florida, and Department of Molecular Physiology and Biological Physics, and Center for Structural Biology, University of Virginia, Charlottesville, Virginia

Received March 15, 2005; E-mail: klsale@sandia.gov; fajer@magnet.fsu.edu

We describe here a method for enhancing the utility of dipolar−EPR distances as constraints in modeling protein structures by explicit incorporation of the spin labels. We show that accounting for the probe conformation and tether length increases accuracy of distance measures 2-fold.

Site-directed spin labeling−electron paramagnetic resonance (SDSL−EPR) provides a powerful analytical tool for measuring distances in proteins and protein complexes. Depending on the particular experimental method, distances ranging from 4 to 70 Å can be accurately measured.[1] Three methods for measuring the dipolar coupling between electron spins have become increasing popular. In the conventional EPR approach (Figure 1A), distances in the 8−20 Å range are derived from the 3−4 G broadening of the spectrum in the presence of the second spin label.[2] Weaker dipolar interactions (0.1−3.5 G) between spins at longer distances (20−70 Å) can be measured using double electron−electron resonance[3,4] and double quantum coherence[5] methods that filter out noninteracting spins. The dipolar field modulates the spin−echo envelope, and the oscillations of this envelope are used to calculate interspin distances (Figure 1B). The third technique, relaxation enhancement of the nitroxide spin label by a paramagnetic metal, measures the T_1 relaxation time by saturation recovery or inversion recovery methods[6] (Figure 1C). In addition to the wide range of distance sensitivity, EPR is also sensitive to the distribution of interspin distances and, thus, can be used to directly probe conformational heterogeneity.

Distance constraints derived from EPR are used for validation and refinement of structural models of proteins and protein complexes in their native environment and of structures produced using homology and threading methods. However, interpretation of SDSL−EPR distances is complicated by the use of extrinsic probes because the distance distribution is derived from the interspin dipolar coupling between probe electrons, which are located in the NO bond of the nitroxide group (Figure 2). The conformational flexibility of the probe-modified side chain increases the uncertainty in the interpretation of the distance between protein atoms during molecular modeling. The problem is especially acute when measuring short distances (∼10−15 Å) where the probe tether length grossly influences the interpretation.

Two reports underscore the importance of this issue. Borbat et al.[5] modeled the widely used MTSSL (Figure 2) spin label with the cysteine disulfide bond parallel to the α-helix to which it was attached, randomized one of the two remaining torsion angles, and varied the other until a distance match between the two nitroxides was achieved. Schiemann et al.[7] used molecular dynamics simulations to account for the possible conformations of a rigid nitroxide spin label (2,2,5,5-tetramethylpyrrolin-1-yloxy-3-acetylene) bound to duplex DNA. As valuable as these approaches are, they are limited to a specific label bound to a specific secondary structure of a molecule.

We previously reported a strategy for searching the conformational space of a spin label attached to a protein structure that accurately predicted the favored conformations of a variety of spin labels, differing in tether length, size, and charge distribution,

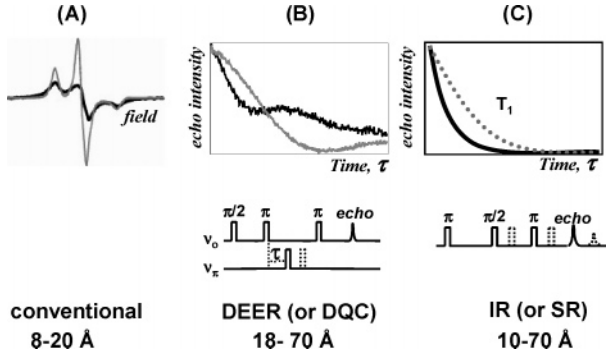


Figure 1. EPR-derived distances are measured by: (A) the broadening of the nitroxide spectrum in the presence of another nitroxide within (8−20 Å), in the gray spectrum of a singly labeled molecule;[2] (B) modulation of the echo height as the neighboring spins (18−70 Å) are saturated by pulses of different frequency, gray: spectra of a biradical with 38 Å interspin separation; black: 25 Å separation, gift of Dr. Jeschke.. The echo modulation was observed with 4 pulse DEER[3] in which a subset of spin is observed with $\pi/2-\pi-\pi$ sequence at frequency, ν_o, creating a refocused echo, which is modulated by neighboring spins that are excited with different frequency, ν_p; (C) cross relaxation of the nitroxides to the faster relaxing paramagnetic metals, gray relaxation in the absence of metal, black in the presence of metal. Relaxation time was measured as an echo decay created by the inversion recovery sequence, $\pi-\pi/2-\pi$.[6]

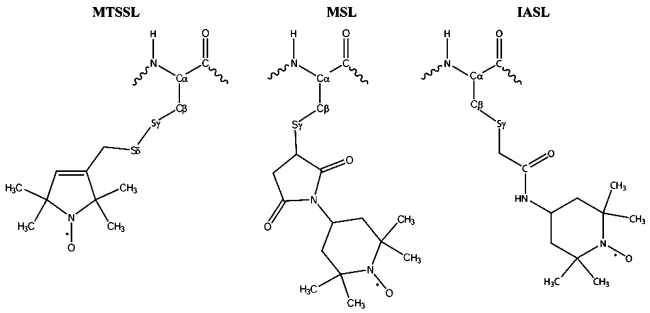


Figure 2. Structures of the spin labels used in this study: 1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-(methyl) methanethiosulfonate (MTSSL); *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) maleimide (MSL); and *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-2-iodoacetamide (IASL).

§ Sandia National Laboratory.
† Florida State University.
‡ University of Virginia.

10.1021/ja051652w CCC: $30.25

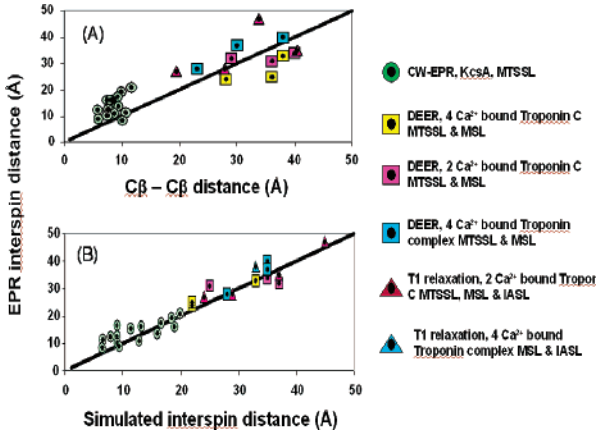


Figure 3. Comparison of EPR spin−spin distances to those measured between Cβ carbons. Distances measured between Cβ carbons have a scatter of 6 Å when compared to the measured values. (B) Correlation between EPR distances and distances between spin labels modeled into the crystal structure using Monte Carlo and molecular dynamics methods; the scatter decreases to 3 Å, and the short distances are linearized.

attached to different sites on a protein. Briefly, Monte Carlo conformational searches on the spin label torsion angles are used to map out the global conformational space of the spin label attached to the protein.[8,9] Short (1 ns at 300 K) molecular dynamics simulations are then used to search the local conformational space and dynamics of the lowest energy spin label conformers. For computational efficiency, all simulations are performed using the CHARMM19 extended atom force field with a distance-dependent dielectric constant, and energy calculations were limited to a 20 Å sphere around both spin labels. The conformational search takes approximately 10 h for the 4000 atom troponin complex on a 2.8 GHz AthlonMP Linux PC.

We have applied this method in several studies, including comparison of solvent accessibilities computed for spin labels modeled into the protein to those measured using EPR, in order to compare differing models of the secondary structure of the inhibitory domain of troponin I.[10] In another study, we determined the myosin regulatory domain orientation in which prediction of probe orientation allowed, for the first time, determination of the absolute orientation of the protein domain using SDSL−EPR.[11]

A natural extension of this approach is the explicit incorporation of spin probes into protein structures for the purpose of using spin−spin distances to validate and refine model structures. Using troponin C, the troponin complex, and the KcsA channel labeled with IASL, MSL, and MTSSL (Figure 2) as model systems, we investigated the correlation between dipolar−EPR distances and distances measured between the corresponding Cβ atoms in the protein structures. Figure 3 shows the correlation between distances determined by dipolar EPR and Cβ−Cβ distances from the corresponding atomic model over the 8−50 Å range. The short (8−20 Å) distances were measured on the KcsA channel using conventional line broadening spectroscopy, while the 20−50 Å distances in troponin were measured using the pulsed EPR methods DEER and metal−nitroxide relaxation between spin labels and Gd^{3+}. Although there is a strong correlation ($R^2 = 0.8$) between the backbone and experimental distances, the clustering in the short distance range illustrates the difficulty in interpreting EPR distances as Cβ−Cβ distances (Figure 3A). In this range, direct modeling of distances is futile because of the lack of correlation ($R^2 = 0.2$) between distances in the protein structure and experimentally measured spin−spin distances. In addition, the agreement between Cβ−Cβ distances and spin−spin distances over the entire distance range is poor with a mean error of 6 Å. Including the spin label in the structural modeling using Monte Carlo conformational searching and molecular dynamics alleviates this problem (Figure 3B). The agreement between the measured and modeled distances improves by a factor of 2 across the full range, having a mean error of only 3 Å, but more importantly, the correlation increases by a factor of 4 over the shorter distance range. The remaining 3 Å average deviation likely derives from factors such as inadequacies in force field parameters, differences between the experimental system and the simulated model system, which is often a homology model, and assumptions made in extracting distances from EPR spectra. An additional benefit of modeling label behavior is the estimate of the heterogeneity in label geometry and its contribution to the heterogeneity of the observed distances. Molecular dynamics trajectories of distances between the labels give a credible estimate of the distance distribution originating from the dynamics of the probes with respect to protein. Significantly wider distributions of observed distances represent the structural heterogeneity of the backbone. Since the EPR experiments are performed on samples that are frozen glass solutions, to some extent, they reflect the protein flexibility that is missed in X-ray crystal structures. The extent of motional damping and narrowing of the distribution is a function of the freezing rate and the "roughness" of the protein energy landscape that is difficult to quantify.

These results have important implications to the design and analysis of dipolar−EPR experiments. For the specific case of orienting a set of transmembrane helices, the number of allowed structures grows exponentially with the error on the distance constraints, decreases exponentially with number of constraints, and deceases exponentially with the radius of the distance graph defining the structure, which is a measure of the way in which the distances connect the helices.[12] Thus, reducing the error in interpreting distances effectively reduces the number of SDSL−EPR experiments required to achieve the same results in terms of number of allowed structures satisfying the distance constraints.

Acknowledgment. Sandia is a multiprogram laboratory operated by Sandia Corp., a Lockheed Martin Company, for the U.S. DOE's National Nuclear Security Admin. (Contract DE-AC04-94-AL85000). P.F. acknowledges support from the National Science Foundation MCB 0346650 and American Heart Assoc. GIA 0455236B. L.S. was an AHA Predoctoral Fellow. The pulsed EPR instrument was funded by NSF CHE-0079649 to P.F.

References

(1) Eaton, S. S.; Eaton, G. R. *Biol. Magn. Reson.* **2000**, *19*, 1−27.
(2) Rabenstein, M. D.; Shin, Y. K. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8239−8243.
(3) Pannier, M.; Veit, S.; Godt, A.; Jeschke, G.; Spiess, H. W. *J. Magn. Reson.* **2000**, *142*, 331−340.
(4) Milov, A. D.; Maryasov, A. G.; Tsvetkov, Y. D. *Appl. Magn. Reson.* **1984**, *15*, 107−143.
(5) Borbat, P. P.; McHaourab, H. S.; Freed, J. H. *J. Am. Chem. Soc.* **2002**, *124*, 5304−5314.
(6) Eaton, S. S.; Eaton, G. R. *Biol. Magn. Reson.* **2000**, *19*, 348−378.
(7) Schiemann, O.; Piton, N.; Mu, Y.; Stock, G.; Engels, J. W.; Prisner, T. F. *J. Am. Chem. Soc.* **2004**, *126*, 5722−5729.
(8) Sale, K.; Sar, C.; Sharp, K. A.; Hideg, K.; Fajer, P. G. *J. Magn. Reson.* **2002**, *156*, 104−112.
(9) *CHARMM19*, force field parameters for IASL, MSL, and MTSSL. http://fajerpc.magnet.fsu.edu/Programs/Molecular_Modelling/Molecular-_Modeling.html.
(10) Brown, L. J.; Sale, K. L.; Hills, R.; Rouviere, C.; Song, L.; Zhang, X.; Fajer, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12765−12770.
(11) Baumann, B. A.; Liang, H.; Sale, K.; Hambly, B. D.; Fajer, P. G. *Biophys. J.* **2004**, *86*, 3030−3041.
(12) Faulon, J. L.; Sale, K.; Young, M. *Protein Sci.* **2003**, *12*, 1750−1761.

JA051652W