Chapter 6

Summary
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The aim of the work described in this thesis was to investigate how residual dipolar couplings can be used to resolve or refine the three-dimensional structure of one of the proteins of the phosphoenol-pyruvate phosphotransferase system (PTS), the main transport system for carbohydrates in Gram-positive and Gram-negative bacteria (see Chapter 1). Dipolar couplings are potentially quite large interactions, caused by the magnetic flux lines of one nucleus (e.g. nucleus P) affecting the magnetic field at the position of another nucleus (e.g. nucleus Q). For a fixed relative orientation of the spins P and Q, the magnetic moment of spin P can decrease or increase the total magnetic field at nucleus Q, depending on whether the magnetic moment of spin P is parallel or antiparallel to the magnetic field $B_0$ (in the case of spin-$\frac{1}{2}$ nuclei). In an ensemble of molecules in thermal equilibrium half of the P nuclei will be parallel to $B_0$ and the other half antiparallel, so Q will show two resonances separated in frequency by the dipolar coupling:

$$D_{PQ} = D_{PQ}^{\text{max}} \langle 3\cos^2\theta-1 \rangle/2$$

where $\theta$ is the angle between the internuclear vector and $B_0$. The brackets $\langle \rangle$ denote time or ensemble averaging and usually, in solution NMR, the $3\cos^2\theta-1$ term for any pair of nuclei averages to zero by the isotropic molecular tumbling and no dipolar couplings can be measured (Prestegard, 1998). This situation changed when Tjandra and Bax demonstrated the use of a liquid crystalline medium to introduce a tunable degree of alignment (Tjandra & Bax, 1997), thereby making it possible to measure residual dipolar couplings in solution NMR of proteins dissolved in such a medium. Since then a number of alternative orienting media has been proposed. An overview of these media and their properties is presented in chapter 2, which will be useful for deciding which medium will be best suited for a certain protein under certain conditions.

Once a suitable medium has been found, residual dipolar couplings can be measured between suitable pairs of nuclei. The modified COCAH pulse sequence described in chapter 3 is expected to be valuable for measurements of scalar and dipolar $C_\alpha H_\alpha$ and $C_\alpha C'$ couplings in proteins. The in-phase-antiphase (IPAP) strategy that has been built in and the use of three dimensions reduces spectral overlap. The modified COCAH will also be helpful if the $N_{i+1}$ resonance can not be measured in an HNCO or (HA)CA(CO)NH based experiment. The $C_\alpha H_\alpha$ and $C_\alpha C'$ coupling of the last residue of a protein and of the residues preceding a proline, for example, can be measured using the modified COCAH but not with an HNCO or (HA)CA(CO)NH based experiment.

After the measurement of residual dipolar couplings in a protein of known three-dimensional structure, an order parameter tensor can be calculated. The order-parameter tensor gives information about the orientation of the biomolecule relative to the magnetic field. Chapter 4 describes the differences between the orientation of HPr-WT and HPr-F48W in KP$_4$-buffers of varying concentrations. We discovered that the orientation of the mutant protein F48W depends on the phosphate concentration in contrast to the...
orientation of the wildtype protein, which is independent of the phosphate concentration. The difference between the orientation of the mutant and the wildtype proteins can be explained by interactions between the bicelles and the protein HP-Pr-F48W. The method used in this study may be useful in studies of weak binding interactions between membrane-bound proteins or protein fragments, embedded in the bicelles, and soluble proteins. The effect of the membrane-bound protein or protein fragment on the orientation of the soluble protein, may yield valuable information about the binding interface.

A partial assignment (1H, 15N, 13C', 13Cα and 13Cβ) for the protein IIB^mtl is presented in chapter 5. 17 Cross-peaks were missing in the HSQC spectrum, presumably due to conformational-exchange broadening. Only one of the cross-peaks in the spectrum could not be assigned. The secondary structure of the protein was predicted using the 13C', 13Cα and 13Cβ secondary chemical shifts. Helices were proposed for residues 36-51, 81-87 and 102-117 and β-strands range from residue 25-31, 57-62, 73-76 and 94-98. Based on a correspondence in secondary structure around the active site, we propose that the active site of IIB^mtl resembles that of IIB^chb. In both proteins, this active site is located at the end of the first β-strand and is followed by a small loop and an α–helix. In addition, another active-site residue (Tyr 105 in our construct, corresponding to Tyr 84 in IIB^chb) takes up the same position in both proteins, both in the primary structure relative to the active site and in the proposed secondary structure. In the next paragraph some suggestions will be given to resolve the three dimensional structure of IIB^mtl.

Prospects

The logical next step in the NMR study of IIB^mtl is the determination of its three-dimensional solution structure. This can be done in a conventional way by collecting NOE data or by measuring residual dipolar couplings. For measuring residual dipolar couplings an orienting medium is necessary (see Chapter 2). Because IIB^mtl is positively charged at pH 7, an uncharged or positively charged medium that is stable at pH 7 is required, e.g. an uncharged or positively charged polyacrylamide gel. Probably, the uncharged phospholipid mixtures are not a good alternative, because proteins with a very flexible part (e.g. a his-tag) have a tendency to interact with the phospholipid bilayers (Gronenborn, 2002). We already tested the usefulness of one other uncharged medium, C8E5-octanol, but in this case the protein was unstable. We expect that the determination of the one-bond 15N-1H, 13Cα-13C' and 13Cα'1Hα backbone dipolar couplings will be sufficient to establish the three-dimensional backbone fold of IIB^mtl. For this purpose a program has recently been developed in our research group (P.F.Buur, to be published) that can be used to search through a database of protein models (obtained by X-ray diffraction or NMR or by any other means) for a model that is compatible with the measured residual dipolar couplings. The program takes into account the possibility of one or more extra stretches of residues that are missing or extra in the database model, compared to the protein under investigation. Of course, a suitable model of a sufficiently similar protein must be present in the database for this approach to be successful.
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Refinement of the model can then be done using the program written by B. Hess (Hess & Scheek, 2003).

References


