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Paramagnetic Ions Provide Structural Restraints in Solid-State NMR of Proteins

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X-ray and NMR can provide structures of proteins in the crystalline state or in solution, respectively. Solid-state (SS) NMR is a growing technique, especially suited for membrane and insoluble proteins and proteins that do not properly crystallize. However, progress in this field has been slow because of the paucity of structural restraints. We show here that protein-bound paramagnetic metal ions can provide a significant number of additional structural restraints, in the form of pseudocontact shifts (pcs). SS NMR on paramagnetic molecules has occasionally been pursued since 1983,[1−5] and more recently, the interaction of substrates with the paramagnetic cytochrome P450 has been studied,[6] and the spectra of a copper(II) protein have been reported.[7]

For our purpose, we used a high-spin cobalt(II) protein; cobalt(II) has total spin quantum number $S = 3/2$, is highly paramagnetic, and possesses sufficient magnetic anisotropy to provide significant pcs in solution.[8] The protein of choice is the 159 AA catalytic domain of matrix metalloproteinase 12 (MMP-12). This protein contains a zinc(II) ion at the catalytic site that can be substituted by cobalt(II).[9] The CP-MAS PDSD[10] spectra of the microcrystalline zinc protein are of excellent quality and permit an essentially complete assignment.[11] The assignment of CoMMP-12 in solution has been also performed in this work, and although its obtainment was not the main objective, it is at the moment more than 90% complete.

The CP-MAS PDSD spectra of microcrystalline CoMMP-12 are also of very good quality and show an increase in the number of observable signals on passing from 8.5 to 11.5 kHz MAS frequency. At MAS frequency of 11.5 kHz, carbon signals from about 85% of the amino acids could be detected (Figure 1a), and more than 70% of these can be assigned by comparison with the solution spectra of CoMMP-12 and by recognizing the spin diffusion pattern for each amino acid (Table S1).

By comparing the SS spectra of the zinc(II) and the cobalt(II) derivatives, it appears that many ^{13}C signals experience shift differences, as illustrated in Figure 1b and Figure S1, which we assign as pcs. This is the first time that pcs are detected for a paramagnetic protein in the solid state. They are apparently easy to measure, and we now aim at assessing whether they can be used as a quantitative structural tool in SS NMR.

We recall here the equation for the pcs:

$$\delta^{pc} = \Delta\chi_{ax}\frac{(3\cos^2\theta - 1)}{r^3} + \frac{3}{2}\Delta\chi_{rh}\frac{\sin^2\theta\cos 2\varphi}{r^3} \qquad (1)$$

where $\Delta\chi_{ax}$ and $\Delta\chi_{rh}$ are the axial and rhombic components of the magnetic susceptibility tensor anisotropy; r is the metal−nucleus distance, and θ and φ are the polar angles describing the orientation of the metal−nucleus vector with respect to the principal axes of the χ tensor. This equation is also expected to hold in the solid state.[12]

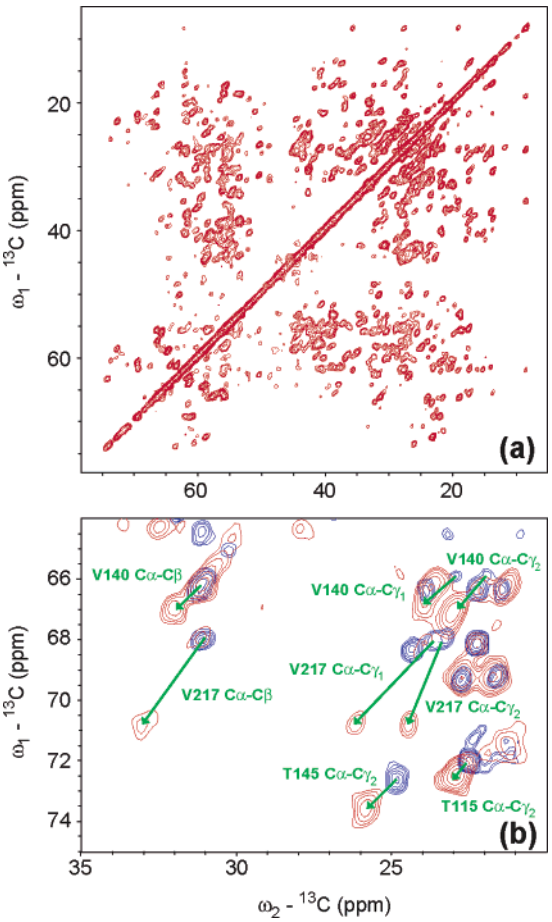

Figure 1. (a) $^{13}C-^{13}C$ CP-MAS PDSD spectrum of a microcrystalline sample of cobalt(II)-substituted MMP-12 (16.4 T, $\omega_R/2\pi = 11.5$ kHz, mixing time 60 ms, 290 K). (b) Superposition of the $^{13}C-^{13}C$ CP-MAS PDSD spectra of the diamagnetic zinc MMP-12 (blue, 16.4 T, $\omega_R/2\pi = 11.5$ kHz, mixing time 15 ms) with the paramagnetic CoMMP-12 (red). Green arrows indicate the paramagnetic shifts. Note the large displacements (ca. 2 ppm) of the V217 Cα−Cβ, Cα−Cγ_1 cross-peaks.

As many as 246 pcs values are measured on ^{13}C signals. They are reported in Figure S2 and in Table S1 and compared with the solution pcs. It appears that many pcs are in good agreement with solution pcs, indicating that they are indeed quantitative structural tools also in the solid state. A large majority of the observed pcs

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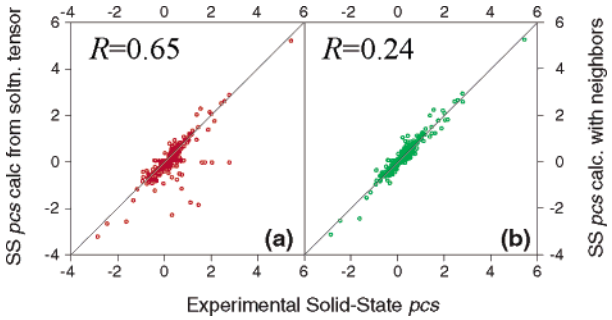

Figure 2. (a) Experimental solid-state pcs against pcs calculated from solution tensor. (b) Experimental solid-state pcs against pcs calculated from solution tensor by taking into account also the intermolecular contributions arising from neighboring molecules. R is calculated as $R = \sqrt{[\Sigma(\delta_{calcd} - \delta_{exptl})^2/\Sigma(\delta_{exptl})^2]}$.

can actually be reproduced well by recalculating them from eq 1 using magnetic susceptibility tensor parameters obtained from the solution data on CoMMP-12 (Figure 2a). However, about 10% of them exhibit substantial deviations. This is because cobalt(II) ions of nearby molecules also contribute to the observed pcs. By assuming that the magnetic susceptibility tensor is the same as that in solution, and by taking the reciprocal dispositions of the other cobalt(II) ions as well as the orientations of the corresponding tensors in the surrounding molecules in the crystal from X-ray data,[13] the additional contributions to the pcs from the surrounding metals can be calculated and summed up. The shifts calculated in this way are reported in Figure 2b, Figure S2, and Table S1.

It is apparent that they are all in very good agreement with the observed ones. Although as many as 50 neighboring cobalt(II) ions have been included in the calculations, only two of them provide most of the additional contributions (see Supporting Information). Therefore, the intermolecular contribution can be sufficiently reduced in paramagnetic-diluted solid solution,[3,4] for example, in this case, cocrystallizing about 30% of the paramagnetic labeled protein with 70% of the diamagnetic, unlabeled protein. We calculate that this dilution would be affordable in terms of sensitivity and would be sufficient, by direct comparison with a sample containing 100% of paramagnetic labeled protein, to recognize cross-peaks that are shifted only by the internal metal ion (see details in the Supporting Information).

The number of restraints per residue used for SS NMR structural determinations ranges from 2−3[14] to 4−5[10] for small proteins to about 7[15] for an 11 AA peptide. They are also difficult to obtain from the experimental data.[15,16] Given these numbers, pcs could sizably increase the number of restraints and be used simultaneously with the other restraints to solve the solid-state structure in the same way as it has been shown in solution,[8,17,18] using programs such as PARACYANA[8] or ParaXplor-NIH.[18]

In our view, these findings may open new perspectives for protein structural determination in the SS NMR because of the following: (i) It is conceivable to use pcs as structural restraints in the solid state by exploiting any metal ion that possesses sizable magnetic anisotropy, such as the very popular lanthanide ions; this also holds for all proteins to which paramagnetic metal binding tags are attached, as this is an expanding field in solution NMR.[19−21] (ii) The effect of paramagnetic centers on nearby molecules is a further source of information. Fibril samples displaying one-dimensional order would be a relevant case, as intermolecular pcs could provide information on the relative arrangement of protein molecules in the fiber. The intrinsic long-range nature of pcs would be ideal for this type of application. (iii) As pcs do not depend on the nucleus, and ^{1}H nuclei are increasingly exploited in the solid state,[22−24] the advantage of using pcs as restraints will be easily extended to protons.

It should be noted that, in solution, an important source of line broadening in paramagnetic molecules, akin to CSA relaxation, is Curie relaxation,[25] which depends on molecular tumbling. Such negative effect is largely not operative in SS NMR (see Supporting Information).

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Supporting Information Available: Experimental Part, Figures S1−6, and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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