EFFECTS OF MULTIPLE BINDING SITES ON STUDIES OF HYDROGEN BONDING BETWEEN NITROXIDE RADICALS AND SOLVENT MOLECULES

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Dedicated to Professor Václav Horák on the occasion of his 70th birthday.

The role of more than one binding site on a nitroxide free radical in magnetic resonance determinations of the properties of the complex formed with a hydrogen donor is examined. The expression that relates observed hyperfine couplings in EPR spectra to complex formation constants and concentrations of each species in solution becomes much more complex when multiple binding sites are present, but reduces to a simpler form when binding at the two sites occurs independently and the binding at the non-nitroxide site does not produce significant differences in the hyperfine coupling constant in the complexed radical. Effects on studies of hydrogen bonding between multiple binding site nitroxides and hydrogen donor solvent molecules by other magnetic resonance methods are potentially more extreme.

Stable nitroxide radicals are important for use as probes of intermolecular interactions with solvent species. Several magnetic resonance-based techniques have been used to characterize the dynamics of transient complex formation between nitroxide radicals and hydrogen donor solvents[1-3]. Important aspects of the use of EPR measurements to determine association constants for the hydrogen-bonded complex have been characterized, including the effects of the polarity of the medium[4], of the acidities of partially fluorinated donors[5], of the role of the inert cosolvent[6], and of donor self-association[7].

A question that arises concerning the use of nitroxide radicals in these studies involves the influence that the structure of the rest of the nitroxide species (other than the N–O moiety) may have on the interaction and on the parameters obtained from measuring the change in observed hyperfine coupling constant with solvent composition. This consideration should be especially important when the radical has more than one potential binding site. This report will examine the effects that arise because of differences in nitroxide probe structure and will explore the role of multiple binding sites in determining observables in magnetic resonance experiments that examine intermolecular interactions between solvent species and nitroxide spin probes.

EXPERIMENTAL

Three different nitroxide free radicals were employed in this comparison. They are di-tert-butyl nitroxide (DTBN), 2,2,6,6-tetramethylpiperidin-oxyl (TMPN), and 4-oxo-2,2,6,6-tetramethylpiperidin-oxyl (TMPO or often TEMPO). Sample preparation and recovery of EPR data followed the same procedure described previously[5].

RESULTS AND DISCUSSION

The hyperfine splittings for the three radicals were measured in both neat benzene and neat carbon tetrachloride. The observed hyperfine coupling constants and the dipole moments for the radicals are listed in Table I. The hyperfine coupling constants for the three nitroxide free radicals in the same inert solvent are in the order a_R(TMPN) > a_R(DTBN) >> a_R(TMPO), the same sequence as is observed for the dipole moments of these three free radicals[8]. Because the unpaired electron in a nitroxide radical is localized on the N–O group[9,10], the hyperfine coupling constant depends on the fraction of the unpaired electron spin density that resides on the ^{14}N atom in the nitroxide, which in turn depends linearly on the reaction field E_R (ref.[11]). This, in turn, is directly proportional to the dipole moment of the solute[12,13], as shown in Eq. (*1*).

$$\Delta a_{obs} = Q_1 \Delta\rho_N + Q_2 \Delta\rho_O = \text{constant}\ (Q_1 - Q_2)\ \mu\ (\varepsilon - 1)/(\varepsilon + 1) \qquad (1)$$

Here, the change in observed hyperfine coupling constant of the nitroxide radical is related to the change in electron spin density on the nitrogen and oxygen atoms, $\Delta\rho_N$ and $\Delta\rho_O$, respectively, which are equal but opposite in sign, the dipole moment of the radical, μ, and the dielectric constant of the solvent, ε. The use of this equation implies that, when a free radical is dissolved in different inert solvents, the dielectric constant of the solvent varies but the dipole moment of the radical remains constant.

The largest change occurs for different nitroxide radicals in the same solvent, showing the dominance of the nitroxide dipole moment in determining the observed hyperfine splitting. The unpaired electron spin density on the ^{14}N atom and conse-

TABLE I
The hyperfine coupling constant a_R for the nitroxide radicals TMPO, DTBN, and TMPN in neat C_6H_6 and CCl_4 and their dipole moments

Radical	a_R (C_6H_6), G	a_R (CCl_4), G	μ, D
TMPO	14.625[a]	14.500[a]	1.36[b]
DTBN	15.550	15.500	3.00
TMPN	15.750	15.600	3.14

[a] All observed hyperfine coupling constants have a probable error of ± 0.025; [b] values are from ref.[8].

quently the observed hyperfine coupling constant are proportional to the reaction field, and the observed hyperfine splittings are larger for the radical with the larger value of the dipole moment. The hyperfine coupling constant for each of the three free radicals in CCl_4 is slightly smaller than the corresponding value in C_6H_6, which is consistent with the change in polarity of the cosolvent, as has been observed in a previous paper[4]. The dielectric constants for C_6H_6 and CCl_4 are nearly equal, being 2.284 and 2.238, respectively[14]. The changes in hyperfine coupling constant for each radical with solvent is consistent with the change in $(\varepsilon - 1)/(\varepsilon + 1)$ which varies from 0.391 to 0.371, particularly given the uncertainty in determining the observed hyperfine coupling constants.

The analysis of data used to characterize nitroxide–hydrogen donor interactions by magnetic resonance methods has focused on the site of the potential hydrogen bond formation as being the nitroxide N–O group. However, for some nitroxide radicals, a second potential binding site exists, and the potential effects of binding at this second site can be examined. On the TMPO radical, hydrogen bond formation may occur to the nitroxide N–O group and to the keto oxygen (labelled O') on the other side of the radical O'--------N–O. The last molecule can be represented simply as R.

The simple expression used to determine the observed hyperfine coupling constant when only one potential bonding site is present is given in Eq. (*2*).

$$a_{obs} = X_R a_R + X_{RD} a_{RD} \qquad (2)$$

X_R and X_{RD} represent the mole fractions of radical free in solution and tied up in a complex with the hydrogen donor, respectively. These mole fractions can be written in terms of the concentrations of the corresponding species, and the equation rewritten in terms of the formation constant for the complex and the initial concentration of the donor species when only one binding site is assumed to be present to give Eq. (*3*).

$$a_{obs} = a_{RD} - (a_{obs} - a_R)/(K_1[D]) \qquad (3)$$

The equilibrium involved is R + D $\rightleftharpoons$ RD (RD is O'--------N–O · · · HO–R), for which the association constant is $K_1 = [RD]/([R][D])$. K_1 has been used in place of K_{assoc} as the association constant for the 1 : 1 complex between the nitroxide site on the free radical and the H-atom on the hydrogen donor to allow association constants for complexes formed at the other site to be distinguished. The initial concentration of the donor $[D]_0$ greatly exceeds the initial concentration of radical so that $[D] \cong [D]_0$. A plot of a_{obs} versus $(a_{obs} - a_R)/[D]_0$ should give a straight line with an intercept of a_{RD} and a slope of $1/K_1$.

The corresponding equilibria for binding at the other site, either with the unassociated nitroxide (a formation of RD' i.e. R–OH · · · O'--------N–O) or a nitroxide already associated with a donor molecule at the nitroxide site (a formation of RDD' i.e. R–OH · · · O'--------N–O · · · H–OR), are given by R + D $\rightleftharpoons$ RD' for which $K_1' =$

[RD']/([R][D]) and RD' + D $\rightleftharpoons$ RDD' for which K_2 = [RDD']/([RD'][D]). Then RD + D $\rightleftharpoons$ RDD' gives K_2' = [RDD']/([RD][D]), for which relation $K_2' = K_2K_1'/K_1$ can be obtained.

The expression for the observed hyperfine coupling constant given in Eq. (*2*) can be modified by including a term involving the mole fraction of each of the four ways that radical species can exist in solution, which are (i) as uncomplexed radical, X_R, (ii) as radical involved in a complex only at the nitroxide end, X_{RD}, (iii) as radical involved in a complex only at the keto oxygen end, $X_{RD'}$, and (iv) as radical involved in a complex at both ends, $X_{RDD'}$, as shown in Eq. (*4*).

$$a_{obs} = X_R a_R + X_{RD} a_{RD} + X_{RD'} a_{RD'} + X_{RDD'} a_{RDD'} \quad (4)$$

These expressions can be used with the expression for the fraction of the radical free in solution, X_R = [R]/([R] + [RD] + [RD'] + [RDD']) and Eq. (*4*) to give Eq. (*5*).

$$a_{obs}([RD] + [RDD']) = a_{RD}[RD] + a_{RDD'}[RDD'] + (a_R - a_{obs})[R] + (a_{RD'} - a_{obs})[RD'] \quad (5)$$

This equation is rearranged by dividing through by ([RD] + [RDD']) and by using the association constants to obtain the expression given in Eq. (*6*).

$$a_{obs} = \{(a_{RD} + a_{RDD'}K_2'[D] - (a_{obs} - a_R)/(K_1[D]) - (a_{obs} - a_{RD'})(K_1'/K_1)\}/Q_D , \quad (6)$$

where $Q_D = (1 + [D]K_2')$. This expression gives a much more complex dependence of a_{obs} on $[D]_0$ (as before, $[D] \approx [D]_0$ because $[D]_0 >> [R]_0$.) This rearrangement of Eq. (*4*) is chosen to give Eq. (*6*) parallel to Eq. (*3*) and indicates that a plot of a_{obs} versus $(a_{obs} - a_R)/[D]$ should no longer give a straight line, as the "intercept" is no longer simply a_{RD} but depends on [D] as $a_{RD} + a_{RDD'}K_2'[D]$, and the "slope" is no longer simply $1/K_1$. Further, the term that multiplies the entire right hand side of Eq. (*6*), Q_D, also depends on [D].

The applicability of Eq. (*6*) can be examined by using the data for CF_3CH_2OH–TMPO samples in C_6H_6, as shown in Fig. 1. Also shown is the best fit of these data obtained by using Eq. (*3*) for which a_{RD} = 15.89 and K_1 = 1.17 (ref.[4]). (In these plots the complication introduced by the variation in dielectric constant with a change in the ratio of the two cosolvents C_6H_6 and CF_3CH_2OH described in a previous paper[4] has been removed.) The other points shown in the figure have been calculated by using Eq. (*6*) with the values of the parameters shown in the figure caption. The high quality of the fit of the experimental points obtained for the simple form of Eq. (*3*) is in direct contrast to the clearly non-linear result obtained from Eq. (*6*). Though the degree of deviation from a straight line is exaggerated by the choice of values for the association constants and hyperfine coupling constants for RD' and RDD' for the sake of producing an easily discernible plot, values of these parameters more nearly in line with what might be expected based on the better established values for these types of donor–radi-

cal systems[4,5,15,16] still produce a noticeable deviation from linearity. Linear plots can be obtained by realizing that the more complex expression given in Eq. (*6*) reduces to the simpler form given in Eq. (*3*) under two sets of circumstances. The first is if K_1' and $K_2' = 0$. In that case no binding occurs at the keto site, so the situation is exactly analogous to the case where only one binding site is possible. But, though binding to the keto site would be expected to be reduced compared to binding at the nitroxide site because the bond dipole moment for the N–O group (4 D, refs[17,18]) is larger than that of the C=O group (≅ 2.4 D, ref.[8]), it should not be zero. In the other case, if $a_{RD} = a_{RDD'}$, $a_R = a_{RD'}$, and $K_1 = K_2$ (which implies that $K_1' = K_2'$), then Eq. (*6*) reduced identically to Eq. (*3*). In other words, if the binding to the second site occurs without changing the hyperfine coupling constant and the probability of binding at each of the two sites is independent of binding at the other, there is no effect on a_{obs}.

The result is that the observation that straight line plots, which have been obtained with correlation coefficients greater than 0.9986 for the solvent–radical pairs given in Table 4 of the first paper in this series[4] and greater than 0.994 for the fluorinated donors given in Table 2 of the second paper[5], do not necessarily indicate that binding does not occur at the keto site of the TMPO radical. Though one possible cause is that no binding occurs at the second site, an equally acceptable explanation is that the hyperfine coupling constant does not change when binding occurs at the distant site and the binding at the two sites remains independent. If $a_{RD'} = a_R$ and $a_{RD} = a_{RDD'}$, and $K_1' = K_2'$ the effect on a_{obs} vanishes and plots according to Eq. (*3*) can be used to obtain a_{RD} and K_1 without complications.

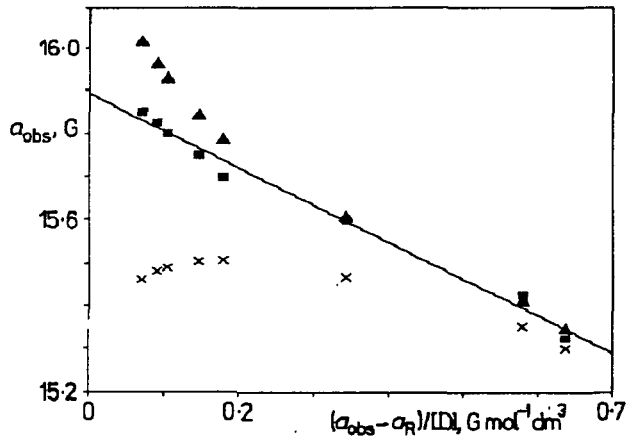

FIG. 1

Plot of a_{obs} vs $(a_{obs} - a_R)/[D]$ for: experimental data for CF_3CH_2OH : TMPO samples (■); sample data generated by using Eq. (*6*) with $K_1 = 1.17$, $K_2 = 0.4$, $K_1' = 0.2$, $a_R = 14.388$, $a_{RD} = 15.889$, $a_{RD'} = 15.139$, and $a_{RDD'} = 16.55$ (▲); sample data generated by using Eq. (*6*) with $K_1 = 1.17$, $K_2 = 0.4$, $K_1' = 0.2$, $a_R = 14.388$, $a_{RD} = 15.889$, $a_{RD'} = 15.139$, and $a_{RDD'} = 15.2$ (×). The straight line is the best fit to Eq. (*3*) of the experimental data with $a_R = 14.388$ and $K_1 = 1.17$. All values of K_i are in mol^{-1} dm^3 and a_i in G

Other magnetic resonance techniques that can be used to examine transient complex formation between nitroxide radicals and hydrogen donors may not be immune to the effects of possible complex formation at the keto site in TMPO under the same circumstances where the EPR measurements are unchanged. Though low field dynamic nuclear polarization studies have been shown to be insensitive to small changes in the structure of the nitroxide probe[19], changes in the correlation time that result from the hindered tumbling of the larger complex may shift the position of the fall-off region at higher magnetic fields and given changes in the observed enhancements. And changes in these rotational correlation times can shift the balance between the relaxation governed by the translational diffusion of the interacting spins versus that governed by the rotational tumbling of the complex formed[20]. In these cases, the added effects caused by hydrogen bond formation to the second site may produce complications that are difficult to extract. But in the current study, the effects of multiple complexation sites on the determination of association constants by using observed hyperfine coupling constants does not seem to be a major factor when the hyperfine coupling constant is unchanged by binding at the second site and the probability of binding at one site is not changed by binding at the other site.

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