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The Structures of some Cobalamins in Solution

O. D. Hensens, H. A. O. Hill, J. Thornton, A. M. Turner and R. J. P. Williams

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The structures of some cobalamins in solution

By O. D. Hensens, H. A. O. Hill, J. Thornton, A. M. Turner, and R. J. P. Williams, F.R.S.
Inorganic and Biophysics Laboratories, South Parks Road, Oxford

Nuclear magnetic resonance spectroscopy has been used to examine the conformations of cobalamins in solution. The perturbation of the resonances in the n.m.r. spectra by lanthanide probes provided particularly valuable information. Except for minor details the conformations are the same as those in the crystalline state. The temperature dependence of the conformation has been reassessed.

We have been interested in determining the structures of the cobalamins (figure 1) in solution for a number of reasons: To compare the structures in the crystalline state (Hodgkin *et al.* 1962; Lenhert 1968) with those in solution; to understand the structural changes (Firth *et al.* 1968)

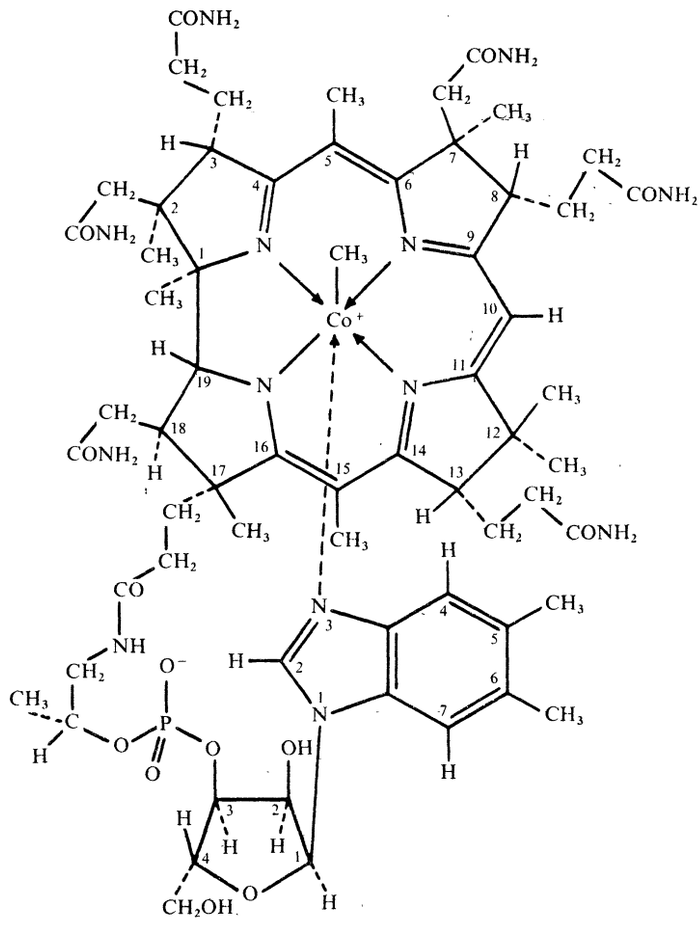

Figure 1

associated with the changes in the spectroscopic properties with temperature; to account for the marked dependence of thermodynamic, kinetic, and spectroscopic properties (Hill 1973; Pratt 1972) on the nature of the axial ligand; eventually to study the structure of the coenzyme in a holoenzyme. At present n.m.r. spectroscopy provides the only method of deriving detailed information on geometry in solution through the use (Barry, Glasel, Williams & Xavier 1974) of lanthanide ions as shift and relaxation perturbants together with the analysis of coupling constants, where relevant.

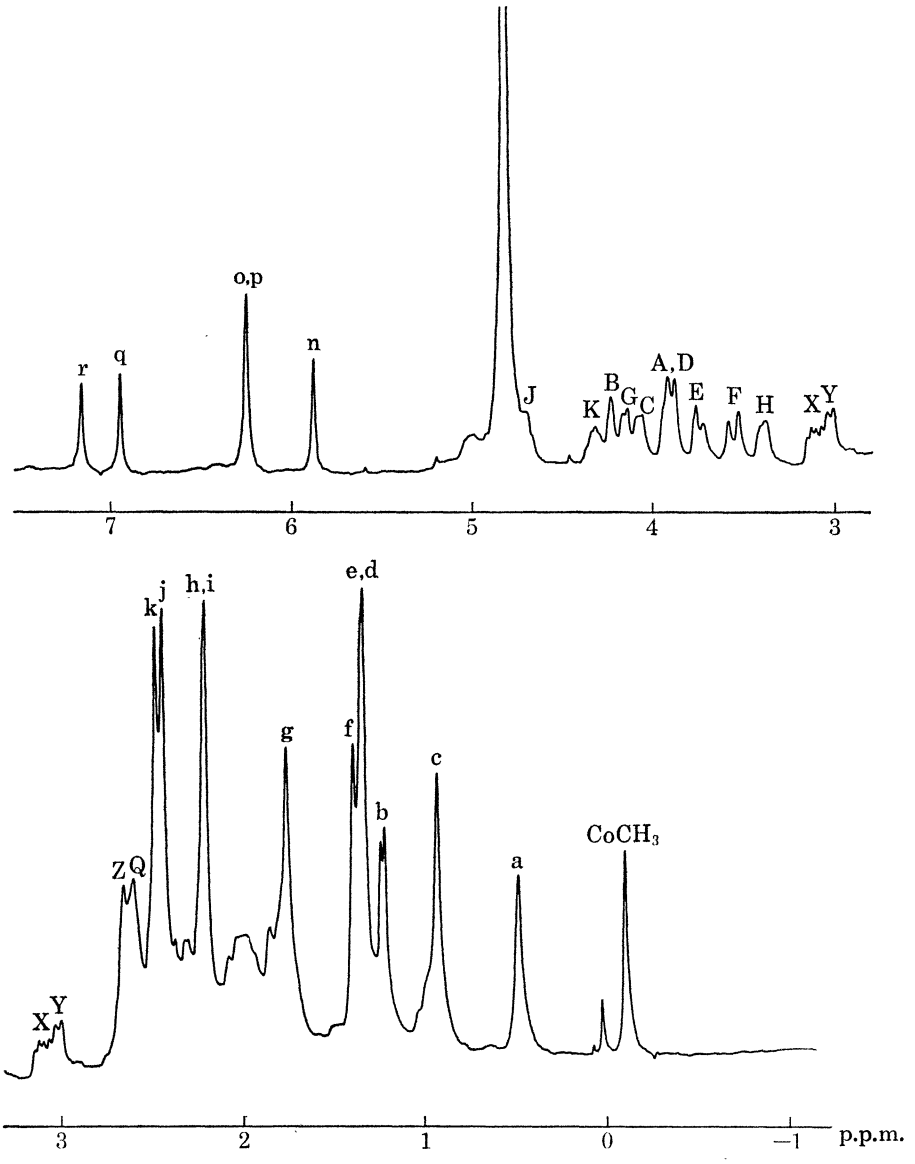

FIGURE 2. The 273 MHz H n.m.r. spectrum of methylcobalamin (10 mM) in D_2O, pH_{obs} 6.5.

The initial requirement is an assignment (Hensens *et al.* 1975) of the n.m.r spectrum to provide a sufficient number of observables which will allow an unambiguous determination of the structure. The assignment of the spectrum of methylcobalamin, shown in figure 2, proceeds as follows. Some resonances, those arising from the Co-CH_3 and the C10-*H* are readily assigned

and that of the ribose C1′-*H* (R1)† is assigned by comparison with related nucleosides. Having assigned R1, conventional decoupling methods lead to assignment of the remaining ribose hydrogens. The propanolamine CH_3, (Pr3) gives a prominent doublet and the assignment of the remaining propanolamine protons follows readily. Substitution at appropriate positions in the 5,6-dimethylbenzimidazole allows assignment of these protons. The ring methyls were assigned by analysis of the pH-dependence and the titration of methylcobalamin with cyanide, both of which result in the formation of 'base-off' forms in which the 5,6-dimethylbenzimidazole is no longer coordinated to the cobalt. The ring current of the 5,6-dimethylbenzimidazole moiety, when the latter is bound to the cobalt, provides an intrinsic perturbation of the chemical shifts of those protons which 'lie below' the plane of the corrin ring. Interconversion between different cobalamins is easily achieved, e.g. ethylcobalamin decomposes to hydroxocobalamin when a solution is heated in air; hydroxocobalamin gives cyanocobalamin on treatment with a slight excess of cyanide. Hence correlation between the assignments in the different cobalamins follows readily. We hope that these assignments, given in table 1, will be of value to studies of the biosynthesis of the corrinoids.

TABLE 1

resonance	chemical shift (parts/10^6)	assignment	coupling constant J/Hz ($\pm$ 0.6 Hz)
r	7.19	B7 – *H*	—
q	6.97	B2 – *H*	—
o	6.27	R1 – *H*	R1 – R2 = 2.4
p	6.27	B4 – *H*	—
n	5.90	C10 – *H*	—
J	4.71	R3 – *H*	R3 – R4 = R3 – P = 7.3
K	4.32	Pr2 – *H*	Pr2 – Pr1′ = 7.3; Pr2 – P = 4.8
B	4.23	R2 – *H*	R2 – R3 = 3.7
G	4.16	C3β – *H*	—
H	3.39	C8β – *H*	—
Y	3.02	C13β – *H*	—
C	4.08	R4 – *H*	R5′ – R5″ = – 14.6
A	3.93	19β – *H*	—
D	3.90	R5′ – *H*	R4 – R5′ = 0
E	3.74	R5″ – *H*	R4 – R5″ = 3
F	3.55	Pr1″ – *H*	Pr1′ – Pr1″ = – 13.4
X	3.11	Pr1′ – *H*	Pr2 – Pr1″ = 0
Z	2.66	C18β – CH_2	—
Q	2.60	C2β – CH_2	—
k	2.48	C5 – CH_3	—
j	2.45	C15 – CH_3	—
h, i	2.21	B5, B6 – CH_3	—
g	1.76	C7α – CH_3	—
f	1.39	C12α – CH_3	—
e	1.34	C2α – CH_3	—
d	1.34	C17β – CH_3	—
b	1.22	Pr3β – CH_3	Pr2 – Pr3 = 6.0
c	0.92	C12β – CH_3	—
a	0.47	C1α – CH_3	—
$CoCH_3$	– 0.81	$CoCH_3$	—

† R indicates a ribose hydrogen, B a 5,6-dimethylbenzimidazole and Pr a propanolamine hydrogen.

^{31}P n.m.r studies show that lanthanide ions bind to the phosphate diester. This permits their use as both chemical shift reagents, increasing the resolution of some regions of the spectra, though we have used them primarily as probe for structure determination in solution. This

requires the magnetic susceptibility tensor to be axially symmetric. Since the shift ratios of all protons were closly similar regardless of the lanthanide employed, the assumption of axial symmetry is probably justified. Use of carboxymethylcobalamin, which contains the Co—CH_2COO^- group, introduces a second major binding site for the lanthanides. A full analysis of the binding of metal ions by standard spectrophotometric procedures allowed the contributions to the paramagnetic shifts due to the binding at the phosphate and carboxylate to be separated. Similarly the use of derivatives of the cobalamins which have the C7 acetamide side-chain hydrolysed to give C7-β-CH_2COO^- introduces a third binding site. In this way a 'triangulation' of the molecular geometry could be conducted. The shifts, using Pr III, Eu III and Dy III, and the line-widths, using Gd III, as probes, were consistent with the initial assignments (q.v). They are also consistent with a molecular structure which is not significantly different from that derived from diffraction studies. In particular, the relative orientation of the 5,6-dimethylbenzimidazole side chain and the corrin ring remains conserved in solution. Modification of the conformation of the ribose phosphate and the propanolamine side chain is required to achieve a good correlation with the paramagnetic shifts and line widths.

Previously we have discussed (Firth 1968; Hill 1969*a*, *b*) the possible changes in the structures of the cobalamins as a function of temperature. In particular it was speculated (Firth 1968) that certain cobalamins, e.g. the coenzyme, 5′-deoxyadenosylcobalamin, existed in a high-temperature form in which the 5,6-dimethylbenzimidazole side chain was not coordinated to the cobalt, i.e. a 'base-off' form. Use of the methods described above at different temperatures shows (Cockle 1970; Cockle, Hensens, Hill & Williams 1975) that the 5,6-dimethylbenzimidazole does not become detached from the cobalt at higher temperatures in any of the cobalamins studied, including the coenzyme. The alteration in the structure which leads to the changes in the spectral properties is probably associated with a change in the 'pucker' of the corrin ring. We understand that other workers (Hogenkamp, Vergamini & Matwiyoff 1975) have come to the same conclusion.

We wish to thank the Medical Research Council for financial support. H.A.O.H. and R.J.P.W. are members of the Oxford Enzyme Group.

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In answer to a question from Professor A. Eschenmoser, Professor R. J. P. Williams replied on behalf of Dr H. A. O. Hill and himself as follows:

The limits of our knowledge of the conformational states of corrinoids present in diol dehydratases and related enzymes are as follows:

The coenzyme. The absorption spectrum of the coenzyme in the holoenzyme is but slightly different from that of the coenzyme in aqueous solution. We conclude that the 5,6-dimethylbenzimidazole is still bound and that only small changes of the corrin ring puckering have taken place on binding to the apoenzyme. The conformation of the 5′-deoxyadenosyl group is not known. We have only preliminary n.m.r data on the corrinoid-binding protein from *C. thermoaceticum*.

The intermediate. The only intermediate which is well defined is a cobalt(II) cobalamin in ethanolamine ammonia lyase, ribonucleotide reductase and the diol dehydratases. The agreement between the results of different groups of workers is very good. Using absorption spectroscopy, we can say using electron paramagnetic resonance spectroscopy that the 5,6-dimethylbenzimidazole is bound, that the conformation of cobalt(II) cobalamin in the enzyme is little different from that in free solution. We have speculated, on the basis of the e.p.r. spectra, that there is a nearby organic radical, 0.5–1 nm distant from the cobalt(II).

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