SPIN PROBES FOR BINDING SITE POLARITY

G.H.DODD, M.D.BARRATT and L.RAYNER
Biophysics Division, Unilever Research Laboratory Colworth/Welwyn, The Frythe, Welwyn, Herts, England

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1. Introduction

Non covalently-bound spin probes provide a means of investigating the dynamic aspects of membrane structures and related systems [1–3]. They give information on membrane conformation which is complementary to that furnished by the spin-labelling approach [4]. For all these experiments it is important to determine the environment of the probe in the membrane.

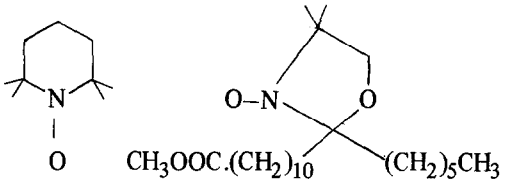
The hyperfine coupling constants, a_N, of most free radicals which contain heteroatoms, are solvent-dependent [5–7]. We have studied this solvent-dependence for selected nitroxide probes. Our results show that a quantitative estimation of the microscopic polarity of binding sites may be obtained from the magnitude of the coupling constants.

2. Experimental

2.1. *Materials*

Methanol, ethanol, 1-propanol, 2-propanol, and 1-butanol (Puriss-grade) were obtained from Fluka, A.G., Basle. Acetonitrile (dry and redistilled), pyridine (dry and redistilled), and isooctane (spectroscopic) were obtained from Hopkin and Williams, Ltd. Dimethylsulphoxide, dichloromethane, chloroform (A.R.), ethylene glycol and sodium dodecyl sulphate were obtained from B.D.H. Ltd., Acetone (dried over Al_2O_3) and dimethylformamide were obtained from May and Baker, Ltd. Egg lecithin and lysolecithin were obtained from Koch-Light, Ltd. Water was distilled and deionised.

The spin probes used in this work were (I),



2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and (II) the 4,4-dimethyloxazolidine-3-oxyl derivative of methyl 12-ketostearate. These were prepared by known methods [8]. 2,2,6,6-Tetramethylpiperidine was oxidised by H_2O_2/sodium tungstate reagent.

2.2. *Methods*

ESR spectra were obtained using a Varian V-4502 X-band spectrometer. Solutions of the probes were made at concentrations of 10^{-3} M and 3×10^{-4} M for probes I and II respectively. Hyperfine couplings were measured between the low-field and the centre lines to within ± 0.05 gauss against standards of the 2,4-dinitrophenylhydrazone of 2,2,6,6-tetramethyl-4-piperidone nitroxide in water and dodecane (16.16 gauss and 14.30 gauss respectively) [9]. All measurements were carried out at room temperature.

3. Results and discussion

Spectroscopic reporter groups using both absorption and fluorescence in the visible range have been used for evaluating the chemical and physical properties of binding sites [10–13]. A variety of empirical solvent polarity scales, based on electronic transitions or linear free energy relationships, are more appropriate for estimating the polarity of

binding sites at the molecular level, than is the bulk dielectric constant [14–16]. For our membrane work we have selected the probes shown as above. TEMPO (I) is soluble in both aqueous and hydrocarbon solvents and distributes itself between the aqueous and hydrocarbon regions of membrane systems [2]. The lipid probe (II) is very insoluble in water and would be expected to be a probe for hydrophobic binding sites.

The hyperfine coupling constants of the probes dissolved in various solvents plotted as a function of the solvent Z value [16] are shown in fig. 1. With the exceptions of chloroform and dichloromethane, a satisfactory linear correlation is found. The reason for the anomalous values given by the halogenated solvents is not clear at this time. The dipolar aprotic solvents correlate well in contrast to what is found with fluorescent probes [16]. The solvent dependence of a_N is usually interpreted as being due to a change of the spin density at the nitrogen nucleus caused by interaction between the solvent and the N-O bond. The difference in a_N values between five- and six-membered nitroxide rings arises from the different bond angles found in the preferred conformations of the molecules.

In our experiments on model membrane systems we have added the lipid probe (II-10^{-4} M) to aqueous dispersions of egg lecithin (1%), lysolecithin (0.5%) and sodium dodecyl sulphate (SDS) (0.5%). The hyperfine couplings in these systems were 14.25, 14.6 and 15.4 gauss respectively. The calculated reorientational correlation times (τ_c) in these systems were 1.1×10^{-9} sec, 1.05×10^{-9} sec and 3×10^{-10} sec, respectively, indicating that although the motion of the probe was restricted compared to a probe solution in a hydrocarbon solvent (e.g. $\tau_c = 4 \times 10^{-11}$ sec

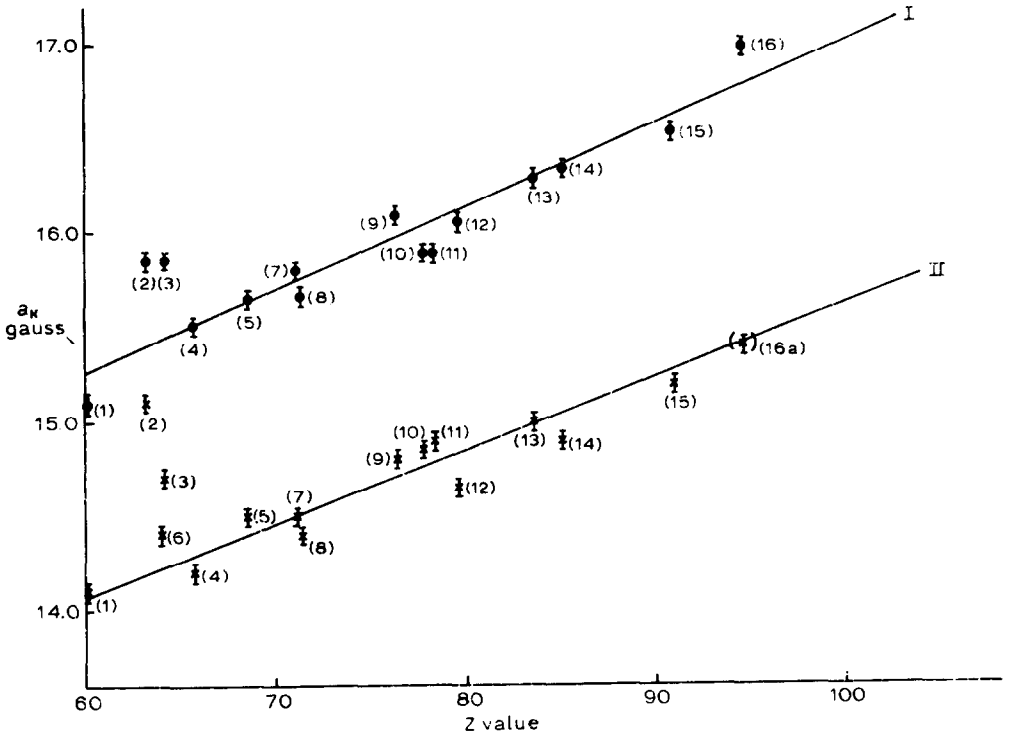


Fig. 1. a_N vs. Z value for nitroxide probes in different solvents. •, probe I; x probe II.
Solvents: (1) isooctane (2) chloroform (3) dichloromethane (4) acetone (5) dimethylformamide (6) pyridine (7) dimethylsulphoxide (8) acetonitrile (9) 2-propanol (10) 1-butanol (11) 1-propanol (12) ethanol (13) methanol (14) ethylene glycol (15) methanol-water (1:1) (16) water (16a) 0.5% SDS in water.

in hexadecane), nevertheless it was still rotating freely.

For egg lecithin in water, the environment of the nitroxide probe (II) has a Z value of 65.0 (derived from a_N). This figure tends to confirm the view that the nitroxide ring is located in the alkyl chain region of the lecithin as has been suggested elsewhere [8].

For lysolecithin in water, the hyperfine coupling of II (14.6 gauss) leads to a Z value of 74.0 which is considerably more polar than that obtained for egg lecithin. Linewidth measurements on high resolution N.M.R. spectra of lysolecithin solutions in D_2O which contain varying concentrations of this spin probe show a considerably greater paramagnetic broadening of the alkyl chain resonances than of the choline resonances [17]. This suggests that the probe is still located in the akyl chain region of the lysolecithin. The higher Z value may be due to the micellar structure of the lysolecithin allowing water molecules to penetrate rather more deeply than is possible in the lecithin bilayer.

The hyperfine coupling of lipid probe, II, in SDS in water (15.4 gauss) corresponds to a Z value of 94.1. This is so close to the value of 94.3 for water, quoted by Kosower [16], that we must conclude that the nitroxide group is in fact located in the water. (We have used the hyperfine coupling of the probe in SDS as the value for water on our graph. Direct measurement was not possible due to the low solubility of the probe in water). The location of the probe in the SDS micelle is shown in fig. 2. It appears that the 18-carbon probe has difficulty in dissolving in a 12-carbon detergent micelle. The energetically unfavourable interaction of the hydrocarbon chains with water is minimised by the probe folding about carbon-12 and lying in the micelle with the nitroxide ring protruding into the water.

These results demonstrate the need for careful evaluation of the location of the nitroxide ring in investigating the structure of model membranes and micelles by the nitroxide probe technique. They also suggest that the spin probe method may be a useful adjunct to the fluorescent reporter group technique for estimating the polarity of membrane sites or enzyme active sites.

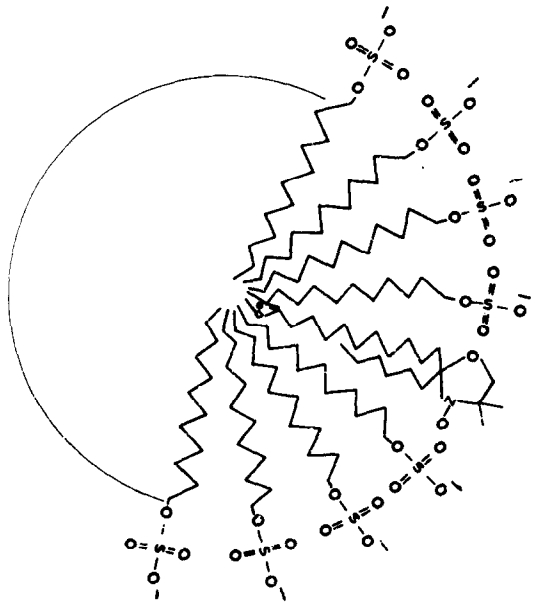


Fig. 2. Diagrammatic representation of the location of spin probe II in the SDS micelle.

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