SOLVENT RELAXATION IN PHOSPHOLIPID BILAYERS: PHYSICAL UNDERSTANDING AND BIOPHYSICAL APPLICATIONS

JAN SÝKORA and MARTIN HOF*
J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic and Center for Complex Molecular Systems and Biomolecules, Dolejškova 3, CZ-18223 Prague, Czech Republic

Solvent relaxation (SR) refers to the dynamic process of solvent reorganisation in response to an abrupt change in charge distribution of a dye via electronic excitation. The temporal response can be monitored through the observation of the dynamic Stokes' shift $\nu(t)$ of the dye's emission maximum frequency. The complete time-dependent Stokes' shift $\Delta\nu$ ($\Delta\nu = \nu(0) - \nu(\infty)$) increases with increasing solvent polarity. Linear proportionality between $\Delta\nu$ and a dielectric measure of the solvent polarity has been experimentally verified [1]. At ambient temperatures, a typical relaxation process $C(t)$ ($C(t) = (\nu(t) - \nu(\infty)) / \Delta\nu$) in an isotropic polar solvent starts with a fast inertial motion on the 0.05 to 0.5 picoseconds (ps) time-range, followed by rotational and translational diffusion occurring on the pico- to sub-nanosecond (ns) time scale [1]. In pure water an average (integral) solvent relaxation time of about 0.3 ps has been determined [2]. It has been known already for almost thirty years that a significant part of solvent relaxation monitored by dyes associated with phospholipid bilayers occurs on the ns time scale [3]. A quantitative and comprehensive picture of SR in the liquid-crystalline phase of phospholipid bilayers, however, has been presented recently [4].

Within the last ten years SR studies in bilayers became of interest for two different motivations: Firstly, a series of publications appeared demonstrating the benefit of this technique in detecting physiological relevant changes in the phospholipid bilayer organization [5-9]. Subsequently, contributions addressing the question on the origin of slow relaxation components probed in lipid membranes were published [10].

A major requirement for valid application or physical interpretation of solvent relaxation studies in bilayers is the knowledge about the location of the used chromophore. It has been demonstrated [4,6-8] that $\Delta\nu$ as well as τ_r are strongly dependent on the location of the chromophore within the bilayer. The solvent relaxation time τ_r of dyes like 6,8-difluoro-4-heptadecyl-7-hydroxy-coumarin is about 0.4 ns in phosphatidylcholine (PC) small unilamellar vesicles at ambient temperature [4]. Those dyes are probing the external interface of the bilayer. SR in the headgroup region probed by 6-propionyl-2-dimethylaminonaphthalene (Prodan) of PC-bilayers is characterized by τ_r value of 1.0 ns [4]. The chromophore of Patman (6-hexadecanoyl-2-(((2-(trimethylammonium)ethyl)-methyl)amino)naphthalene chloride) is identical to the one of Prodan but is

* E-mail: hof@jh-inst.cas.cz

located deeper in the bilayer [8]. The obtained value for τ_r is significantly larger (1.7 ns) than obtained for Prodan [4].

Solvent relaxation within the hydrophobic backbone region has been monitored by a set of n-(9-anthroyloxy)stearic acids (n-AS) [4,6]. Again, SR is occurring on the ns time scale. For example, the relaxation time assigned to the solvent relaxation for 9-AS is about 4.0 ns [4]. Interestingly, the solvent relaxation becomes slower with deeper location within the phospholipid bilayer. We believe that a decreasing number of water molecules solvating the anthroyloxy-chromophore is leading to a slower solvent response with deeper location within the hydrophobic backbone of the bilayer. In summary, we observe a slowing down of SR when starting from bulk water (sub-ps), passing the external interface (sub-ns) and the headgroup region (ns), and finally reaching the hydrophobic backbone (several ns) of the bilayer.

A comparison of $\Delta\nu$ values for different membrane labels is only valid if the labels contain the identical chromophore. Comparison of three "Prodan" like dyes [4] and five n-AS dyes [4,6] demonstrates a decreasing $\Delta\nu$ with deeper location, which gives evidence for a polarity gradient within the bilayer in the liquid-crystalline phase.

Recent studies aiming for an understanding of the origin of the "slow" relaxation are based on experiments using dyes with rather undefined location [10]. It is proposed that ionic headgroups make the nearest water molecules nearly immobile and that those "bound" water molecules undergo slow exchange with "unbound" water molecules. This sounds plausible, but it only considers the role of the water molecules, but not the direct influence of the charged headgroups on the solvent relaxation kinetics. The influence of the chemical structure of the phospholipid headgroups on the SR monitored by Patman and Prodan has been demonstrated [8].

The solvent relaxation technique has been shown to detect micro-"viscosity" (τ_r) and -polarity ($\Delta\nu$) changes in the bilayer due to temperature [5,7,8], ethanol addition [9], membrane curvature [5], and lipid composition variations [6-9] as well as due to the binding of calcium ions [6,8] and blood coagulation proteins [8]. The defined location of the mentioned dyes provides the possibility to investigate selectively the protein interaction with different domains of the bilayer. The demonstrated high sensitivity of this technique for the binding of peripheral proteins led to a better understanding of the prothrombin-calcium-membrane interactions than it was achievable by standard fluorescence techniques, like anisotropy or excimer formation studies [8].

In summary, it has been shown, that the application of the solvent relaxation approach in biomembrane research is in several aspects superior to standard fluorescence techniques. On the other hand, a thorough understanding of the origin of the evident slow relaxation in bilayers is not at all reached yet.

Acknowledgements. The Center for Complex Molecular Systems and Biomolecules is supported by the Czech Ministry of Education, Youth and Sports via grant number LN 00A032.

REFERENCES

1. Horng, M.L., Gardecki, J.A., Papazyan, A. and Maroncelli, M. Subpicosecond measurements of polar solvation dynamics: coumarin 153 revisited. **J. Phys. Chem.** 99 (1995) 17311-17337.
2. Jimenez, R., Fleming, G.R., Kumar, P.V. and Maroncelli, M. Femtosecond solvation dynamics of water. **Nature** 369 (1994) 471-473.
3. Easter, J.H. and Brand, L. Nanosecond time-resolved emission spectroscopy of a fluorescence probe bound to L-α-egg lecithin vesicles. **Biochem. Biophys. Res. Commun.** 52 (1973) 1086-1092.
4. Sýkora, J., Kapusta, P., Fidler, V. and Hof, M. On what time-scale does solvent relaxation in phospholipid bilayers happen? **Langmuir** 18 (2002) 571-574.
5. Hof, M., Hutterer, R., Perez, N., Ruf, H. and Schneider, F.W. Influence of vesicle curvature on fluorescence relaxation kinetics of fluorophores. **Biophys. Chem.** 52 (1994) 165-72.
6. Hutterer, R., Schneider, F.W., Lanig, H. and Hof, M. Solvent relaxation behaviour of n-anthroyloxy fatty acids in PC-vesicles and paraffin oil: a TRES study. **Biochim. Biophys. Acta** 1323 (1997) 195-207.
7. Hutterer, R., Schneider, F. W. and Hof, M. Time-resolved emission spectra and anisotropy profiles for symmetric diacyl- and dietherphosphatidyl-cholines. **J. Fluor.** 7 (1997) 27-33.
8. Hutterer, R., Schneider, F.W., Hermens, W.T., Wagenvoord, R. and Hof, M. Binding of prothrombin and its fragment 1 to phospholipid membranes studied by the solvent relaxation technique. **Biochim. Biophys. Acta** 1414 (1998) 155-164 and references 12, 13, 15, and 29 therein.
9. Hutterer, R. and Hof, M. Probing ethanol-induced phospholipid phase transitions by the polarity sensitive fluorescence probes Prodan and Patman. **Z. Phys. Chem.** 216 (2002) 333-346.
10. Bhattacharyya, K. and Bagchi, B. Slow dynamics in constrained water in complex geometries. **J. Phys. Chem. A** 104 (2000) 10603-10613 and reference 51 therein.