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COMMUNICATIONS

Published on Web 10/14/2003

MAS NMR Structure of a Microcrystalline Cd-Bacteriochlorophyll *d* Analogue

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Recent studies demonstrate that modern MAS NMR techniques allow the structure determination of uniformly labeled aggregates, disordered systems, and polypeptides.[1−4] Similar to established methodology for structure elucidation of proteins in the dissolved state, distances or torsion angles between the nuclei can be measured and used for restraining of possible conformations. In solution, however, the packing of molecules is of little interest, and "families" of molecular conformations are resolved. This contrasts with a genuine solid state, where the space filling arrangement is essential. Thus, while the structure determination of molecules in solution can follow a purely bottom-up approach, in the microcrystalline solid state the top-down restrictions following from the requirement to fill up space in an orderly fashion have to be taken into account in the construction of a structural model from MAS NMR data.[3]

Here, we acquire MAS NMR data of a uniformly ^{13}C- and ^{15}N-labeled Cd-bacteriochlorophyll *d* analogue (Figure 1), a derivative of the natural BChl *c* in the chlorosomes of green photosynthetic bacteria.[5] Ultimately, the study of these modified aggregates will help to explain the self-organization process in vivo. Various spectroscopic techniques have provided converging evidence for Cd···OH···O═C bonding, similar to the Mg···OH···O═C moieties in the natural system.[6] From low-resolution diffraction data, a distance between the molecular planes of 6.4 Å was resolved.

First, an assignment of the ^{1}H and ^{13}C resonances was obtained with 2D and 3D dipolar correlation spectroscopy. The ^{13}C NMR line widths before apodization are 220−300 Hz in a magnetic field of 17.6 T and are essentially the same in a field of 9.4 T. This shows that there is little heterogeneous line broadening due to bulk susceptibility effects. In addition, a single set of peaks is detected. These observations imply local crystalline order with the same unique structural environment for every molecule.

From the MAS NMR signal from ^{113}Cd, which has a natural abundance of 12%, the chemical shift anisotropy (CSA) principal elements were extracted. The CSA shows that the Cd is strongly interacting with a fifth ligand, and the asymmetry parameter $\eta \approx 0.25$ reveals a nonaxial coordination.[7−9] In addition, the ^{1}H and ^{13}C aggregation shifts, defined as the chemical shifts in the solid state relative to the corresponding shifts of the monomer in solution, reveal ring current effects that are very useful for structure determination.[10] The ^{1}H aggregation shifts are mainly due to ring currents and are exceptionally strong, up to 10 ppm in the upfield direction. These results can be accommodated by forming parallel stacks with the 3^{1}-OH groups coordinated to the Cd of the neighboring molecule and sheets due to hydrogen bonding between the 3^{1}-OH functionalities of one stack and the 13-C═O groups of another. The structure was optimized by the MM+ force field, since ab initio methods do not yet describe the weak interactions governing the intermolecular arrangement of this system satisfactory, while calculations at the force field level have been validated for large chlorophyll aggregates.[11,12] Subsequently, a ^{1}H ring current shift calculation was done, where contributions of all macrocycles within a radius of 24 Å were added. The rings were approximated by circular loops, where the shielding of a single macrocycle was calculated by a DFT calculation at the B3LYP/6−311 g(d,p) level. The calculation reproduces the experimental ring current shifts of the ^{1}H atoms with a standard deviation of the calculated versus the observed values of 1.2 ppm on a range of 11.6 ppm, while the correlation coefficient equals 0.98. In this way, the shift data reveal a unique densely packed structure of overlapping macrocycles arranged in sheets of parallel inclined stacks (Figure 3A). This structure was confirmed by a CHHC experiment (Figure 2). Here ^{13}C−^{13}C correlations are generated by ^{1}H spin diffusion, and close intermolecular contacts can be detected, providing direct evidence about the molecular arrangement.[13] For instance, a correlation between the 12-Me and the 3-CH_2OH moieties is in excellent agreement with the proposed sheet structure, since the 3^{1}-OH's are

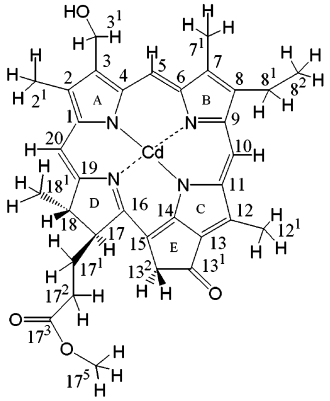

Figure 1. Chemical structure of the Cd-bacteriochlorophyll *d* analogue.

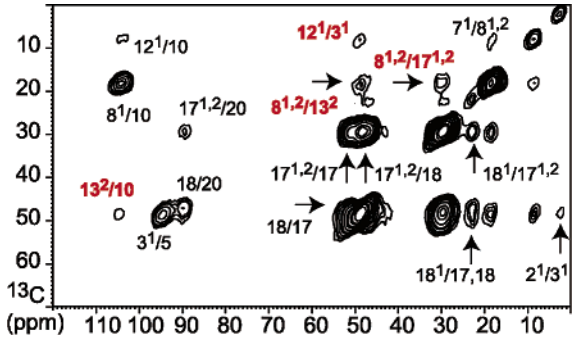

Figure 2. ^{13}C−^{13}C CHHC spectrum recorded in a field of 9.4 T using a mixing time of 200 μs. The intermolecular correlations are indicated in red.

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10.1021/ja0367492 CCC: $25.00

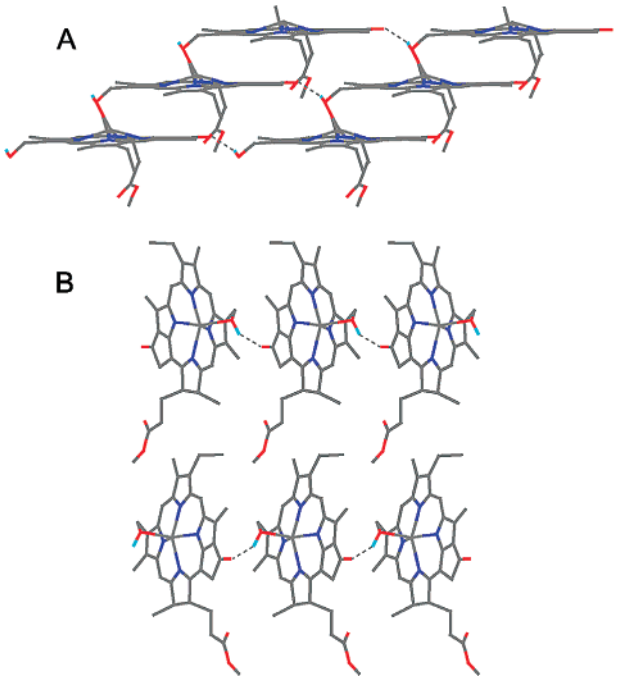

Figure 3. Crystalline structure projected along the *b*-axis (A) and the *c*-axis (B).

close to the 12-Me when sheets are formed by OH···O═C hydrogen bonding (see also Figure 3B). The CHHC experiment also reveals close contacts between the 8-Et region and the 17-propionate region. This points to a head-to-tail arrangement of the sheets.

In a final modeling step, multiple layers were brought within van der Waals contact in a head-to-tail orientation as indicated by the NMR results. A large aggregate containing 60 molecules was optimized. Two possible arrangements transpire that are both in accordance with the NMR, where the mutual orientation of adjacent sheets is either similar, corresponding to a triclinic structure, or with a 180° screw axis, corresponding to a monoclinic structure. Both structures were found to be stable in the modeling. The former, however, is energetically less likely due to unfavorable electric dipole interactions. According to our calculations, the electric dipole moment in the aggregates has an estimated strength of 5.1 Db and is directed ca. 30° out of the ring plane. With the screw axis, the components perpendicular to the rings are antiparallel in neighboring sheets. In the force field approximation, the energy of the monoclinic structure is a modest ∼0.5 kcal per molecule lower than for the triclinic structure.

From the optimized monoclinic 60-mer, the crystal cell parameters were extracted. It has space group $P2_1$ with $a = 14.3$ Å, $b = 27.3$ Å, $c = 6.4$ Å, $\beta = 147.2°$ and $Z = 2$. A projection along the *b*-axis is depicted in Figure 2, which corresponds to one layer of Chlide. The Cd−Cd distance is 6.4 Å, which has also been observed by electron diffraction.[6] Figure 3 represents a projection along the *c*-axis, showing the contact between layers. The structure of a layer is well determined and the error in the position is estimated to be 0.2 Å. The error in the relative position of weakly bound neighboring layers is more difficult to quantify within the force field approach. The total structural model is provided as Supporting Information in CIF format. The structure determined for the Cd-bacteriochlorophyll *d* analogue compares well with structures of well-ordered chlorophyllide crystals that have been resolved with X-ray diffraction. For instance, in hydrated chlorophyllide *a* the rings are in a pseudohexagonal net, where ring A of one molecule overlaps with rings C and E of its translationally equivalent neighbor.[14] Water molecules act as cross-linkers both by coordinating to a central Mg metal and by hydrogen bonding to a neighboring ring E carbonyl. Here, the 3-hydroxymethyl group introduces an additional functionality, leading to the network in Figure 3A. Since water is not present as a cross-linker and due to the strong interaction, the volume per molecule has the relatively small value of ∼667 Å^3, compared to, for example, 864 Å^3 for ethyl chlorophyllide *a* dihydrate. In the chlorophyllide *a* crystals, the absence of long tails also results in a back-to-tail orientation as visible in Figure 3B, and two-fold or three-fold screw axes are observed there between the neighboring layers.[14] For self-assembled chlorophyll *a*/H_2O, on the other hand, the sheets orient back-to-back as a result of the long farnesyl tails that interdigitate in bilayers.[13]

In conclusion, we have shown that a detailed structural model of a microcrystalline structure can be obtained with MAS NMR refined with molecular modeling, and we expect that this study is relevant to future applications in nanotechnology or structural research.

Acknowledgment. Financial support is acknowledged from the Human Frontier Science Program RG0184/1997-M, Japanese MEXT and JSPS, and Demonstration Project BIO4-CT97-2101 of the Commission of the European Communities. We thank J. Hollander, C. Erkelens, and T. Miyatake for assistance during the experimental work. J.M. acknowledges a Casimir-Ziegler award of the Academies of Sciences in Amsterdam and Düsseldorf. H.J.M.d.G. is a recipient of a PIONIER award of The Netherlands Organization for Scientific Research (NWO).

Supporting Information Available: Crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0367492