by membrane bound ATPases. *Bioelectrochem. Bioenerg.* 15:457–476.

Weaver, J. C., and R. D. Astumian. 1990. The response of living cells to very weak electric fields: the thermal noise limit. *Science.* 247: 459–462.

Weaver, J. C., and R. D. Astumian. 1992. Estimates for ELF effects: noise-based thresholds and the number of experimental conditions required for empirical searches. *Bioelectromagnetics.* 1:S119–S138.

Analyzing the Structure of Polypeptides in Membranes by Fluorescence Quenching

Erwin London

Department of Biochemistry and Cell Biology, State University at Stony Brook, Stony Brook, New York, 11794 USA

One of the most difficult problems in membrane biophysics is the determination of the location of molecules within the membrane. For membrane peptides and proteins the problem is especially severe, as formidable barriers remain to the crystallographic and nuclear magnetic resonance techniques that are so useful in solution. Therefore, the development of alternate methods is of great interest. It is becoming increasingly attractive to use fluorescence to locate groups within membranes. Such a strategy requires a reliable method to determine the depth of a fluorescent group within the membrane. This has proven to be much harder than expected. Among the more powerful methods is the use of membrane-bound fluorescence quenchers such as brominated or nitroxide-labeled lipids. Nitroxide-labeled lipids are coming into wide use for such applications because they have the ability to quench a wide range of fluorophores, and their location in the membrane has been fairly well defined. In addition, a simple equation that allows the calculation of depth from the amount of quenching has been developed, and the validity of the resulting analysis has been tested using a wide variety of membrane-bound fluorophores. This "parallax analysis" approach involves the comparison of the intensity of fluorescence in two different samples in which phospholipids that carry a quenching group at different, but defined, depths have been incorporated into the bilayer (Chattopadhyay and London, 1987; Abrams and London, 1993).

To solve the structure of a peptide or protein by fluorescence quenching a unique fluorescent site must be introduced in the form of a single Trp residue or single fluorescence-labeled Cys. By localizing the membrane location for a series of such mutants, the membrane penetration of an entire polypeptide chain could potentially be mapped out in detail. In this issue Jones and Gierasch (1994) begin to look at the insertion of a signal sequence peptide by combining introduction of a single Trp with fluorescence quenching by nitroxides. Other groups have recently applied this approach to both peptides and membrane proteins (Chung et al., 1992; Palmer and Merrill, 1994).

Jones and Gierasch (1994) start with control experiments using both Lys-Trp-Lys, and a peptide with a 20-residue-long poly-Ala-Val stretch in which a Trp has been incorporated near either the center or the ends. They demonstrate the basic reliability of the approach by obtaining the expected surface location for the Trp in the former case, and the expected nearly transmembranous conformation in the latter.

The main experiments involve the LamB signal sequence peptide with a Trp fixed at either toward the middle (W18) or C-terminal (W24) of the peptide. Quenching detects a deep insertion of these peptides. Jones and Gierasch (1994) interpret these results in terms of a model in which an equilibrium exists between a predominant nontransmembranous but membrane-penetrating helix that is tilted from the membrane surface and a less favorable transmembranous structure. Introduction of ionizable residues toward the center of the peptide tend to decrease the overall depth of penetration, perhaps decreasing the degree of penetration by decreasing the tilt in the nontransmembranous form.

One important observation made by Jones and Gierasch (1994) is that the wavelength of maximum emission is correlated with depth much more weakly than would be hoped. The well known observation that fluorescent groups emit at progressively shorter (blue-shifted) wavelengths as polarity is decreased could potentially be used to measure the depth of a residue in the membrane. It might be hoped that the deeper a residue is located in the hydrophobic core of the membrane, the more blue-shifted it would fluoresce. However, Jones and Gierasch (1994) find introduction of an Asp residue within the most hydrophobic regions of the LamB signal peptide largely abolishes the blue shift of Trp in the 18 position, despite the fact that nitroxide quenching shows the Trp remains deeply embedded in the membrane. Furthermore, the introduction of an Arg residue has only a weak effect on the emission wavelength, despite resulting in a perceptibly shallower Trp depth. The authors mention several possible explanations for the surprisingly reduced blue shift in the Asp peptide, favoring increased hydration near the Trp residue due to the presence of Asp. Intriguingly, low pH reestablishes the expected blue shifts of Trp fluorescence in these peptides. Whatever the explanation, the dangers of using an indirect method such as maximum emission wavelength to estimate depth are clearly highlighted by these results.

As might be expected, the observations of Jones and Gierasch (1994) raise almost as many questions about the structure of the LamB polypeptide as they answer. Is the weak quenching of the Asp peptides related to some phenomenon, such as peptide aggregation within the membrane, that could also explain the lack of a Trp blue shift? What is happening to the structure of the Asp containing peptides at low pH where the blue shift returns to expected values? Does the introduction of Trp affect peptide structure? Furthermore, Jones and Gierasch (1994) correctly point out that the absolute values of the depths obtained from the parallax analysis are subject to uncertainties at this stage of our understanding. Are

Received for publication 4 August 1994.

0006-3495/94/10/1368/03 $2.00

there effects on quenching due to exclusion of the zwitterionic PC-based quenchers from the vicinity of the cationic peptide studies by anionic PG? Are there peptide-induced perturbations in the depths of nitroxide groups that influence the depth values obtained from quenching? The answers to all these questions will only become apparent once polypeptide segments are examined in which the fluorescence group is systematically substituted at a variety of positions. Nevertheless, it is becoming increasingly clear that fluorescence quenching will be an increasingly useful tool in the analysis of membrane protein structure.

REFERENCES

Abrams, F. S., and London, E. 1993. Extension of the parallax analysis of membrane penetration depth to the polar region of model membranes. *Biochemistry*. 32: 10826–10831.

Chattopadhyay, A., and E. London. 1987. Parallax method for direct measurement of membrane penetration depth utilizing fluorescence quenching by spin-labeled phospholipids. *Biochemistry*. 26:39–45.

Chung, L. A., J. D. Lear, and W. F. DeGrado. 1992. Fluorescence studies of the secondary structure and orientation of a model ion channel peptide in phospholipid vesicles. *Biochemistry*. 31:6608–6616.

Jones, J. D., and Gierasch, L. M. 1994. Effect of charged residue substitutions on the membrane interactive properties of signal sequences of the *Escherichia coli* LamB Protein. *Biophys. J.* In press.

Palmer, L. R., and Merrill, A. R. 1994. Mapping the membrane topology of the closed state of the colicin E1 channel. *J. Biol. Chem.* 269:4187–4193.