ELECTRON TRANSFER AND WATER PENETRATION IN BIOLOGICAL SYSTEMS: UNCONVENTIONAL USE OF NITROXIDES

Itzhak Bilkis,[a] Antonio Barbon,[b] Tamar Eliash,[c] Mordechai Sheves,[c] and Lev Weiner[c]

[a] Fac. of Agric., Food and Environ., The Hebrew U., Rehovot, POB 12, 76100, Israel (Itzhak.bilkis@mail.huji.ac.il)

[b] Dept. of Chemical Sciences, U. of Padova, Via Marzolo 1, 35131 Padova, Italy

[c] Dept. of Organic Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

It is possible to induce electron transfer (ET) in many biological systems by introducing donor-acceptor pairs, i.e. metal ions [1], or by integrating systems into solid-state junctions [2]. We have recently proposed and validated a novel methodology. It takes advantage of a redox reaction between a nitroxyl radical and a metal Ru(III) center, both bound at selected specific positions within suitable biological systems [3]. The applicability of the method has been demonstrated by using bacteriorhodopsin (BR) and time-resolved EPR. Site-directed spin-labelled mutants of BR were prepared, and a spin-labelled analogue of BR retinal was synthesized and incorporated into the active site of the apoenzyme (BR_{art}). The Ru(II)-bipyridyl complex was also bound at a specific coordination site. ET was followed by monitoring the decrease in the intensity of nitroxyl EPR signal. It was shown that the rates of photo-stimulated ET were significantly lower for the buried retinal analogue in BR than for mutants in which the spin label was located at external sites. The crystal structure of BR was used to interpret the rates of ET observed. It was subsequently found that this methodology can be conveniently utilized to study water penetration to specific sites in the protein at which the nitroxide spin label is bound. We have used isotopic labelling water ($H_2{}^{17}O$) to demonstrate that it can penetrate to a buried spin-labelled site, and convert an EPR-silent oxoammonium cation to a nitroxide displaying a detectable EPR signal. This provides a novel and unique approach for measuring site-specific water penetration into biological systems.

[1] H. Gray, J. Winkler, Chem. Phys. Lett. 2009, 483, 1–9.

[2] I. Ron, L. Sepunaru, S. Itzhakov, T. Belenkova, N. Friedman, I. Pecht, M. Sheves, D. Cahen, J. Am. Chem. Soc. 2010, 132, 4131 –4140.

[3] T. Eliash, A. Barbon, M. Brustolon, M. Sheves, I. Bilkis, L. Weiner, Angew. Chem. Int. Ed. 2013, 52, 8689 –8692