John von Neumann Institute for Computing

A Study on the Structure and Dynamics of Spin Labeled Proteins by MD Simulations and EPR Spectroscopy

Prasad Gajula, Igor V. Borovykh, Christian Beier,
Peter Gast, Martina Huber, Heinz -Jürgen Steinhoff

published in

NIC Workshop 2006,
From Computational Biophysics to Systems Biology,
Jan Meinke, Olav Zimmermann,
Sandipan Mohanty, Ulrich H.E. Hansmann (Editors)
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **34**, ISBN-10: 3-9810843-0-6,
ISBN-13: 978-3-9810843-0-6, pp. 145-148 , 2006.

http://www.fz-juelich.de/nic-series/volume34

A Study on the Structure and Dynamics of Spin Labeled Proteins by MD Simulations and EPR Spectroscopy.

Prasad Gajula[1], Igor V. Borovykh[1], Christian Beier[1], Peter Gast[2], Martina Huber[2], and Heinz -Jürgen Steinhoff[1]

[1] Dept.of Physics, University of Osnabrueck,Barbarastr-7, 49076 Osnabrück, Germany
[2] Dept.of Bio/Mol.physics, Huygens Laboratory, Leiden University, Leiden, The Netherlands
E-mail: prasad.gajula@uos.de

Investigations of conformational changes is a prerequisite to understand a protein's biological function. To explore these conformational changes a strategy is developed combining molecular dynamics (MD) simulations and electron paramagnetic resonance (EPR) spectroscopy of spin labeled proteins. The reaction center (RC) of *Rhodobacter sphaeroides*, a photosynthetic protein, is chosen as a model system to study the structure and dynamics that determine electron transport function. Only in combination EPR experiments and MD simulations positively identify the spin label linker position as Cys156 on subunit H. The distance between the primary acceptor (QA) and the bound spin label determined by EPR experiments and MD simulations deviate by about 7% indicating that the conformations of RC in the crystallized and in the solubilized states are slightly different.

1 Introduction

Proteins execute and control essentially all functions in living organisms by changing into different conformational sub-states[1]. Understanding the underlying mechanisms involved in these conformational transitions is one of the main challenges of biophysical research. EPR spectroscopy is one of the few techniques available to study the structure and dynamics of a protein under physiological conditions. To overcome experimental limitations and to improve the analysis of the experimental results molecular dynamics simulations are applied. In the present report, the combination of MD simulations with EPR spectroscopy serves as a powerful tool to explore the conformation and conformational dynamics of the reaction center (RC) protein of *Rhodobacter sphaeroides*, a protein involved in photosynthesis. A cysteine specific spin probe, a so called methanethiosulfonate spin label which contains an unpaired electron on its nitroxide group, is attached to RC (for a detailed review on site-directed spin labeling see[2]). The EPR spectrum, which is sensitive to the spin label reorientational dynamics, reports on the interaction of the spin label side chain with the protein backbone or neighboring side chains, the conformational dynamics of the protein, and the rotational diffusion of the protein. Inter-residue distances can be determined by analysis of the dipolar interaction of two unpaired electrons, e.g., a spin label and a native radical center. MD simulations of the spin labeled RC, on the other hand, provide trajectories of the spin label side chain dynamics from which EPR spectra are calculated[3].

2 Methods and Materials

The photosynthetic RC is an integral membrane protein complex, that is composed of three polypeptide subunits (L, M and H) and ten non covalently bounded cofactors[4]. This

complex (Fig.1)with particular spatial configuration plays a central role in the process of energy conversion. EPR experiments were performed on spin labeled RC. MD simulations were performed to identify which of the native cysteines the spin label binds preferentially. Simulation of EPR spectra based on MD trajectories allows a direct comparison of experimental spectra with the simulated one. For a detailed review on the method, see[3].

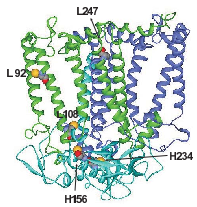

Figure 1. Reaction centre of Rb. Sphaeroides with 5 native cysteines at positions 92, 108, 247 on subunit L and at 156, 234 on subunit H. The spin label can only bind to one of these 5 cysteines.

3 Results and Discussion

3.1 Identification of the spin label linker position

Biochemical analysis and EPR experiments on the isolated LM complex shows that the spin label is bound to either of the two cysteines on subunit H. Further MD simulation analysis was performed to identify the exact position of the spin label on subunit H.

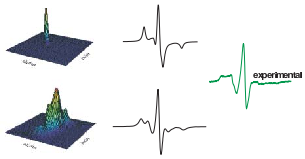

Figure 2. Orientation distribution of the nitroxide ring(left) and calculated EPR spectra (middle) for spin labels attached to position 234, C234R1, (top) and 156, C156R1, (bottom). The experimental spectrum is shown in green (right). The population distributions projected onto the alpha-beta Euler angle plane reveals the different restrictions of the reorientational dynamics of the nitroxides at, two sites. These different nitroxide dynamics lead to distinguishable degrees of motional averaging of the g- and hyperfine tensor anisotropies and are reflected in the EPR spectral shapes.

The conformational space occupied by the nitroxide of spin label C234R1 during a 6 ns MD simulation run shows considerable restriction of the spin label motion. Consequently the EPR spectrum calculated for an isotropic distribution of protein orientations and with the potential determined from the shown orientation distribution(Fig.2) of the nitroxide coincides with a powder spectrum. In contrast, the space covered by the nitroxide of the spin label side chain at position C156 reveals considerable dynamics. The Euler angle projection shows a broad distribution of the angle beta with distinct population maxima.

The apparent hyperfine splitting and the spectral shape of C156R1 are similar to that of the experimental spectrum. Based on this result we conclude that cysteine 156 was labeled in the present experiment[5].

3.2 Distance Measurements

Changes in RC conformation related to electron-transfer events could, in principle, be detected by measuring the distance between a native cofactor and a spin label. For that purpose a two-frequency pulsed electron paramagnetic resonance method (double electron electron spin resonance - DEER) was used[6].

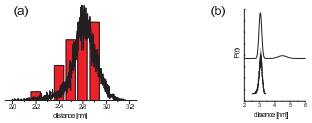

Figure 3. Distance between oxygen, O5 of QA and the centre of the N-O bond of the spin label for all frames of the MD run represented as distance distribution and the histogram of results of energy minimization on 30 structures (shown in red). (b) Distance distribution determined from analysis of experimental data (DEER). To facilitate comparision the distance distribution determined by MD simulation shifted by + 0.28 nm is also shown(bottom).

A distance of 3.05 nm is found in between the light generated semiquinone anion radical of the primary acceptor (QA) and the spin label at position H156 from DEER experiments. MD simulations are performed to interpret the distance. A 6 ns MD run comprising the entire RC protein yields a distance distribution that is close to the experimental one. However, the average distance value found by the MD simulation is smaller than that obtained by DEER by approximately 0.2 nm. Energy minimization on randomly selected points of the MD trajectory confirms this result yielding a slightly broader distribution width. The possible reasons for the difference in the distance values determined by two methods could be(i) incomplete sampling of the nitroxide accessible space during the 6ns MD or (ii) small differences of the conformations of RC in the frozen samples used in experiments and of the crystal structure used for MD simulations.

4 Conclusion and Outlook

Combining the molecular dynamics simulations and EPR spectroscopy we identify the spin label binding site at position 156 of subunit H of RC protein. The average distance between the native cofactor QA and the spin label bound to position 156 determined by EPR and MD deviate by about 7% indicating that the conformations of RC in the crystallized and in the solubilized states are slightly different.

References

1. Frauenfelder H. and McMahon B. , PNAS **95**, 9 (4795-4797)1998.
2. Steinhoff, H.-J. Frontiers in Bioscience 7, c97-110 (2002)
3. Steinhoff,H.-J.,Hubbell,W.L. Biophysical Journal **71** (2201)1996.
4. Stowell,M.H.B., *et.al.* Science 276, 812-816 (1997)
5. Gajula, P., Borovykh, I.V., Beier, C. , Gast, P., Steinhoff , H.-J. (in preparation)
6. Borovykh, I.V., Ceola, S., Gajula, P., Gast, P., Steinhoff , H.-J., Huber, M. JMR **180**, 178-185 (2006)