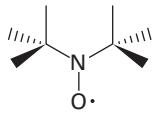
IMPACT 19 …ON BIOCHEMISTRY AND NANOSCIENCE: Spin probes

The anisotropy of the *g*-value and of the nuclear hyperfine interactions can be observed when a radical is immobilized in a solid. Figure 1 shows the variation of the lineshape of the EPR spectrum of the di-*tert*-butyl nitroxide radical (**1**) with temperature. At 292 K, the radical tumbles freely and the isotropic hyperfine coupling to the ^{14}N nucleus gives rise to three sharp peaks. At 77 K, motion of the radical is restricted. Both isotropic and anisotropic hyperfine couplings determine the appearance of the spectrum, which now consists of three broad peaks.



1 Di-*tert*-butyl nitroxide

A **spin probe** (or *spin label*) is a radical that interacts with a molecular assembly (a biopolymer or a nanostructure) and has an EPR spectrum that depends on the structural and dynamical properties of the assembly. The ideal spin probe is one with a spectrum that changes significantly in response to its environment: nitroxide radicals are very useful in this regard. For example, nitroxide spin probes have been used to show that the hydrophobic interiors of biological membranes, once thought to be rigid, are in fact very fluid and that individual lipid molecules move laterally through the sheet-like structure of the membrane.

Benzyl *tert*-butyl nitroxide (**2**) and dibenzyl nitroxide (**3**) readily form host–guest complexes with species such as β-cyclodextrin (**4**), and it is found that the EPR spectra from the free and bound nitroxide are significantly different (Fig. 2). The magnitude of the hyperfine coupling to the N atom appears to be sensitive to the degree of exposure of the N–O group to solvent, so a complex in which the N–O group is buried within the host (and so shielded from the solvent) has an EPR spectrum significantly different from the free nitroxide, or from a complex in which the N–O group is only partially exposed to solvent. In addition, the value of the hyperfine coupling to the benzylic H atoms (the H atoms of the CH_2 groups attached the benzene ring) is sensitive to the dihedral angle between these C–H bonds and the N–O bond. For free nitroxide it is likely that there is free rotation about the C–N bond, leading to an averaged value for the coupling. Once bound in a cyclodextrin, the rotation is more constrained, resulting in a different value for the coupling.

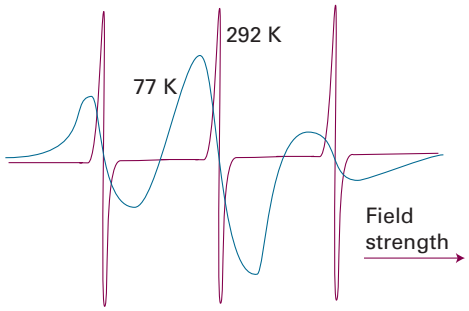


Figure 1 EPR spectra of the di-*tert*-butyl nitroxide radical at 292 K (purple) and 77 K (blue). Adapted from J.R. Bolton, in *Biological applications of electron spin resonance*, H.M. Swartz, J.R. Bolton, and D.C. Borg (eds.), Wiley, New York (1972).

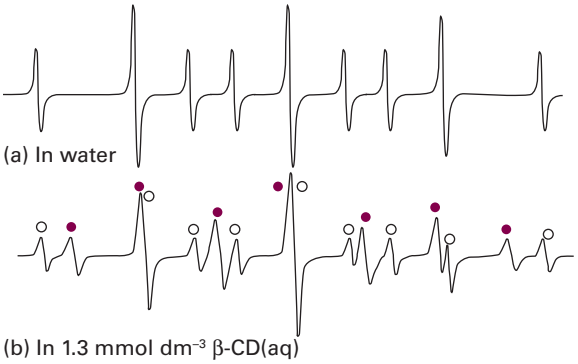
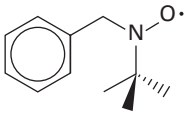
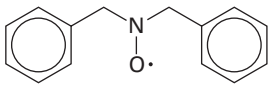


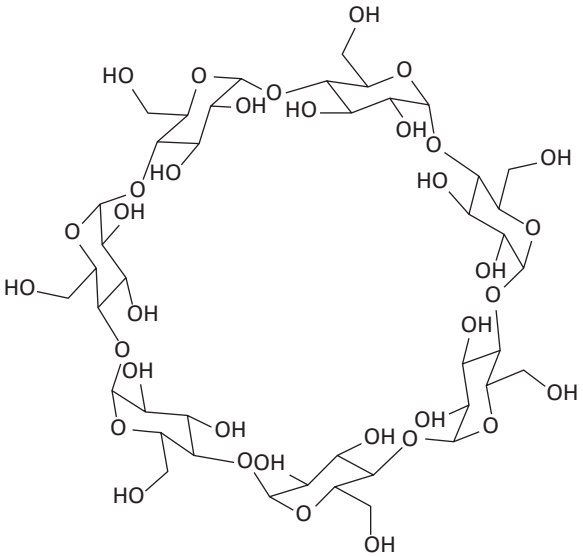
Figure 2 The EPR spectra of benzyl-*tert*-butyl nitroxide radical (a) in water and (b) in the presence of β-cyclodextrin. Spectrum (a) shows splittings due to coupling with one N atom and two equivalent H atoms. Spectrum (b) is a superposition of the spectrum from the radical in water (peaks marked with o) and the spectrum from the radical bound to the host (peaks marked with •), for which the hyperfine couplings are different. (Based on P. Franchi et al., *Curr. Org. Chem*. **8**, 1831 (2004).)



2 Benzyl *tert*-butyl nitroxide



3 Dibenzyl nitroxide



4 β-Cyclodextrin

The fact that separate spectra are seen for free and bound nitroxide, rather than an averaged spectrum, implies that the rate of exchange between the two environments is slow on the EPR timescale. This slowness is advantageous because it means that by recording spectra with different concentrations of nitroxide and guest, and at different temperatures, it is possible to determine values of the equilibrium constant, the rate constants for binding and dissociation, the (standard) enthalpy change of the complexation process, and the activation energies.

The symmetrical nitroxide (**3**) can bind with two cyclodextrin molecules to form a 2:1 host–guest complex. The observed value of the hyperfine coupling to the N atom is consistent with low exposure of the N–O group to solvent, as expected for a complex in which the group is protected on both sides by cyclodextrin molecules.