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³¹P-NMR STUDIES OF COBALAMINS

³¹P-NMR observations of aquocobalamin (H₂OCbl) in H₂SO₄/H₂O mixtures show that both the base-on and base-off species may be observed and quantitated. Correlation of the base-off ³¹P-chemical shifts with a generalized acidity function [1] gives values of -1.59 and -0.09 for

the two macroscopic pK_a's for phosphodiester deprotonation, virtually identical to the values previously obtained for base-off CNCbl and CH₃Cbl (Table, [2]). Similar observations of several other base-off cobalamins (Table) show that all the base-off cobalamins have the same phosphodiester macroscopic pK_a's (-1.59 ± 0.02 and -0.04 ± 0.02) and the same chemical shifts for the phosphodiester protonated (-339.88 ± 3.80 Hz) and deprotonated (-36.70 ± 2.80) base-off species. However, the base-on species of these cobalamins have chemical shifts that vary (from -50.16 to +9.37 Hz) in a regular way with the apparent free energy of coordination of the dimethylbenzimidazole ligand (ΔG_{Co}, Table) so that Δδ_{31P} (the difference in chemical shift between the base-on and base-off species) is a linear function of -ΔG_{Co} (slope = 7.93 ± 0.40 Hz kcal⁻¹mol, intercept = -28.06 ± 1.90 Hz, correlation coefficient r² = 0.964). In addition, both the chemical shift of the base-on species and the value of Δδ_{31P} are linearly related to the axial Co-N bond length for adenosylcobalamin (AdoCbl), d_{Co-N(Bz)} = 2.24 Å [3], CH₃Cbl, 2.14 Å [4] and CNCbl, 2.06 Å [5].

These results may be interpreted in terms of the

Table
³¹P-Chemical Shifts and Phosphodiester Macroscopic pK_a's of Cobalamins ^{a)}

| Cobalamin | pK _{base-off} ^{b)} | ΔG _{Co} ^{c)} | pG ₄ | pG ₅ | Base-Off Species | | Base-on δ _{31P} ^{d)} | Δδ _{31P} ^{e)} |
|---|--------------------------------------|--------------------------------|-----------------|-----------------|--|--|---|---------------------------------|
| | | | | | Phosphodiester Protonated δ _{31P} ^{d)} | Phosphodiester Deprotonated δ _{31P} ^{d)} | | |
| CH ₃ (CH ₂) ₂ Cbl | 4.10 | - 1.97 | -1.58 | -0.05 | -337.76 | -38.10 | -50.16 | -11.98 |
| AdoCbl | 3.67 | - 2.57 | — | — | — | -36.70 | -47.15 | -10.44 |
| NC(CH ₂) ₃ Cbl | 3.50 | - 2.81 | -1.60 | -0.02 | -342.14 | -36.63 | -43.66 | - 7.03 |
| CH ₃ Cbl | 2.89 | - 3.64 | -1.62 | -0.02 | -335.43 | -38.47 | -35.66 | 2.81 |
| CF ₃ CH ₂ Cbl | 2.60 | - 4.04 | -1.56 | -0.02 | -345.50 | -33.51 | -32.91 | 0.60 |
| CF ₂ HCbl | 2.15 | - 4.66 | -1.61 | -0.03 | -342.31 | -33.47 | -25.58 | 7.89 |
| NCCH ₂ Cbl | 1.81 | - 5.19 | -1.60 | -0.02 | -342.14 | -34.33 | -25.91 | 8.42 |
| CF ₃ Cbl | 1.44 | - 5.62 | — | — | — | -36.70 | -11.99 | 24.71 |
| CNCbl | 0.10 | - 7.44 | -1.57 | -0.04 | -335.81 | -37.40 | - 1.72 | 35.68 |
| H ₂ OCbl | -2.13 | -10.48 | -1.59 | -0.09 | -337.10 | -41.64 | - 0.37 | 51.01 |
| Average: | | | -1.59 ± 0.02 | -0.04 ± 0.02 | -339.88 ± 3.80 | -36.70 ± 2.80 | | |

a) 25 ± 1°C.

b) Apparent pK_a of the base-on-base-off reaction.

c) Apparent free energy of coordination of the free-base benzimidazole ligand.

d) In Hz, from external 85% H₃PO₄ (negative shifts upfield from the reference) at 80.988 MHz.

e) Difference in chemical shift (in Hz) between the base-on and base-off species.

work of GORENSTEIN [6-8] who has shown that the ^{31}P -chemical shift of phosphate compounds is controlled by O-P-O bond angles. Thus, in base-on CH_3Cbl or $\text{CF}_3\text{CH}_2\text{Cbl}$ (Table) the conformation of the phosphodiester is apparently the same as that of all the base-off species and there is no difference in chemical shift between the base-on and base-off species. When coordination of the axial ligand is weaker (e.g., AdoCbl), lengthening of the axial Co-N bond causes a decrease in the RO-P-OR' bond angle and an upfield shift of the base-on ^{31}P -resonance. When the axial ligand coordination is tighter (e.g., CNCbl) the shortened axial Co-N bond causes an increase in the RO-P-OR' bond angle and a downfield shift in the base-on ^{31}P -resonance.

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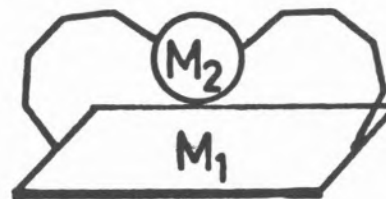
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SYNTHETIC PORPHYRINS CONTAINING ADJACENT, REDOX-ACTIVE SPECIES; MODELS OF BIOLOGICAL ELECTRON TRANSFER SITES

The remarkable diversity of function of hemes and chlorophylls in biochemistry is due, in large part, to changes in the surrounding protein environment. In systems which are involved in electron transport the nearby protein environment often includes other metal centers held in close proximity to the tetrapyrrole.

In an attempt to mimic some of the electron transport and photochemical charge separation properties of these centers we have prepared a series of model systems in which transition metals are covalently held a short distance from a metalloporphyrin.



The first binucleating ligand involves a 2,2'-bipyridine unit strapped across the face of a porphyrin. 5,5'-bis(3-hydroxypropyl)-2,2'-bipyridine (**1**) is prepared in six steps from β -picoline. Reaction of **1** with mesoporphyrin-II diacid chloride under high dilution conditions yields the bipyridine-bridged porphyrin **3** in good yield. Heterobinuclear complexes of **3** can be readily formed in a two step sequence. Refluxing **3** with $\text{Ru}(\text{bipy})_2\text{Cl}_2$ in