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Solid-state NMR study of the SH3 domain of α-spectrin: application of $^{13}C-^{15}N$ TEDOR and REDOR†

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ABSTRACT: A fully $^{13}C-^{15}N$-labeled and a selectively alanine-$^{13}C^{\beta}$ tryptophan-$^{15}N^{ring}$-labeled sample of the Src homology region 3 (SH3) domain of α-spectrin (chicken), a 62 residue protein, were biosynthesized and studied by solid-state cross-polarization magic angle spinning (CP/MAS) NMR, $^{13}C-^{15}N$ rotational echo double resonance (REDOR) and $^{15}N-^{13}C$ transferred echo double resonance (TEDOR) spectroscopy. In the first part of the study it is shown that spectral editing with the TEDOR sequence leads to a drastic simplification of the ^{13}C MAS spectrum of the fully labeled sample, allowing the resolved spectroscopy of groups of ^{13}C nuclei, according to their distance to neighboring ^{15}N nuclei. In the second part of the study the inter-residual distance between the alanine residue Ala55 and the tryptophan residue Trp42 was determined by the measurement of the dipolar coupling between Ala-$^{13}C^{\beta}$ and Trp-$^{15}N^{ring}$, yielding a dipolar coupling of 48 ± 8 Hz, which after correction for fast molecular vibrations gives a value of 53 ± 8 Hz, corresponding to a CN distance of 3.85 ± 0.25 Hz. The result is compared to the CN distances obtained by x-ray diffraction and liquid-state NMR. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: SH3 domain; α-spectrin; protein; TEDOR; REDOR; spectral editing; chemical editing; CN distances; dipolar solid-state NMR

INTRODUCTION

The biological function of a protein is mainly determined by two factors, the primary structure, i.e. its amino acid sequence, and the geometrical arrangement of the amino acid chain and the side groups, i.e. its secondary and higher structures.[1] The primary structure of peptides and proteins of interest is known in general or can be determined by standard techniques. The determination of the higher structures is more elaborate. If suitably crystallized samples of the protein are available, it is possible to determine these higher structures by x-ray diffraction. An alternative approach is multi-dimensional liquid-state NMR spectroscopy of dissolved molecules.[2,3] Both of these methods have their limitations: on the one hand, many proteins are not available in single-crystal form, for example owing to poorly crystallizing behavior. On the other hand, proteins often exceed the range of molecular weights suitable for liquid-state NMR studies, are only poorly soluble or tend to aggregate. Furthermore, the important group of membrane embedded proteins cannot be analyzed by x-ray diffraction or solution NMR.

Solid-state NMR spectroscopy[4,5] can help in structural studies of these classes of proteins. Because of the special experimental requirements of this type of NMR, this approach is still under development. Two major problems have to be solved before a successful solid-state structural analysis of a protein is feasible. First, the spectral resolution has to be increased to achieve a resolution comparable to the resolution in liquid-state NMR. Second, techniques for monitoring neighbor relationships of different amino acid residues have to be developed, applied and combined with efficient techniques for distinguishing between the contributions from different types of interactions.

One approach for the study of proteins with solid-state NMR relies on orienting the sample with respect to the magnetic field B_0, e.g. by embedding the protein in a membrane. Orientation constraints are derived from dipolar couplings and chemical shift interactions, the values of which depend on the relative alignment of the molecule with respect to the external field.[6,7] This technique is restricted to a limited class of proteins. Therefore, magic angle spinning (MAS)[8,9] NMR techniques and in particular the cross-polarization (CP) MAS experiment[10,11] have provided the major means to solve the resolution problem. The linewidths typically obtainable by MAS are in most cases still an order of magnitude worse than those typically found in liquid-state NMR. Only very recently with the development of high-speed ^{13}C CP/MAS probes and high-field solid-state NMR spectrometers do linewidths suitable for real high resolution in the solid state seem to be achievable.

† This paper is dedicated to Professor Dr Harald Günther on the occasion of his 65th birthday.

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