A New Electron Spin Resonance Spectrum for an Exchange- and Dipole-Dipole coupled Superoxocobalamin ··· Free-radical Pair occurring in Adenosylcobalamin-containing Systems

Abbas Pezeshk* and Robert E. Coffman
Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242, U.S.A.

Laser-induced photolysis of highly purified, polycrystalline adenosylcobalamin generates, under anaerobic conditions, an e.s.r. spectrum characteristic of a strongly exchange-coupled Co^{II} ··· free-radical pair. On exposure to oxygen, a new spectrum appears, concurrent with the disappearance of the previous spectrum. The band structure of the new spectrum is that of a triplet, and can be shown to be the result of exchange and dipole–dipole coupling between two unpaired spins separated by a distance of *ca.* 8.7 Å. We suggest that the new biradical state is a $Co–O_2^-$ ··· $R^•$ pair ($R^•$ = adenosyl radical).

The cobalt–carbon bond in adenosylcobalamin cleaves, under the influence of applied radiation or due to thermal effects when enzyme bound, by a homolytic mechanism leading to the generation of either Co^{II} ··· $R^•$ (radical) pairs or $R^•$ ···· $R^•$ pairs. We recently have reported that when highly polycrystalline adenosylcobalamin is partially dehydrated (by application of a vacuum) and then irradiated with a He/Ne laser, a new spectrum resembling a stabilized intermediate between stable adenosylcobalamin and separate cob(II)alamin and free radical is obtained.[1] In a recent series of experiments, we found that the new complex is reactive to gaseous oxygen, and that another new type of spectrum is obtained. The analysis of the data, guided by a general program which calculates the rigid-lattice e.s.r. lineshapes to be expected for any given set of *g* tensors, rotation angles of the second *g* tensor relative to the first, and the relative 'magnetic geometry' of two free-radical molecules,[2] showed clearly that the new spectrum is that of a radical pair derived from the original 'exchange-coupled' pair, and that it has a unique structure as well as a unique set of *g* values which are of interest because of their unusual properties.

Experimental

Samples of adenosylcobalamin were purchased from Sigma and purified by two-stage gel filtration as reported earlier.[1]

The samples of pure adenosylcobalamin were prepared in conventional e.s.r. tubes, to which was attached a graded seal so that the tube could be removed from the vacuum line while still under vacuum. The samples were partially dehydrated by evacuation at room temperature for 15—30 min. They were then irradiated for *ca.* 15 min with a Metrologic ML855 He/Ne laser which produces about 5—8 mW of power at λ = 632.8 nm. The resulting e.s.r. spectra were then studied. The effect of oxygen on the spectrum was observed by gradually introducing air into the tube while stored in a freezer overnight and then pumping off the oxygen with a vacuum system. All operations were carried out under subdued light.

Results and Discussion

When highly purified, anaerobic polycrystalline adenosylcobalamin (prepared as above) is irradiated with a low-power He/Ne laser, the previously reported[1] spectrum was obtained, Figure 1(*a*). This spectrum exhibits features assigned to cob(II)alamin, a signal at g = 2.006 assigned to adenosyl radical ($R^•$) and a broad signal at g = 2.119 assigned to a strongly exchanged-coupled system, which is held together by lattice forces. The relatively small high-field lines were not assigned. A similar spectrum could only be calculated with our simulation program by assuming that the distance between the respective molecules, each bearing an unpaired spin, is *ca.* 5.0 Å. (The geometrical co-ordinates obtained in this case are tentative; a separate experimental and theoretical study of this lineshape is planned.)

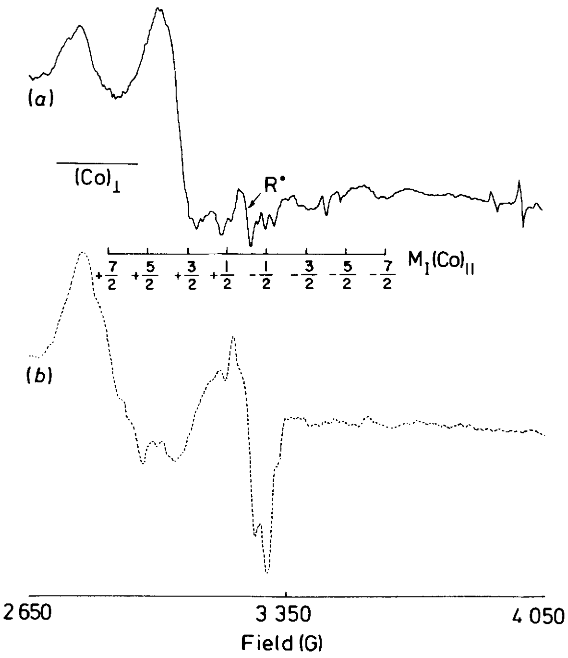

Figure 1. E. s. r. spectra of photolyzed polycrystalline adenosylcobalamin at 77 K: (*a*) showing features of cob(II)alamin, adenosyl radical ($R^•$), and the exchange-coupled cob(II)alamin and adenosyl radical at g = 2.119; (*b*) after exposure to oxygen showing a new signal at g = *ca.* 2.0

When the sample is exposed to oxygen, the e.s.r. signal interpreted as an exchange-coupled Co^{II} and the radical $R^•$ at g = 2.119 is replaced by a signal at g = *ca.* 2.0, Figure 1(*b*). The new signal at g = *ca.* 2.0 is expanded and illustrated in Figure 2(*a*). This spectrum could only be simulated by carefully examining the background, and then doing a background subtraction. Although there appear to be five peaks in the

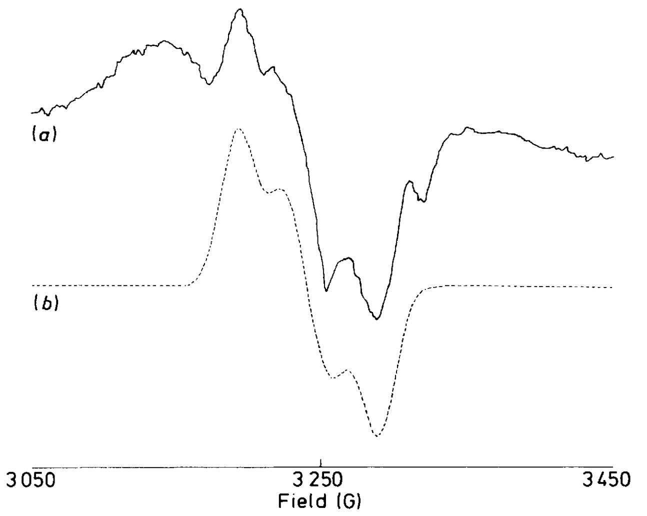

Figure 2. (*a*) E.s.r. spectrum of the exchange-coupled superoxocobalamin and adenosyl radical recorded at 77 K. (*b*) Computed best fit with parameter values as given in the Table

derivative spectrum, both the low-field and high-field peaks are part of the cobalt parallel features, a fact which is easily verified by pumping off all of the oxygen. In this case, the new component disappears, leaving behind the indicated low- and high-field peaks.

Simulation of this spectrum by searching for a reasonable set of g-tensor components and relative geometry factors (the distance R, and the geometric angles ζ and η; see Figure 3) showed that an approximate solution could be obtained for a distance (between centres of unpaired spins) of *ca.* 8.7 Å and for relative angles of the order of the magic angle values, namely $\zeta = 54.74$ and $\eta = 45.0°$. Computer simulation of this spectrum is shown in Figure 2(*b*), and the e.s.r. data are summarized in the Table. We refer to this spectrum as a 'triplet,' as it superficially resembles the derivative of an absorption lineshape with three overlapping peaks.

The spin-Hamiltonian used in our analysis is given by equation (1), where g_1 is the diagonal g tensor for spin system 1,

$$\mathscr{H} = \beta\vec{B}\cdot g_1\cdot\vec{S_1} + \beta\vec{B}\cdot g_2\cdot\vec{S_2} + \mathscr{H}_{dd} + \mathscr{H}_{ex} \qquad (1)$$

and g_2 is the non-diagonal g tensor for spin system 2. The magnetic dipole–dipole and exchange operators are assumed to have the simple forms given by equations (2) and (3) respectively.

$$\mathscr{H}_{dd} = \sum_{i,j=1}^{3,3} J_{ij}^{dd}\hat{S}_{1i}\hat{S}_{2j} \qquad (2)$$

$$\mathscr{H}_{ex} = J_0(R)\vec{S_1}\cdot\vec{S_2} \qquad (3)$$

The dependence of J_0 on R, $J_0(R)$, was chosen as the limit function value.[3] As is well known, the exchange interaction operator is biquadratic in the spin operators $\vec{S_1}$ and $\vec{S_2}$. We have purposely dropped the vector terms ('Dzialoshinsky–Moriya'[4,5] or antisymmetric) and symmetric, traceless tensor terms (pseudo-dipolar) in order to simplify the analysis, and because these additional terms are expected to be small when one member of the free radical pair is a carbon-based free radical with only a small amount of angular momentum involved in the wavefunction. The general lineshape calculation which we perform is similar to that in our previous paper.[2]

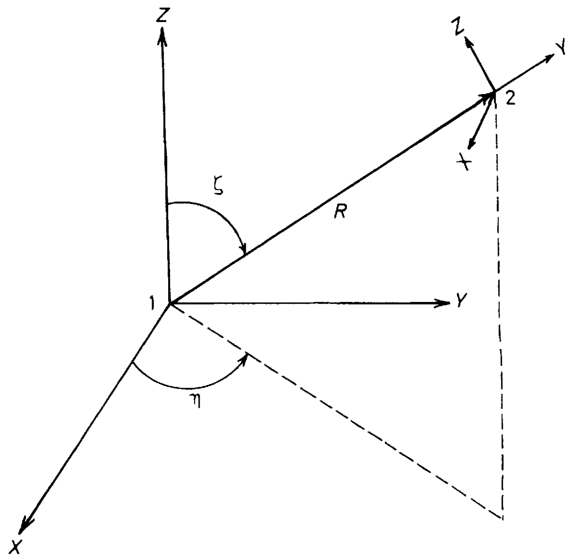

Figure 3. Co-ordinate system used to define angles ζ and η and distance R. The X, Y, and Z axes are parallel to g_{xx}, g_{yy}, and g_{zz} of spin system 1

Table. Parameters for the computer best fit

Superoxocobalamin g values	$g_x = 1.980$ $g_y = 2.01$ $g_z = 2.05$
Radical g values	$g_x = g_y = 2.005$ $g_z = 1.990$
Dipolar parameters[a]	$R = 8.70$ Å $\zeta = 54.74°$ $\eta = 45.0°$
Linewidth	$\Delta H_{pp} = 20.0$ G[b]

[a] Tentative estimates. [b] G = 10^{-4} T.

The chemical identification of the species giving rise to the new spectrum of Figure 2(*a*) is the result of the initial set of experimental data (leading to the discovery reported here) and the programs used for the spin–spin lineshape analysis. Refinement of the parameters of the Table is planned, which will require e.s.r. spectra at two or three microwave frequencies and at liquid helium or higher (*i.e.* 4—77 K) temperatures. These spectra will then be fitted to our lineshape programs using non-linear least-squares techniques. The parameter values reported in the Table are thus labelled 'tentative,' since the final set of these values will surely be quantitatively different from our initial set of values.

According to our results, the homolytic cleavage of the Co–C bond leads to the appearance of an activated intermediate which we interpret as a strong exchange-coupled state between cobalt and adenosyl radical at $g = 2.119$. When oxygen is admitted to the e.s.r. tube, the resulting long-lived triplet state reacts with O_2 to produce a new signal, with a g value of *ca.* 2.0. We propose the mechanism of Figure 4 to account for these observations.

It is known that molecular oxygen binds reversibly both to cobalt(II) porphyrin[6] and vitamin B_{12r}.[7] Therefore, we suggest that the oxygen molecule binds to cob(II)alamin and forms a superoxide radical bound to the cobalt atom. As a result, the adenosyl radical, R$^{\bullet}$, is pushed away by O_2, and is presumably trapped by hydrogen bonds to the lattice in a pocket at a longer distance with respect to the cobalt atom. We are, therefore,

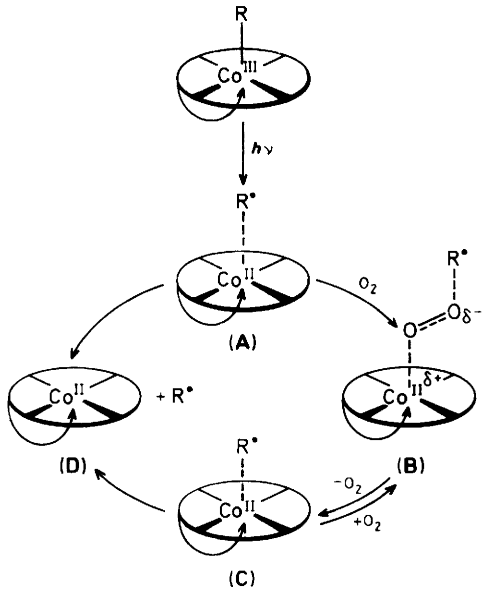

Figure 4. Scheme showing reaction intermediates for anaerobic photolysis of adenosylcobalamin and its reaction with oxygen; R• = adenosyl radical, X = H_2O

dealing with the interaction of the unpaired electron of the superoxide bound to cobalt and the unpaired electron of the radical R• [intermediate (**B**)]. This interaction will result in the formation of a new long-lived triplet state, *i.e.* $Co-O_2^- \cdots R^\bullet$ pair. When the sample tube is deaerated under vacuum, the new e.s.r. signal is replaced by a low-intensity signal at $g = 2.0$ which corresponds to the radical R• and the characteristic e.s.r. spectrum of cob(II)alamin which is left unchanged [intermediate (**C**)]. Moreover, our findings show that the oxygen molecule binds to the cobalt atom reversibly, as reported previously for the cobalt(II) porphyrin and vitamin B_{12r}. Intermediate (**C**) most likely converts to the intermediate (**D**) where the cobalt ion and the radical R• are far apart and are not interacting (Figure 4). We have also considered the possibility of formation of peroxyl radical, $RO_2^\bullet$, which could be exchange-coupled with another radical R•. This interaction is ruled out due to the fact that if an oxygen molecule binds to the radical R• to form $RO_2^\bullet$, the covalent bond formed is so strong that the oxygen cannot be removed under reduced pressure.

These results show that exchange-coupled complexes are formed in this system with distances between the radical pairs which are less than the value of *ca.* 9.0 Å found in the well known 'doublet' type of e.s.r. spectrum.[8] The doublet spectrum is characteristic of a weakly exchange-coupled spectrum ($J_0 \ll g\beta B$), while the type of spectrum observed for more tight binding is characteristic of a high exchange frequency ($J_0 \gg g\beta B$).

Our interest in this spectrum is also stimulated by the observation of a similar spectrum recently reported by Rao and Symons,[9] which was discovered in a methylcobalamin system. This spectrum may also be a triplet, but it is possibly more complex. Our general analysis shows that in the strong-exchange domain, the randomly averaged derivative spectrum may contain up to six major peaks. This is easily understood, by realising that when the $S = 1$ triplet of spin states is well separated from the singlet $S = 0$ state, then there are two possibly allowed $\Delta M = 1$ transitions, which will have slightly different positions with respect to the external magnetic field, corresponding to the three X, Y, and Z turning points in the spherically averaged spectrum. So the triplet spectrum is merely a highly symmetrical case, in which the general six lines have collapsed to three. For truly short distances, the effect of relaxation on the spectrum is unknown (*e.g.* cross relaxation may be important), so it is not at all clear that our calculation is directly applicable in that case. This is a matter for further investigation, but it is interesting both that unusual spectra occur and that unusual quasi-stable complexes exist in this system. It is possible that similar quasi-stable complexes may be found in other related systems also. Evidently, lineshape simulation can contribute considerably to the understanding of such systems.

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