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NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF COBALT(II) PORPHYRIN-QUINONE COMPLEXES

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SUMMARY

Weak intermolecular complexes of cobalt(II) and nickel(II) mesoporphyrin dimethyl ester with 1,4-naphthoquinone, vitamin K-3, vitamin K-1 and ubiquinone 30 have been detected by ^{1}H-NMR spectroscopy in chloroform solutions. The relative orientation of the components of complexes is discussed.

INTRODUCTION

Respiration and photosynthesis both require highly organized electron transport chains for the absorption and transfer of energy. It has been suggested [1] that the efficiency of these chains may depend on the formation of molecular complexes between chlorophyll and the heme group of cytochrome on the one hand and quinone on the other. Molecular complex formation is often readily detected by the appearance of an intense "charge transfer" band in the ultraviolet or visible spectrum. However, such bands have been difficult to detect for 1 : 1 porphyrin complexes with both nitroarenes [2–4] and quinones [3–5]. The latter complexes are very weak and complex formation is accompanied by only a slight broadening and drop in intensity of the porphyrin absorption bands. Our initial observation [5], using NMR spectroscopy, indicated the usefulness of paramagnetic metal porphyrins both for detecting complex formation and obtaining structural [6–8] information in solution. We were interested therefore in applying these techniques to molecular complexes of quinones, perhaps the most biologically relevant example.

MATERIALS AND METHODS

Materials

Cobalt(II) and nickel(II) mesoporphyrin IX dimethyl esters were prepared [9] by insertion of the divalent ions into the metal-free porphyrin. 1,4-Naphthoquinine, vitamin K-3, ubiquinone, ex-baker's yeast, were obtained from Koch-Light Ltd., and

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vitamin K-1 from Mann Research Laboratories Inc. All quinones were protected from light as much as possible.

Methods

^{1}H-NMR spectra of naphthoquinone and vitamin K-3 were recorded on a JOEL JNM-4H-100 spectrometer at 100 MHz at 33 °C. All other spectra were recorded on a JEOL-C-60HL instrument at 60 MHz at 25 °C. A standard quinone solution (A), in C^2HCl_3 containing about 1 % tetramethylsilane was prepared for the titrations at constant quinone concentration and its spectrum recorded. The porphyrin was weighed into another standard flask and made up to the mark with solution A. The spectrum of this solution was also recorded. A series of additions was then made up to 0.5 ml of both A and B in two separate NMR tubes to obtain a set of spectra at increasing porphyrin concentrations. Oxygen-free nitrogen was bubbled through the C^2HCl_3 before use. Electron paramagnetic resonance spectra were recorded on a JEOL JES-3BSX instrument at about 9.15 GHz at −150 °C. The required amounts of porphyrin and quinone were weighed into a special vacuum EPR cell fitted with a Thunberg head. Analar $CHCl_3$ (1 ml) was degassed by repeated freezing, evacuation and thawing and was then distilled on to the sample.

RESULTS AND DISCUSSION

We have previously shown [4] that a weak 1 : 1 molecular complex is formed between Co(II) mesoporphyrin IX dimethyl esters and vitamin K-3, with a solvent-dependent formation constant which varies between 3.8 ± 4 $(\mathrm{l} \cdot \mathrm{mol}^{-1})$ in dichloromethane to 16 ± 5 $(\mathrm{l} \cdot \mathrm{mol}^{-1})$ in diethyl ether. Complex formation between Co(II) mesoporphyrin IX dimethyl esters and vitamin K-3 is reflected in a dependence of the chemical shifts of all resonances in the ^{1}H-NMR spectrum [10] of the latter on the presence of the former, Fig. 1. All resonances are shifted to higher field and broadened.

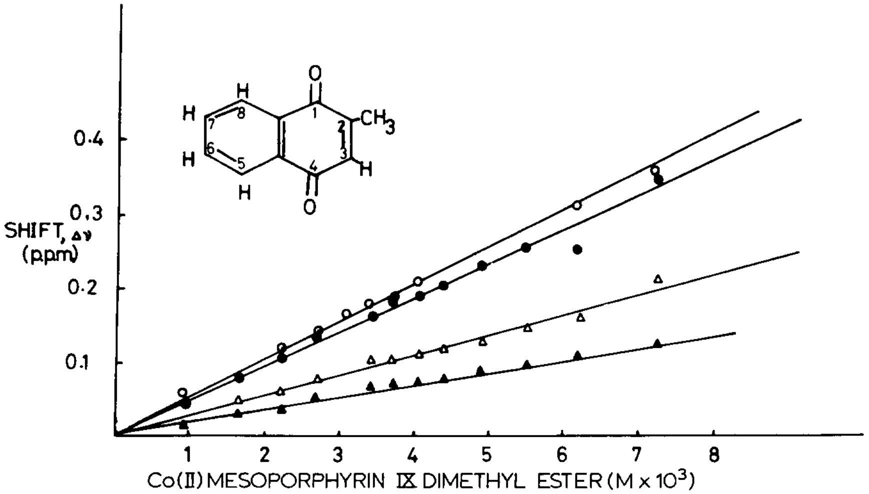

Fig. 1. The shifts of the ^{1}H resonances of vitamin K-3 (0.06 M) with increasing concentration of Co(II) mesoporphyrin IX dimethyl ester in C^2HCl_3. ○, $H_{5,8}$ and ●, $H_{6,7}$, △, H_3, ▲, $H_{2\text{-}CH_3}$.

The shifts in the presence of an equivalent concentration of Ni(II) mesoporphyrin IX dimethyl esters are less than 10 % of those in the presence of Co(II) mesoporphyrin IX dimethyl esters. In previous publications [6–8] we have shown that the observed shifts are due to the dipolar interaction between the magnetic dipole centred on the metal ion and the magnetic dipole of each proton, with a negligible contribution from the shift associated with the porphyrin ring current. Provided it can be shown that the magnetic susceptibility tensor, or failing that the g tensor, has axial symmetry, then it is possible to derive "structures" in solution which would have paramagnetic shifts consistent with the observed shift ratios. The dipolar shift in such a case is given [11] by:

$$\Delta H_d/H_o = (\chi_{\parallel}-\chi_{\perp})\,(3\cos^2\Omega-1)/3r^3 \qquad (1)$$

where $\Delta H_d/H_o$ is the dipolar shift, $\chi_{\parallel}$ and $\chi_{\perp}$ are the parallel and perpendicular (with respect to an axis passing through the metal ion and perpendicular to the plane of the porphyrin; the approximate C_4 axis) components of the magnetic susceptibility tensor, Ω is the angle between the axis and the direction vector of length r, from the metal ion to the nucleus whose resonance is being observed. It is unnecessary to derive the shifts in the fully-formed complex since the ratios of the reciprocal gradients in Fig. 1 correspond [6] to the ratios of the shifts in the fully-formed complexes. The ratios with respect to a standard proton, i, are of the form: $\Delta H_j/\Delta H_i = [(3\cos^2\Omega_j-1)/r_j^3]/[(3\cos^2\Omega_i-1)/r_i^3]$. The geometrical factors are to be averaged over all motions which are rapid on the NMR time scale.

Axial symmetry is confirmed in the present case by the observation of the low-temperature EPR spectrum of the Co(II) mesoporphyrin IX dimethyl ester which in the presence of a twenty-fold excess of vitamin K-3, shows spectra of both complexed and uncomplexed Co(II) mesoporphyrin IX dimethyl ester ($5 \cdot 10^{-3}$ M), characterised by $g_{\parallel} = 2.0$, $g_{\perp} = 3.3$, for the former and $g_{\parallel} = 2.1$ and $g_{\perp} = 2.5$, for the latter. As in other examples [6–8], this is typical of a situation where aggregates of Co(II) mesoporphyrin IX dimethyl ester are broken up by the addition of molecules such as vitamin K-3, steroids and nitroarenes with the concomitant formation of molecular complexes.

The method [6] of calculating structures depends on varying the position and direction of the major axis centred on the cobalt with respect to vitamin K-3 and at chosen intervals, calculating $\Omega_{i,j}$ and $r_{i,j}$ and hence shift ratios. In the present case, the ratios, taking $H_{5,8}$ as 100, are $H_{5,8} : H_{6,7} : H_3 : H_{2\text{-}CH_3}$; 100 : 90 : 54 : 34. The search procedure is as follows: Coordinates [12] for the vitamin K-3 were provided with C4 centred at the origin i.e., x, y, z equal to 0, 0, 0, x being perpendicular to the yz plane of the quinone and z defined as the direction along the C4-C5 bond and H8 lies in the negative quadrant of both y and z. The determination of the x, y and z coordinates of the metal requires [6] a search over various distances, r, of the metal from C4 and of the four angles which define the magnetic symmetry axis and the angle of orientation of the quinone plane. Only solutions which agree with the observed ratios within a preset tolerance are acceptable. Initially a rough scan for solutions with high tolerances i.e., a 10 % error on all shift data, was undertaken and the search was made from $r = 5$ Å to $r = 7$ Å in steps of 0.5 Å and over all angles in increments of 10°. Within these tolerances, thirty-four solutions were obtained; at 5 Å, zero; 5.5 Å, zero; 6.0 Å, six; 6.5 Å, seventeen; 7.0 Å, eleven. As there were no solutions with r less

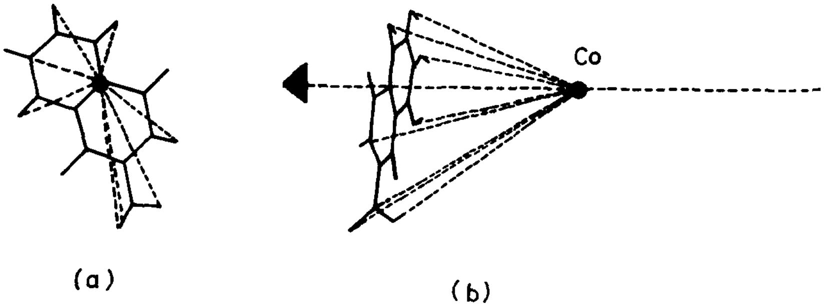

Fig. 2. Two views showing the orientation of vitamin K-3 with respect to the magnetic susceptibility axis which is assumed to be perpendicular to the plane of Co(II) mesoporphyrin IX dimethyl ester and pass through the cobalt(II). In (a) the view is along the symmetry axis; in (b) it is perpendicular to it.

than 5.5 Å, subsequent searches were restricted to 5.5 Å to 8.0 Å. The increments in the angles were altered to 5°, and the tolerances on the shift ratios reduced to 6 %. There were solutions with $r = 5.5$ Å, zero; 6.0 Å, four; 6.5 Å, twenty-seven; 7.0 Å, twenty-three; 7.5 Å, twenty-one; 8.0 Å, fourteen. The solutions fall into two families: both have the symmetry axis in the range from 70° to 90° i.e., "perpendicular" to the quinone plane. The metal atom is below the benzenoid rather than the quinoid ring e.g. centrum of coordinates of the families for $r = 7.0$ Å are $\pm x = 6.8$ Å; $y = +0.5$ Å; $z = -0.6$ Å. The families at different values of r are all similar. Fig. 2 shows the metal position and orientation of the axis with respect to vitamin K-3. The two families differ in that one is for $+x$ and the other for $-x$. The relationship of the metal to the quinone is not symmetrical about the quinone plane as the crystallographic data do not indicate an exactly planar vitamin K-3 structure. The family with the 3-CH_3 group tilted away from the metal is four times as heavily populated.

It must be emphasized that these structures are weighted averages of all those which interconvert on a time short compared to the NMR time scale. However, if there were no preferred orientations of the Co(II) mesoporphyrin IX dimethyl ester with respect to vitamin K-3, there would be no differential shifts, i.e. no dipolar shifts. As has been stated previously, the concept of a "structure in solution" is ill-defined, given the role of the solvent and the rapid thermal motion. The method used herein senses the dominant "correlation of orientations" which are thought [13] to be an important part of the structures of molecular liquids. A unique structure need not emerge from these and similar investigations. However, the following conclusions can be drawn from the results obtained: the planes of the porphyrin and the quinone are approximately parallel in the complex and the oxygen atoms of the quinone are not coordinated to the metal ion. Indeed the "structure" suggests that the interaction in the complex occurs between the porphyrin ring and the quinoid ring. Crystalline molecular complexes with *p*-benzoquinone as the "acceptor" display structures in which the carbonyl groups are situated over the centre of the benzene ring of the "donor", an arrangement which is thought to maximize the contribution of the polar carbonyl groups to dispersion forces [14]. A similar interaction could occur within the structure indicated in Fig. 2. Any interaction at such a large interplanar distance must be weak and indeed the formation constants of the complexes are very small.

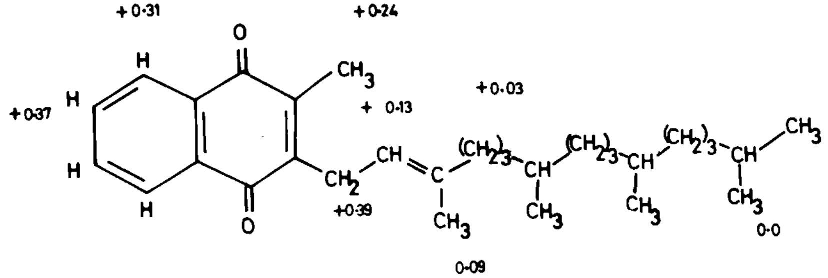

Fig. 3. The shifts induced in the ^{1}H-NMR spectrum of vitamin K-1 (0.065 M) by Co(II) mesoporphyrin IX dimethyl ester (0.012 M) in C^2HCl_3.

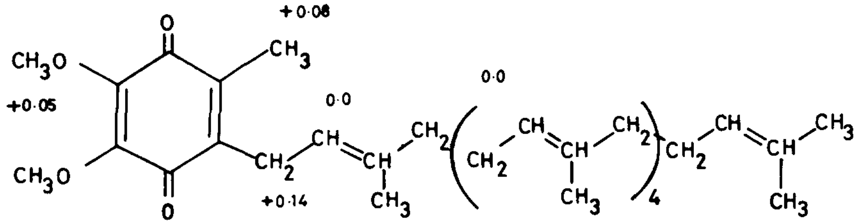

Fig. 4. The shifts induced in the ^{1}H-NMR spectrum of ubiquinone-30 (0.026 M) by Co(II) mesoporphyrin IX dimethyl ester (0.014 M) in C^2HCl_3.

These "structures" would not appear to be significantly influenced by the 2-CH_3 group since the shift ratios for 1,4-naphthoquinone are similar: $H_{5,8} : H_{6,7} : H_{2,3} = 100 : 94 : 48$. However, the ratios of the shifts [15], which are again linearly dependent on Co(II) mesoporphyrin IX dimethyl ester concentrations of vitamin K-1 (Fig. 3), are somewhat different with $H_{5,8} : H_{6,7} : H_{3CH_2} : H_{2CH_3} = 100 : 120 : 126 : 77$ which suggests that the long side chain does influence the porphyrin-quinone complexes with the metal moved slightly towards the quinone ring. The shifts of the resonances of the side-chain are small suggesting that it is extended away from the metal. The CH_2 group bound to the quinone ring in ubiquinone-30 (Fig. 4) appeared to be the nearest protons to the metal.

These results indicate that the structures of the complexes are similar with the quinonoid ring placed over the porphyrin and with the benzene ring closer to the metal and the chain, where present, extended away from the metal. The structures suggest that electron transfer between a quinone and the heme unit or chlorophyll could take place via the porphyrin (or chlorin) moiety since they are arranged in a suitable orientation. While the structures of complexes between these components are unknown in vivo, the first step of electron transfer in the photosynthetic unit [16] of bacteria may well be from a photo-activated chlorophyll pigment to a naphthaquinone.

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