DOI: 10.1002/anie.200603901

Structural Analysis of Biomolecules

Novel Approaches to the Experimental Charge Density of Vitamin B_{12}**

*Birger Dittrich, Tibor Koritsanszky, Anatoliy Volkov, Stefan Mebs, and Peter Luger**

Knowledge of the steric, dynamic, and electronic contributions to intermolecular interactions is of fundamental importance to the modeling of biochemical processes. X-ray diffraction has become a widely applied experimental tool for gaining this knowledge, since the analysis of high-resolution diffraction data can lead not only to the solid-state structure, but also to the corresponding electron density (ED).[1] In spite of the potential applicability to larger systems of biological interest,[2–5] X-ray ED studies have been almost exclusively restricted to small molecules,[6] because the assumption of perfect periodicity becomes less realistic for large unit-cell structures. This issue is already a problem for systems of intermediate size, such as the alkylcobalamines (R−Cbl), whose crystal structures have been described as notoriously inaccurate,[7] owing to the disorder of side chains and solvent molecules.

Alkylcobalamines are cofactors of mammalian enzymes that catalyze methyl-transfer, isomerization, and redox reactions. The first two reactions involve the breaking and reforming of the Co−C_R bond, presumably through a reversible heterolytic or homolytic cleavage.[8,9] Since the alkylcobalamin coenzymes are inactive, and their binding to the protein does not involve any change in the equatorial ligation of the cobalt atom by the corrin ring, the main issue in cobalt-enzyme research is to understand how the axial Co−C_R bond is labilized by the apoenzyme.[10] A detailed analysis of the electronic configuration of the cobalt center and the covalent character of the Co−C_R bond should provide some insight into the destabilization of the bond through intermolecular forces.

Quantum-chemical studies on cobalt corrinoids have been limited to geometry-optimized models for the coenzymes (alkyl corrin complexes), the sizes of which were reduced through the truncation of side chains.[11] More recent studies have been based on density functional theory (DFT) calculations and have utilized experimental structures determined from relatively high-resolution diffraction data. Calculations on CN−Cbl,[12] Me−Cbl,[13] and Ado−Cbl (Ado = 5′-deoxy-5′-adenosyl)[14] have shown that the polarity of the Co−C_R bond increases in the order R = CN, Ado, Me, even though the equatorial ligation of the cobalt cation (that is, the Co−N_{eq} bonds) remains unchanged upon variation of the axial R group.

Modern X-ray ED studies on transition-metal complexes[6] have combined d-orbital population analyses[15] with density-based topological analyses,[16] allowing a quantitative characterization of the metal–ligand interactions.[17] Since, for these interactions, the ED at the bond critical point (BCP) is low, whereas the bond-parallel curvature (λ_3) is very high, the effects of the radial basis functions[18] can be pronounced; the limitations of the standard multipole model[19] are becoming increasingly evident.

The present study has the following novel components:

a) We were able to crystallize a new solvate of vitamin B_{12} with a low solvent content (twelve water and three propanol molecules per molecule of cyanocobalamin in

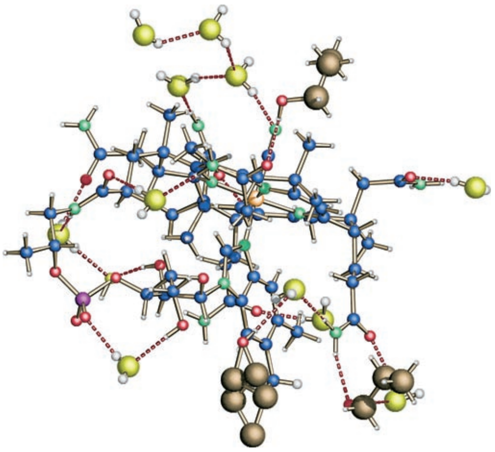

Figure 1. SCHAKAL[33] representation of the molecular structure of the solvate of vitamin B_{12} (C blue, H white, Co orange, N green, P purple, O red) with twelve water (O yellow) and three propanol molecules (C bronze), one of which is partially disordered. The dashed red lines represent hydrogen bonds.

---

[*] Dipl.-Chem. S. Mebs, Prof. Dr. P. Luger
Freie Universität Berlin
Institut für Chemie und Biochemie/Kristallographie
Freie Universität Berlin
Fabeckstrasse 36a, 14195 Berlin (Germany)
Fax: (+49) 30-838-53464
E-mail: luger@chemie.fu-berlin.de

Dr. B. Dittrich
Chemistry M313, School of Biomedical and Chemical Sciences
University of Western Australia
35 Stirling Highway, Crawley, WA 6009 (Australia)

Prof. Dr. T. Koritsanszky
Department of Chemistry
Middle Tennessee State University
P.O. Box 68, Murfreesboro, TN 37132 (USA)

Dr. A. Volkov
Department of Chemistry
State University of New York at Buffalo
747 Natural Sciences Complex, Buffalo, NY 14260-3000 (USA)

[**] This work was supported by the Deutsche Forschungsgemeinschaft (grants SPP1178, Lu222/29-1, and Ko1350/4-1) and by the Australian Synchrotron Research Program, which is funded by the Commonwealth of Australia under the Major National Research Facilities Program. B.D. thanks the ASRP for a postdoctoral fellowship.

the asymmetric unit, of which only one propanol molecule was partially disordered; Figure 1). Crystals of this solvate are stable for weeks in the mother liquor, but decompose immediately upon removal. The sample was, thus, oil-protected and shock-frozen in situ on a Bruker SMART 1 K diffractometer. A high-resolution data set was then collected at 100 K using conventional $Mo_{K\alpha}$ radiation.[20]

b) Our recently developed invariom approach[21,22] was used to generate a starting model for the initial aspherical-atom refinement according to the Hansen–Coppens formalism[19] (XD program[23]). Through this approach, the convergence of the multipole refinement could be accelerated, and the disordered sites could be better resolved. The invariom density was constructed, starting from the converged structure refined with SHELXL-97,[24] through the superposition of 53 chemically unique and transferable pseudoatoms. These pseudoatoms were extracted from the ab initio densities calculated for 29 model compounds,[25] which were geometry-optimized using the B3LYP functional and the D95++(3df,3pd) basis set.[26] Invarioms for the atoms of the corrin ring were extracted from the ED of the entire ring system, because the delocalized system could not be described with simple invariom model compounds (which consider only nearest neighbors). The conventional parameters (x, y, z, and U_{ij}) were first refined against all the diffraction data, with fixed scattering factors deduced from the invariom model and idealized lengths for the bonds involving hydrogen atoms.[27] In subsequent least-squares cycles, the multipole parameters of all the chemically independent pseudoatoms, except those of the solvent molecules, were refined to full convergence.

c) To model the ED of the cobalt center, three types of radial density functions (RDF) were tested: standard single-zeta Slater functions with energy-optimized Hartree–Fock exponents (SZHF);[28] the same functions, but with exponents fitted against the theoretical (B3LYP/TZVP) structure factors of the *Co*α-cyano-*Co*β-aminocobalt(III) corrin (CACo(III)C) model compound (SZFIT); and RDFs for all monopoles derived through the direct-space spherical-harmonics projection of the cobalt stockholder atom in the CACo(III)C model compound[29] and expressed in terms of three Slater functions (STOCK).

Figure 2 shows that the nodeless SZFIT functions routinely used in standard multipole refinements are inappropriate for the description of the density of the chemically bound atoms. The differences between the SZFIT and STOCK functions are especially pronounced for the odd-order *l* terms, which are clearly more structured for the STOCK functions than for the SZFIT functions.

The deformation ED maps in the plane of the corrin ring for the SZFIT refinements on the data simulated for the CACo(III)C model compound and on the experimental data for the vitamin B_{12} solvate are shown in Figures 3a and b. Both maps, as do the corresponding Laplace distributions, clearly reveal a lock-and-key arrangement, which is characteristic for an octahedral cobalt-ion coordination environment; the regions of electron excess in the equatorial plane (the *XY* plane) are concentrated on the lines bisecting the N-Co-N angles between *cis* nitrogen atoms, while the regions of electron deficiency are located along the Co–N bonds (along the *X* and *Y* axes). This situation is also reflected in the d-

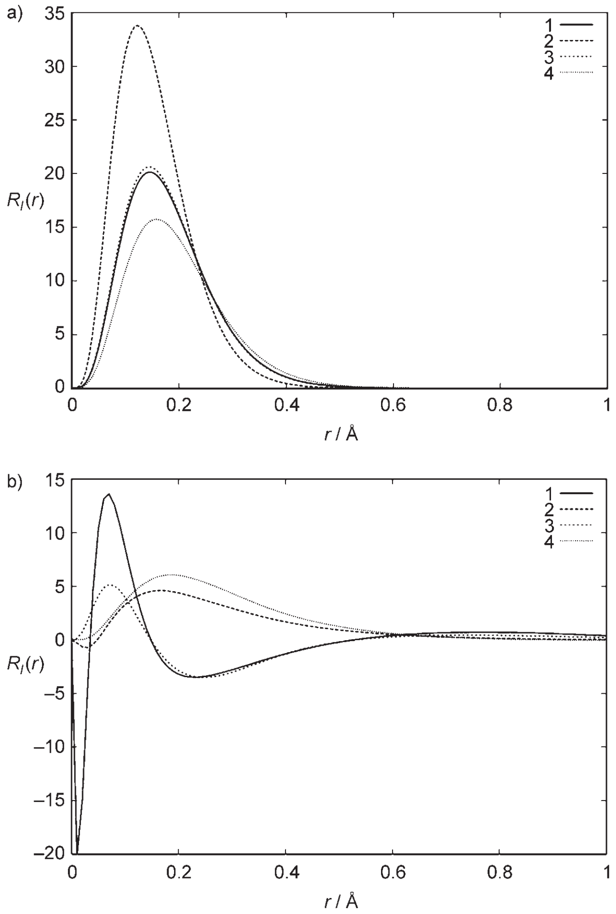

Figure 2. a) SZFIT and b) STOCK radial density functions ($R_l(r)$, for l = 0–4) of the Co^{III} ion, extracted from the theoretical (B3LYP/TZVP) electron density of the CACo(III)C model compound. For explanation of the function types, see text.

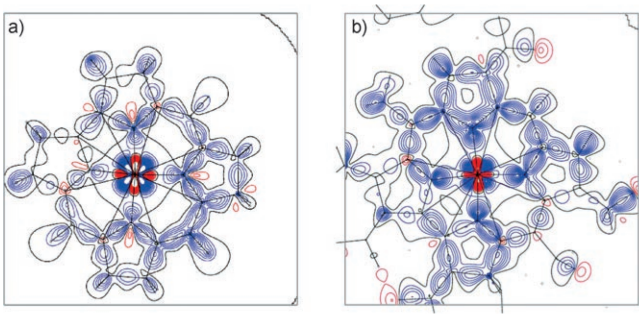

Figure 3. a) Deformation electron density extracted from the theoretical (B3LYP/TZVP) structure factors of the CACo(III)C model compound. b) Experimental static deformation electron density in the corrin ring of the vitamin B_{12} solvate, obtained after multipole refinement with SZFIT radial density functions. Positive, zero, and negative contour lines in blue, black, and red, respectively; contour interval is 0.1 e Å^{-3}.

orbital populations of the cobalt atom obtained using the different RDFs (Table 1). While both the SZFIT and the STOCK functions lead to the preferential occupation of the field-stabilized t_{2g} orbitals for both molecules, the contribution of the e_g orbitals to the bonding of the metal center is markedly stronger in the vitamin B_{12} solvate than in the model compound (Table 1). The relatively high populations of the d_{z^2} orbitals (the Z axis is along the Co–CN bond) obtained for the vitamin B_{12} solvate using both the SZFIT and the STOCK functions suggest a significant σ contribution to the axial bonds. This hypothesis is supported by the topological analysis, which gives ρ(BCP) values of decreasing magnitude for the Co–CN, Co–N_{eq}, and Co–N_{ax} bonds (Table 2), in accord with the results of the DFT calculation on CACo(III)C. The difference between the ρ(BCP) values of the two axial bonds is the most pronounced for the STOCK functions; the relatively high ED concentration in the Co–CN bond explains its stability, although its BCP lies in the region where the Laplace function is positive, which is characteristic of metal–ligand interactions.

Table 1: d-Orbital populations [%] derived from the theoretical electron density of the CACo(III)C model compound and from the X-ray diffraction data of the vitamin B_{12} solvate, using different radial density functions.

	CACo(III)C, B3LYP/TVZP		B_{12}, Experiment	
d Orbital	SZFIT	STOCK	SZFIT	STOCK
z^2	8	0	17	14
x^2-y^2	0	10	10	7
e_g	**8**	**10**	**27**	**21**
xy	50	26	23	25
yz	40	42	26	28
zx	2	22	24	26
t_{2g}	**92**	**90**	**73**	**79**

Table 2: Theoretical and experimental properties at the bond critical points of the Co–N and Co–C bonds.[a]

Bond	ρ(BCP) [e Å^{-3}]	$\nabla^2\rho$(BCP) [e Å^{-5}]
Co–C_{ax}	0.89	3.8
	0.83	13.6
	0.93	10.5
Co–N_{ax}	0.65	8.7
	0.41	9.3
	0.37	10.9
Co–N_{eq}	0.76	8.6
	0.66	14.1
	0.71	13.6

[a] For each bond type, first row: theory (B3LYP/TZPV); second row: experiment (SZFIT); third row: experiment (STOCK). The standard uncertainties for ρ(BCP) and $\nabla^2\rho$(BCP) are 0.01 e Å^{-3} and 0.1 e Å^{-5}, respectively. Entries for the Co–N_{eq} bond type are average values for the four equatorial bonds.

Herein, we have shown that a conventional X-ray diffraction experiment can, indeed, yield data of sufficient quality for the charge density to be determined, even for a relatively large structure, such as that of the present solvate of vitamin B_{12}, which has more than 250 atoms in the asymmetric unit. The interpretation of the data benefited from a combination of the pseudoatom formalism and quantum-chemical methods. The initial invariom refinement led to reliable atomic coordinates and displacement parameters not only for the vitamin B_{12} molecule, but also for the solvent molecules, including the disordered propanol molecule. More importantly, we demonstrated that, for chemically bound atoms, theoretically derived RDFs are superior to those obtained from calculations on isolated atoms, even if the differences in the basis sets do not manifest themselves markedly in the usual figures of merit. Efforts are also in progress to evaluate atomic properties of vitamin B_{12} on the basis of the ED of the solvate. A comparison with analogous results for the coenzyme Ado-Cbl, for which single crystals with sufficiently good diffraction properties must be grown, is in preparation.

Experimental Section

Single crystals were obtained by slow diffusion of *n*-propanol into a saturated aqueous solution of vitamin B_{12}. A series of 14 runs, each consisting of 606 frames, was collected on a CCD diffractometer (Bruker) using $Mo_{K\alpha}$ radiation from a sealed 2.4 kW tube.[20] ω Scans were performed at 2θ settings (and exposure times) of 0 (10), −30 (20), −60 (83), and −90° (180 s). To increase the redundancy of the data, an additional ϕ scan at a 2θ setting of 60° was added. The step width was 0.3° in ω or ϕ. The measurement strategy was determined with ASTRO,[30] the measurement was controlled with SMART,[30] and the integration and reduction of the data were carried out with SAINT[30] and SORTAV.[31]

660822 reflections were measured: 98204 were unique (redundancy 6.7), of which 70663 had $F_o > 3\sigma(F_o)$. An analytical absorption correction was carried out with the program Euhedral,[34] which gave an internal R factor of 0.036. The structure was solved with SHELXS-97[32] and spherically refined with SHELXL-97[24] to an R_1(spherical) value of 0.048. The multipole refinement yielded an R_F value of 0.041 and an R_w value of 0.034. CCDC-614012 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Received: September 22, 2006

Keywords: charge density · cobalamins · computer chemistry · vitamin B_{12} · X-ray diffraction

---

[1] P. Coppens, *X-ray Charge Densities and Chemical Bonding*, Oxford University Press, Oxford, **1997**.

[2] C. Jelsch, V. Pichon-Pesme, C. Lecomte, A. Aubry, *Acta Crystallogr. Sect. D* **1998**, *54*, 1306–1318.

[3] D. Housset, F. Benabicha, V. Pichon-Pesme, C. Jelsch, A. Maierhofer, S. David, J. C. Fontecilla-Camps, C. Lecomte, *Acta Crystallogr. Sect. D* **2000**, *56*, 151–160.

[4] C. Jelsch, M. M. Teeter, V. Lamzin, V. Pichon-Pesme, R. H. Blessing, C. Lecomte, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3171–3176.

[5] C. Lecomte, B. Guillot, N. Muzet, V. Pichon-Pesme, C. Jelsch, *Cell. Mol. Life Sci.* **2004**, *61*, 774–782.

[6] T. Koritsanszky, P. Coppens, *Chem. Rev.* **2001**, *101*, 1583–1627.

[7] C. Kratky, B. Kräutler, *Chemistry and Biochemistry of B_{12}*, **1999**, Wiley, New York.

[8] R. G. Matthews, *Acc. Chem. Res.* **2001**, *34*, 681–689.

[9] L. G. Marzilli in *Bioinorganic Catalysis* (Eds.: J. Reedijk, E. Bouwman), Marcel Dekker, New York, **1999**, p. 423.

[10] B_{12} (Ed.: D. Dolphin), Wiley, New York, **1982**.

[11] See, for example: S. H. Kim, H. L. Chen, N. Feilchenfeld, J. Halpern, *J. Am. Chem. Soc.* **1988**, *110*, 3120–3126; M. F. Summers, P. J. Toscano, N. Bresciani-Pahor, G. Nardin, L. Randaccio, L. G. Marzilli, *J. Am. Chem. Soc.* **1983**, *105*, 6259–6263; L. Randaccio, N. Bresciani-Pahor, E. Zangrando, L. G. Marzilli, *Chem. Soc. Rev.* **1989**, *18*, 225–250.

[12] L. Ouyang, L. Randaccio, P. Rulis, E. Z. Kurmaev, A. Moewes, W. Y. Ching, *J. Mol. Struct.* **2003**, *622*, 221–227.

[13] E. Z. Kurmaev, A. Moewes, L. Ouyang, L. Randaccio, P. Rulis, W. Y. Ching, *Europhys. Lett.* **2003**, *62*, 582–587.

[14] L. Ouyang, P. Rulis, W. Y. Ching, G. Nardin, L. Randaccio, *Inorg. Chem.* **2004**, *43*, 1235–1241.

[15] A. Holladay, P. Leung, P. Coppens, *Acta Crystallogr. Sect. A* **1983**, *39*, 377–387.

[16] R. F. W. Bader, *Atoms in Molecules: A Quantum Theory*, Oxford University Press, Oxford, **1990**.

[17] P. Macchi, L. Garlaschelli, S. Martinengo, A. Sironi, *J. Am. Chem. Soc.* **1999**, *121*, 10428–10429.

[18] A. Volkov, Yu. Abramov, P. Coppens, C. Gatti, *Acta Crystallogr. Sect. A* **2000**, *56*, 332–339.

[19] N. K. Hansen, P. Coppens, *Acta Crystallogr. Sect. A* **1978**, *34*, 909–921.

[20] Crystal and experimental data for the solvate of vitamin B_{12} (cyanocobalamin·12 water·3 propanol): $C_{63}H_{88}CoN_{14}O_{14}P\cdot 12\,H_2O\cdot 3\,C_3H_7OH$, 100 K, crystal size: $0.4\times 0.26\times 0.2\ mm^3$, orthorhombic, space group $P2_12_12_1$, $a=15.831(4)$, $b=22.374(5)$, $c=25.304(6)$ Å, $V=8963(4)$ Å^3, $Z=4$, $\rho_{calcd}=1.30\ g\,cm^{-3}$, $\mu(Mo_{K\alpha})=0.29\ mm^{-1}$, $\lambda(Mo_{K\alpha})=0.7107$ Å, $(\sin\theta)/\lambda_{max}=1.22$ Å^{-1}.

[21] B. Dittrich, T. Koritsanszky, P. Luger, *Angew. Chem.* **2004**, *116*, 2773–2776; *Angew. Chem. Int. Ed.* **2004**, *43*, 2718–2721.

[22] B. Dittrich, C. B. Hübschle, M. Messerschmidt, R. Kalinowski, D. Girnt, P. Luger, *Acta Crystallogr. Sect. A* **2005**, *61*, 314–320.

[23] T. Koritsanszky, T. Richter, P. Macchi, A. Volkov, C. Gatti, S. Howard, P. R. Mallinson, L. Farrugia, Z. Su, N. K. Hansen, XD, A Computer Program Package for the Multipole Refinement and Topological Analysis of Electron Densities from Diffraction Data, **2003**.

[24] G. M. Sheldrick, SHELXL-97, A Program for the Refinement of Crystal Structures, University of Göttingen (Germany), **1997**.

[25] Supporting information on the invariom application is available upon request. It contains a comparison of the figures of merit for the spherical and invariom refinements, invariom names, and the model compounds used.

[26] Gaussian 98 (Revision A.7), M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. G. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Replogle, J. A. Pople, Gaussian, Inc., Pittsburgh (USA), **1998**.

[27] F. H. Allen, *Acta Crystallogr. Sect. B* **1986**, *42*, 515–522.

[28] E. Clementi, C. Roetti, *At. Data Nucl. Data Tables* **1974**, *14*, 177–478.

[29] T. Koritsanszky, A. Volkov, *Chem. Phys. Lett.* **2004**, *385*, 431–434.

[30] ASTRO, **1995–1996**, SMART, **1996**, SAINT, **1994–1996**, Bruker-AXS Inc., Madison (USA).

[31] R. H. Blessing, *Acta Crystallogr. Sect. A* **1995**, *51*, 33–38.

[32] G. M. Sheldrick, SHELXS-97, A Program for Crystal Structure Solution, University of Göttingen (Germany), **1997**.

[33] E. Keller, SCHAKAL97, A Fortran Program for the Graphical Representation of Molecular and Crystallographic Models, University of Freiburg (Germany), **1997**.

[34] M. Lutz, Euhedral Tutorial, Universität Utrecht, **2003**.