Preparation and X-ray Analysis of Crystals of Azido- and Chlorocobalamin Containing LiCl: A Structural Model for the Interactions of the Corrin Ring with Ionic Species

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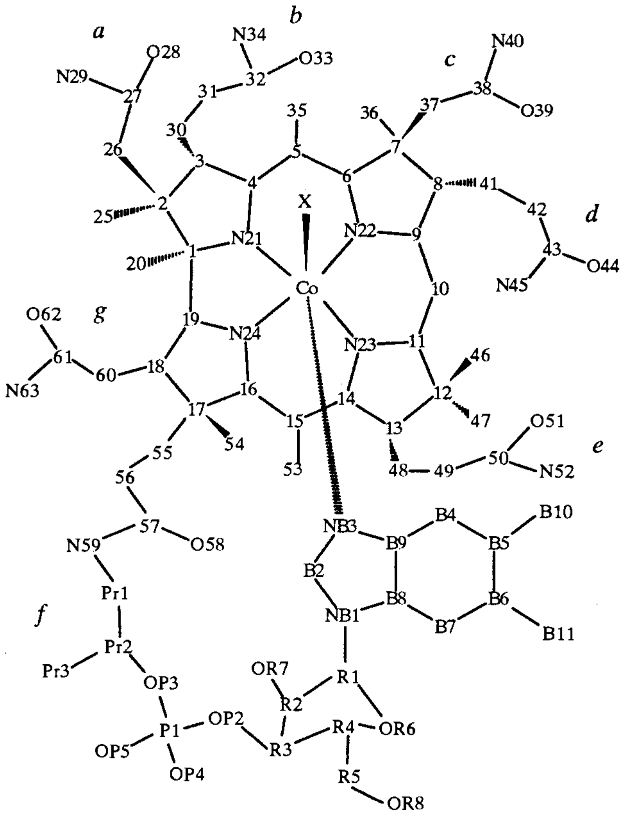
Introduction

Recently, the reactions of adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl) (for XCbl see Chart 1) with cyanide have been reinvestigated.[1,2] The AdoCbl has been shown[1] to react by attack of the cyanide ion on the β-adenosyl side. On the contrary, no cyanation reaction between MeCbl and cyanide has been observed under the same conditions. ^{1}H NMR results[2] suggested the formation of an ion-pair species MeCbl·CN$^-$, with the potentially π-interacting cyanide ion located close to the corrin ring, on the side of dimethylbenzimidazole ligand. Association between MeCbl and charged species, particularly anions such as PtX_4^- or $AuCl_4^-$, has been reported in a few cases[3] and ion-pair formation, prior to substitution, has been suggested[4] in the displacement of the β-H_2O ligand in aquocobalamin. NMR studies on the dimerization of RCbl, with several R groups, indicated that all the RCbl, whether forming dimers (R = Me, Et) or not (R = n-Pr, Ado, CN, H_2O), showed NaCl specific (although not easily interpretable) shifts of some resonances.[5] All these results suggest the existence of weak bonding interactions between corrinoids and charged species, which influence the corrinoid solution behavior. The interest in the study of the interactions, involving corrinoids and charged species, resides in the possible role that they could play in determining the organo-group transfer and electron-transfer reactions of B_{12} compounds.[2,5,6] Furthermore, such interactions are potentially important in the binding of corrinoids to B_{12} proteins, via charged amino acid residues. With the aim to obtain, at least in the solid state, a structural picture of the interactions between corrinoids and charged species, we have tried to crystallize cobalamins in the presence of highly concentrated salts, using the well-known hanging-drop technique, usually employed for obtaining protein single crystals. We report here the preparation and the X-ray analysis, based on synchrotron data, of crystals of chloro- and azidocobalamin containing LiCl, **1** and **2**, respectively.

Chart 1



X = Cl (**1**), N1-N2-N3 (**2**)

Experimental Section

Preparation of the Crystals. Crystals were grown by the hanging-drop method of vapor diffusion. Crystallization of **1**: 2 μL of a (H_2-OCbl)Cl solution at 25 mg/mL were mixed with 2 μL of a precipitant solution containing PEG and LiCl. Typical ranges of concentration of the precipitants are 25−30% PEG and 2.0−3.0 M for LiCl. The droplets were suspended over the same precipitant solution and allowed to equilibrate at room temperature. Parallelepiped-shaped red crystals appeared in the drop whithin 2 days. Crystallization of **2**: Red parallelepiped-shaped crystals were obtained with the same procedure of **1**, with precipitant solutions also containing 0.02 M NaN_3.

Crystal Structure Determination. The data collections were carried out at the X-ray diffraction beamline of the Elettra Synchrotron (Trieste, Italy), using the rotating crystal method with 0.8 Å monochromatic wavelength and a 30 cm MAR image plate with maximum resolution of 0.89 Å. Friedel equivalent reflections were not merged. Crystal data for **1** and **2** are given in Table 1. The structures were solved by conventional Patterson and Fourier methods and refined by full-matrix least-squares on F^2. No absorption correction was applied. Anisotropic refinements of all non-hydrogen atoms with full occupancy (H atoms were included at calculated positions with fixed thermal parameters) gave a final $R1$ of 0.0486 for **1** and of 0.0512 for **2**, calculated with 10543 (995 variables) reflections with $I > 2\sigma(I)$ for **1** and 11683 (1016 variables) reflections with $I_o > 2\sigma(I_o)$ for **2**. Calculations have been carried out using the SHELXL programs.[7]

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Table 1. Crystallographic Data for Compounds **1** and **2**

compound	**1**	**2**
formula	$C_{62}H_{103}Cl_3CoLi_2N_{13}O_{21.5}P$	$C_{62}H_{106.60}Cl_2CoLi_2N_{16}O_{23.3}P$
fw	1584.7	1623.71
T, K	100(2)	100(2)
λ, Å	0.8000	0.8000
cryst syst, space group	orthorhombic, $P2_12_12_1$	orthorhombic, $P2_12_12_1$
a, Å	15.468(5)	15.434(1)
b, Å	22.588(8)	22.679(1)
c, Å	23.561(4)	23.450(1)
V, Å^3	8232(4)	8208.1(7)
Z, D_{calcd}, Mg/m^3	4, 1.279	4, 1.314
μ, mm^{-1}	0.398	0.372
$F(000)$	3348	3436
cryst size, mm	0.4 × 0.2 × 0.2	0.3 × 0.2 × 0.2
no. of reflcns collcd	10 621	11 828
no. of data/parameters	10 543/995	11 683/1016
GOF (F^2)	1.037	1.098
$R1(F_o)$, $R_w(F_o^2)$ [$I > 2\sigma(I)$][a]	0.0486, 0.1462	0.0513, 0.1450
$R1(F_o)$, $R_w(F_o^2)$ (all data)[a]	0.0489, 0.1467	0.0519, 0.1457
absolute struct parameter	−0.079(16)	0.009(15)

[a] $R1 = \sum||F_o| - |F_c||/\sum|F_o|$, $R_w = [\sum w(|F_o| - |F_c|)^2/\sum|F_o|^2]^{1/2}$.

Table 2. Co−X and Co−N Axial Coordination Distances in Cobaloximes, in Cobalamins, and in 10-Cl-cobalamins

	$XCo(DH)_2py$[a]		cobalamin		10-Cl-cobalamin	
X	Co−X	Co−N	Co−X	Co−N	Co−X	Co−N
H_2O	1.916(3)[b]	1.926(3)[b]	1.952(2)[c]	1.925(2)[c]	1.948(3)[d]	1.967(4)[d]
Cl	2.229(1)[e]	1.959(2)[e]	2.262(1)[f]	1.990(4)[f]		
Cl			2.259(5)[g]	1.954(13)[g]		
N_3	1.950(2)[h]	1.973(1)[h]	1.985(3)[f]	1.999(3)[f]		
CN	1.937(2)[i]	1.995(2)[i]	1.858(12)[l]	2.011(10)[l]	1.97(2)[d]	2.04(1)[d]
CH_3	1.998(5)[m]	2.068(3)[m]	1.99(2)[l]	2.19(2)[l]	1.979(7)[d]	2.200(7)[d]
ado	2.015(2)[n]	2.072(2)[n]	2.023(10)[o]	2.214(9)[o]		
adePr			1.959(10)[p]	2.212(8)[p]		

[a] DH = monoanion of dimethylglyoxime. [b] Reference 16. [c] Reference 12. [d] Reference 13a. [e] Reference 17. [f] Present work. [g] Reference 8. [h] Reference 18. [i] Reference 19. [l] Reference 15. [m] Reference 20. [n] Reference 21, X = (5-deoxy-β-D-ribofuranos-5-yl)methyl. [o] Reference 11; ado = 5′-deoxy-5′-adenosyl. [p] Reference 13b; adePr = adenylpropyl.

Results and Discussion

To obtain single crystals, many attempts were carried out by adding either NaY (Y = Cl, CN) or LiY (Y = Cl, Br and I) to water solutions of several cobalamins. When LiCl was added to $(H_2OCbl)Cl$, good-quality crystals were obtained. The structure analysis (see below) revealed that the crystallized compounds was actually $ClCbl{\cdot}2LiCl{\cdot}nH_2O$ (**1**), formed from the starting aquocobalamin by displacement of the axially coordinated water molecule by Cl^-.[8] Recently, it has been shown that Cl^- substitutes water in the aquocobalamin at high chloride concentration.[4,9] By addition of NaN_3 to the aquocobalamin water solution, in the presence of LiCl good quality crystals of $N_3Cbl{\cdot}2LiCl{\cdot}nH_2O$ (**2**) were obtained using the same technique. The X-ray diffraction data for **1** and **2** were collected at 100 K, using the synchrotron radiation.[10] The present structural determinations have high resolution, and, among the previous cobalamin X-ray analyses, only those of the aquocobalamins $(H_2OCbl)ClO_4$[12] and $[(H_2O)(10\text{-}Cl{-}Cbl)]ClO_4$[13] (10-Cl-Cbl = 10-chlorocobalamin) are of comparable accurancy. Since the crystal structures of **1** and **2**[14] are very similar, if the X axial ligand is excluded, the description of **1** applies also to **2**. The ORTEP diagrams of **1** and **2** are given in Figure 1. With the exception of the acetamide side chain *c*, involved in an intramolecular H-bond with the X axial ligand, all the other side chains interact with Cl^- and Li^+ ions (Figure 2). The distances between N40 of the *c* chain and the Cl ligand in **1** and the N donor of the azido group in **2** are 3.312(6) and 3.131(6) Å, respectively. An intramolecular H-bond between the *c* chain and the X axial ligand was also observed in $(H_2OCbl)ClO_4$,[12] involving the O39 atom of the *c* chain and the coordinated water molecule. The cations are coordinated to the amidic O atoms of the chains *a*, *d*, *e*, and *g* and to OP4 of the chain *f*, whereas the anions are held by NH···Cl H-bonds to the amidic N atoms of the chains *a*, *b*, *e*, and *g*, as shown in the sketch of Figure 2. The side chains *a*, *e*, and *g* interact both with Li^+ and Cl^- ions, *d* and *f* only with Li^+ and *b* only with

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(10) Diffraction data were collected for **1** and **2** also at 275 K. Comparison of the refined parameters at 275 K with those at 100 K, showed a significant increase in the thermal parameters essentially of the side chains, but their order is maintained. A similar influence of the temperature was observed when the AdoCbl structures, determined at 279 and 15 K, were compared.[11] Only slight differences in the esd's of the coordination geometry were detectable at the two temperatures. A shrinkage of about 3% in the unit cell determined at the lowest temperature is found. Diffraction data for **2** at room temperature were also collected using a conventional X-ray source (Mo Kα). A detailed comparison of the five data collections will be given elsewere.

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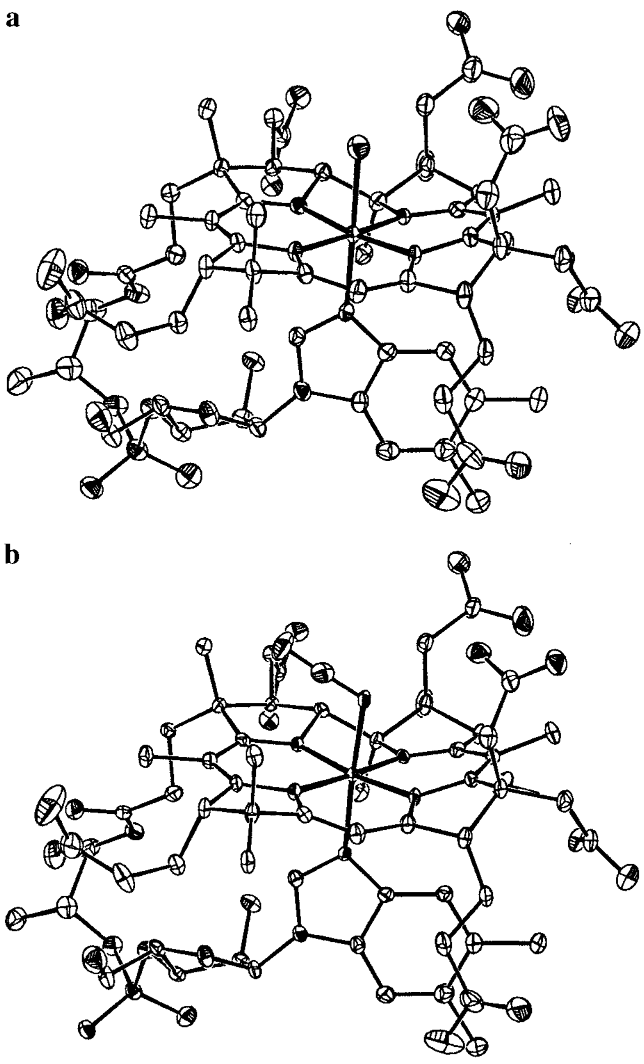

Figure 1. ORTEP drawings of the cobalamin in **1** (a) and in **2** (b).

Cl$^-$. Eight ions bind each corrin, three of which (one Li$^+$ and two Cl$^-$) lie above the β face and five (four Li$^+$ and one Cl$^-$) of which lie above the α face of the corrin (Figure 2). This could suggest that the α face is more negatively charged than the β one. The H-bonding scheme, involving also the ordered[11] water molecules with full occupancy, is shown in Figure 2. Chains *a* and *g* are bridged by the Cl1 ion, whereas chains *e* and *f* are held together by a H-bond between the sugar OR8 hydroxyl group and the amidic O51 atom (2.810(6) Å in **1** and 2.827(6) Å in **2**). Because of these interactions, all the side chains, including the sugar moiety, are found completely ordered.[10] The side chain conformations differ from those found in the free salt ClCbl, which exhibits however a similar intramolecular H-bond of 3.31 Å.[8] The total water content is significantly less than that found in the free salt cobalamins.[8,11–13,15] This is probably due to the occupation by anions and cations of the sites occupied by some ordered water molecules in the crystal structures of the free salt cobalamins.

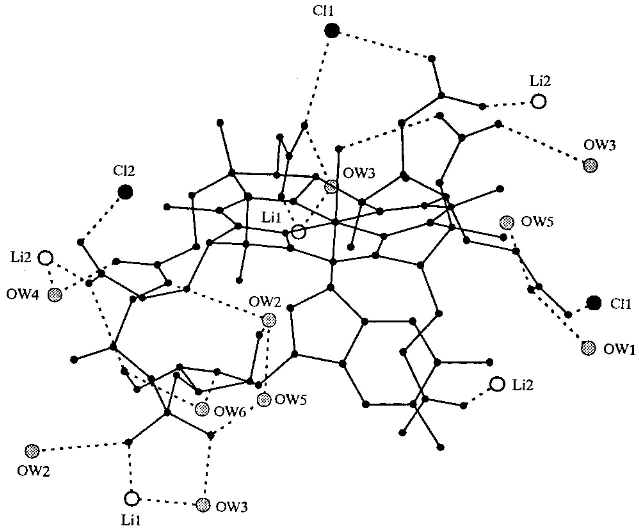

Figure 2. Sketch of the structure of **1**, which evidentiates the interactions of ions and water molecules with the amide side chains. The intramolecular H-bond, involving the *c* side chain is also shown. A similar sketch applies to **2**. The corrin O atoms and water molecules make a distorted tetrahedral geometry around Li ions and the Li−O distances range from 1.895(10) to 2.103(16) Å in **1** and from 1.880(7) to 2.041(8) Å in **2**. The shortest distance involves OP4, and the longest involves the water molecule OW3. In **1**, Cl1 completes the distorted trigonal geometry with one more amido N atom (N34) of a symmetry-related corrin, whereas Cl2 a distorted pyramidal geometry with two water molecules. The Cl···HN distances range from 3.125(6) to 3.340(11) Å, and Cl2···O distances are 3.125(6) and 3.232(13) Å. The sum of the angles around Cl1 and Cl2 are 356.6(2) and 266.5(2)°, respectively. Very similar figures are found in **2**.

The distortion of the corrin ring from planarity, as measured by the fold angle[12,13] (17.8(1) and 16.7(1)° in **1** and **2**, respectively), is close to those found in other cobalamins, where the fold angle ranges from 10.9 to 18.7°, generally increasing with the shortening of the Co−NB3 axial bond.[12,13] The high accurancy of the equatorial Co−N distances found in **1** and **2** together with those reported for (H$_2$OCbl)ClO$_4$[12] and [(H$_2$O)(10-Cl-Cbl)]ClO$_4$[13] allows us to observe that the two Co−N bonds, involved in the corrin five-membered ring, are significantly shorter than the other two Co−N bonds (Table 1S). The axial coordination distances for several cobalamins are given in Table 2. It is apparent that the axial Co−NB3 distance increases by about 0.3 Å with the increase in the electron donating ability of the trans ligand from H$_2$O to adenosyl. The comparison of the axial distances in cobalamins and in the analogous cobaloximes, XCo(DH)$_2$py (DH = monoanion of dimethylglyoxime, py = pyridine), a vitamin B$_{12}$ model is also of interest.[16–19] The Co−N axial distances in the latter follow the same, although less enhanced, *trans*-influence trend. With the exception of the cyano derivatives, the Co−X distances have similar values in cobaloximes and in cobalamins, although for nonorganometallic derivatives these distances appear to be longer in cobalamins. The latter differences may be a consequence of the combined effect of the different *cis*-influence of the equatorial ligand and of the intramolecular H-bond, present only in cobalamins.

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These results illustrate a first structural example for the interaction between corrin and ionic species. Thus, the corrin acts as a molecular “octopussy” which “clutch” anions and cations through its “tentacles”, the amide side chains. The ion distribution about the macrocycle is such that the crystal packing shows no hint for corrin pairing in the solid state.[5] Similar basic features for ion-corrin interactions should be expected in the MeCbl analogue, although the side chain *c* is no longer involved in the intramolecular H-bond, as in **1** and **2**. On this basis, similar kind of interactions can be suggested for the MeCbl$\cdot$CN$^-$ species,[2] since the cyanide ion should be able to make H-bonds with the NH_2 amidic groups. Efforts to prepare MeCbl, containing LiCl and NaCN, are in due course.

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Supporting Information Available: Tables of atomic coordinates, anisotropic thermal factors, calculated hydrogen atom coordinates, bond lengths and angles, and H-bonds and Table 1S, where equatorial coordination bond distances for the cobalamins, whose structures have been refined to a high accuracy, are compared, are available (16 pages). X-ray crystallographic files, in CIF format, are also available on the Internet only. Ordering and access information is given on any current masthead page.

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