Chapter 5

EPR Spectroscopic Ruler: the Method and its Applications

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Abstract: The distance measurement method based on Fourier deconvolution of dipolar coupling in spin-labeled EPR spectra provides a new way of examining the structure and function of biological macromolecules. In this chapter, we describe a new approach that has been developed for effective and reasonably accurate data analysis, followed by discussions of several successful applications to interesting biological problems on membrane-associated proteins. This method of EPR spectroscopic ruler has emerged as a powerful tool to investigate the functions of membrane-associated proteins.

1. INTRODUCTION

X-ray crystallography and NMR have made important contributions to our understanding of the structure and function of many soluble proteins. Recent determination of crystal structures of ribosomes truly represents the success of protein crystallography (Ban et al., 1999; Clemons et al., 1999; Cate et al., 1999). Currently, there are more than 11,000 entries in the protein data bank (PDB), and the number is expected to increase rapidly in the future in pace with the swift progress of the structural genomics project.

In contrast, the progress in determining the structure of membrane proteins, which are as many as one third of the total encoded proteins in an organism (Gerstein, 1998), has been very slow. Structures of only a handful
of membrane proteins have been determined. Although several promising crystallization procedures such as antibody conjugation (Iwata et al., 1995) and the cubic phase method (Pebay-Peyroula et al., 1997) have been developed, the generality of these methodologies has not been proven.

Furthermore, conformational changes triggered by ligands or membrane potentials are essential for the regulatory functions of membrane proteins. There is compelling evidence that membrane receptors, ion pumps, and ion channels are mechanically analogous to simple machines composed of basic components such as shafts, hinges and rotors. "Piston", "scissors", "see saw", "screw", "tilt" and "rotation" are the hypothetical terms describing movements in the membrane proteins. Despite extensive efforts, all of these models have not been experimentally tested, largely because high-resolution techniques remain ineffective for such dynamic problems of membrane proteins.

Could spin labeling EPR be an effective tool for these important, but difficult problems, although maybe with lower resolution? In this chapter we will try to provide some answers to this question. We will describe first the EPR dipolar distance measurement method (Rabenstein and Shin, 1995), followed by discussions of several successful applications to interesting biological problems on membrane-associated proteins (Poirier et al., 1998b; Ottemann et al., 1999; Thorgeirsson et al., 1997).

Modern molecular biology techniques are so advanced that native residues in a protein can be routinely replaced by cysteines, which provide specific labeling sites for thiol specific spin labels. This labeling method is often called site-directed spin labeling (SDSL) (Hubbell and Altenbach, 1994). We use SDSL to attach a pair of nitroxides to specific positions of a protein. The two nitroxides then experience dipolar interaction, which has an inverse cube dependence on the inter-spin distance (Figure 1). We are interested in the analysis of the dipolar broadened EPR spectra to determine the interspin distance.

The method discussed in this chapter is largely limited to 9 GHz continuous wave (CW) EPR spectroscopy. As an initial step towards the establishment of the methodology, an EPR spectroscopic ruler, a series of $\alpha$-helical polyalanine peptides (Marqusee et al., 1989) containing the two nitroxides, was synthetically made in order to probe the accuracy, precision, and measurable distance range of the EPR method. Moreover, a new simplified dipolar interaction theory, called Fourier convolution-deconvolution (FCD), which requires no adjustable parameters, has been developed for an effective and reasonably accurate data analysis. In this chapter, we will start with the description of the theory. Next, we will explain the step-by-step data analysis using the FCD method. In particular, we will put some emphasis on the procedure by which we treat the problem of incomplete labeling. The problem of incomplete labeling is the major source of error existing in most double spin labeling EPR experiments. This