Journal of Biomolecular NMR, **14:** 23–32, 1999.
KLUWER/ESCOM

Use of organic solvents and small molecules for locating binding sites on proteins in solution*

Claudio Dalvit**, Philipp Floersheim, Mauro Zurini & Armin Widmer
NOVARTIS Pharma AG, CH-4002 Basel, Switzerland

Received 18 January 1999; Accepted 16 February 1999

Key words: FK506 binding protein, multiple solvent suppression, organic solvents, protein–ligand interactions, 3D NMR structure

Abstract

Application of a modified ePHOGSY and other novel NMR experiments to an H_2O-DMSO solution of the protein FKBP12 identified the presence of one molecule of DMSO bound in the substrate binding site. It occupies the same spatial region occupied by the pipecolidine moiety of the immunosuppressive drugs FK506 and Rapamycin complexed to the protein. The binding constant K_D for this DMSO molecule was only 275 mM. A substructure search of small molecules similar to DMSO resulted in the identification of molecules with improved binding affinity. This work represents a clear example of the powerful interplay of molecular modelling and NMR.

Introduction

Structural studies of the interactions of organic solvents and small molecules with a target protein are of crucial importance in the so-called rational-based drug design (Allen et al., 1996; Shuker et al., 1996). The organic solvents contain functional groups which are present in more complex molecules. Neutron and X-ray diffraction studies have shown that organic solvents can bind in the substrate binding sites, inhibiting the catalytic activity of an enzyme, and in other pockets which are not occupied by the substrates. The knowledge of the intermolecular interactions in these secondary pockets is important in the design of inhibitors with greater specificity for the target protein. Several physical–chemical techniques have been used for deriving the thermodynamical and structural properties of the interaction of organic solvents with proteins (Douzou and Petsko, 1984; Lehmann et al., 1985; Lehmann and Stansfield, 1989; Mattos and Ringe, 1996). Recently NMR has emerged as a powerful technique for studying these interactions (Liepinsh and Otting, 1997; Otting, 1997; Ponstingl and Otting, 1997; Dalvit, 1998). A limiting factor which has delayed the application of NMR in this type of investigation was the presence of intense multiple solvent signals in the ^{1}H NMR spectrum of proteins dissolved in solvent mixtures. The dipolar intermolecular interactions are usually very weak and they are easily masked by the residual solvent signals if the suppression of these resonances is not optimal. Owing to the recent developments of efficient multiple solvent suppression techniques (Ponstingl and Otting, 1997; Dalvit, 1998; Dalvit et al., 1999), it is now possible to detect and quantify these weak interactions without the interference of solvent signals.

We report the use of these NMR techniques and other novel NMR experiments in the identification of interactions of DMSO and other similar molecules with the protein FKBP12 (FK506 binding protein). The choice of this solvent was dictated simply by experimental observations. At the maximum DMSO concentration used in our studies (1.54 M), only one binding site for DMSO was found. DMSO binds in the active site of the protein and occupies the spatial region occupied by the pipecolidine ring of the immunosuppressive drugs FK506 and Rapamycin.

*Dedicated to the memory of Ezio Dalvit.
**To whom correspondence should be addressed. Present address: Pharmacia & Upjohn S.p.A., Viale Pasteur 10, I-20014 Nerviano (Mi), Italy. E-mail: Claudio.Dalvit@eu.pnu.com.

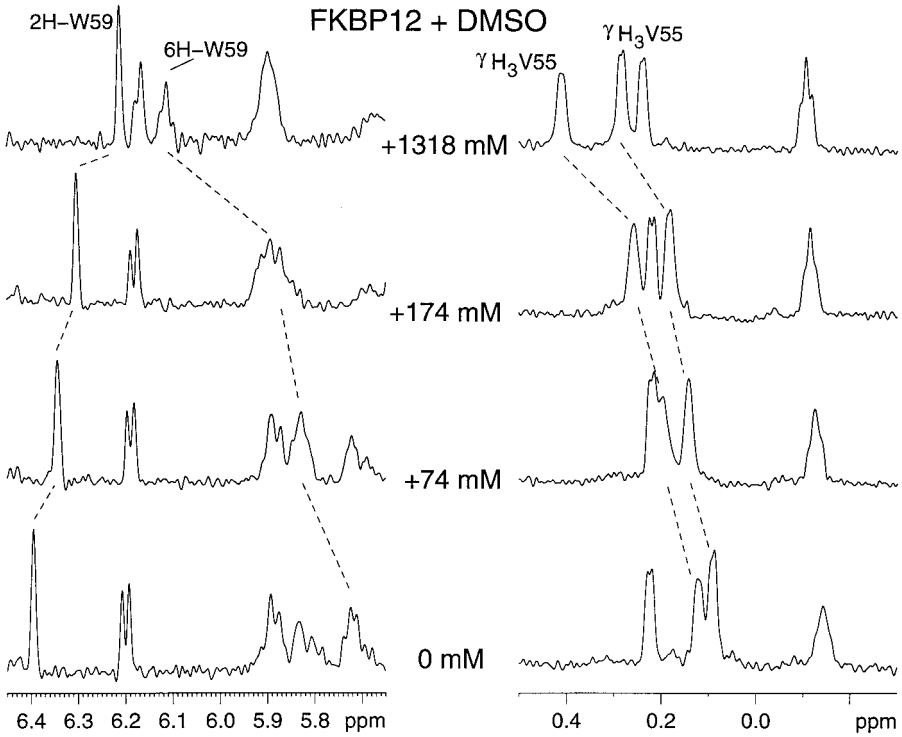


Figure 1. One-dimensional spectra recorded for a 0.3 mM solution of FKBP12 (50 mM phosphate buffer, pH 6.6) in H_2O as a function of the DMSO concentration. The spectra were acquired with the modified excitation sculpting sequence for multiple solvent suppression. The ^{13}C satellite signals of DMSO were suppressed with ^{13}C decoupling applied during the two soft ^{1}H 180° shifted laminar pulses (SLP) (Boyd and Soffe, 1989; Patt, 1992). A total of 32 scans with a repetition time of 2.8 s were acquired for each spectrum. The length of the two doubly selective soft rectangular 180° pulses and of the four sine-shaped PFGs was 3 and 0.6 ms, respectively. The gradient recovery time was 200 μs. The concentration of DMSO is given and the assignments for some resonances are labelled.

Materials and methods

NMR experiments

All NMR experiments were recorded with a 600 MHz spectrometer comprising a Bruker actively shielded magnet and an Avance Bruker console. The gradients were generated with an ACCUSTAR^{TM} unit connected to a 5 mm triple-resonance inverse probe equipped with actively shielded x,y,z-gradient coils. All spectra were acquired at T = 296 K.

The protein solutions were in 50 mM phosphate buffer, pH 6.6, and the protein concentration ranged between 0.3 and 0.5 mM. DMSO (**1**) was purchased from Pierce, methyl (methyl sulfinyl) acetate (**2**) was purchased from Aldrich and the compounds 5′-methyl-2′-methylsulfinyl acetophenone (**3**) and 3-methyl-3-(2-methyl-1,3-dioxolan-2-YL)-1-methylsulfinyl-2-butanone (**4**) were purchased from Salor.

Reported proton resonance assignments for uncomplexed FKBP12 (Rosen et al., 1991) were used in these studies. Distance constraints were classified according to the intensities of the intermolecular NOEs in three classes: strong (<3 Å), medium (<4 Å) and weak (<5 Å).

Structure calculations

Structural calculations were performed using a three-stage procedure with the program X-PLOR (Brünger, 1992). Topology and parameter files for X-PLOR calculations were generated with the graphical program WITNOTP (A. Widmer, unpublished). Initially, ligand coordinates were randomized in a 5×5×5 Å cube around an arbitrary point in the binding site of the X-ray structure of the FKBP12/Rapamycin complex (Van Duyne et al., 1991). The PDB code is 1fkb (Bernstein et al., 1977). The Rapamycin molecule was removed before calculations. These random coordinates were then converted into a stereochemically reasonable ligand structure using molecular dynamics (1.1 ps at 300 K). In the second stage the FKBP12/ligand complex was refined by restrained simulated anneal-

ing (7.5 ps at 900 K, followed by linear cooling to 100 K during 37.5 ps, Tripos force field (Clark et al., 1989) and intermolecular distance constraints derived from the NMR experiments). Finally, the resulting structures were energy minimized by 700 steps of conjugate gradient refinement. Protein coordinates were kept fixed throughout the whole procedure. The final results are independent of the choice of the arbitrary ligand position in stage one. This was confirmed for molecule (**3**) by a series of structure calculations starting from three different points within 15–20 Å of the center of mass of the ligand in 1fkb.

Selection of sulfoxides

In order to select commercially available sulfoxides with a potential to bind to FKBP12, we proceeded as follows. The CS(=O)C substructure was used for a substructure search in the Available Chemicals Directory (ACD library) with Isisbase (ISIS client/server software system and ACD Finder, version 2.0.1, with 250 282 entries, in database ACD-3D 98.1, MDL Information Systems, Inc.). Three-dimensional models of all resulting 70 sulfoxides with a molecular weight (MW) less than 300 were generated with Concord version 4.0.2 (Pearlman, 1998). Each model was superimposed with respect to its S and O atoms of the SO group on the SO group of the above NMR structure of DMSO bound to FKBP12. For each structure, these starting geometries were used for several independent runs of high-temperature dynamics with X-PLOR (Brünger, 1992) while the SO group was restrained in its initial position and the atoms of FKBP12 were fixed. Subsequent simulated annealing and final minimisation of each structure were carried out with only the FKBP12 atoms fixed. All energy calculations involved TAFF parameters (Clark et al., 1989) including electrostatic interactions with MPEOE charges (Momany and Rone, 1992; A. Widmer, unpublished). The resulting structures were ranked with respect to the computed interaction energy between FKBP12 and the ligand. The interaction energies ranged from −34 to −14 kcal/mol. Sulfoxides **2**, **3** and **4** with respective interaction energies of −20.8, −24.7 and −26.7 kcal/mol (all in R-configuration) were selected by inspection of the intermolecular interactions of the modelled complexes and by inspection of the structural variation of the candidate ligands. Another criterion used in the selection was the immediate availability of the compounds.

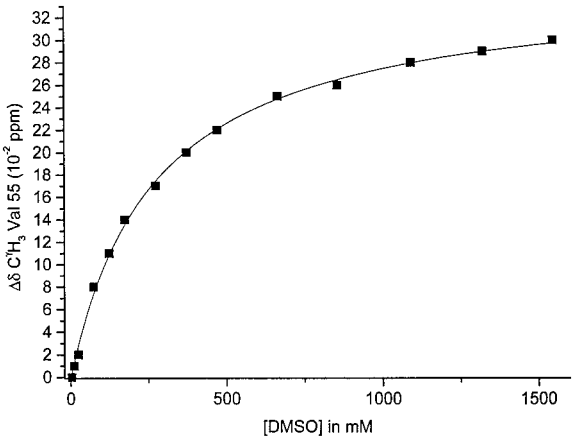


Figure 2. Chemical shift change for one of the C$^{\gamma}$H3 resonances of Val^{55} as a function of DMSO concentration. The spectra were recorded as described in Figure 1. The curve represents the best fit obtained using Equation 1. The K_D extracted from this fit is 275 mM and the chemical shift change $\Delta\delta_M$ upon formation of a 1:1 complex is 0.35 ppm.

Results and discussion

DMSO

DMSO is often used as the solvent of choice for preparing solutions of molecules to be tested in biological assays. During our work aimed at the identification of molecules binding to the protein FKBP12 using NMR we have noticed that the addition of DMSO to the protein solution resulted in characteristic chemical shift changes for some proton resonances (see Figure 1). This represented an experimental proof that DMSO was binding to the protein. Evidence for DMSO binding to the protein was also reported by others (Burkhard, 1995). Prompted by these findings we decided to carry out detailed work in order to characterize the interactions between the organic solvent and the protein and identify the binding site. DMSO is a good hydrogen-bond acceptor and has one side which can attach to accessible hydrophobic parts of the protein. The exchange of the protein between its free and complexed state with DMSO is fast on the NMR time scale, as evident in Figure 1. The chemical shift change for one of the C$^{\gamma}$H$_3$ methyl group resonances of Val-55 as a function of the DMSO concentration is shown in Figure 2.

Analysis of the data in Figure 2 permits, assuming a simple ligand binding mechanism, the calculation of the dissociation constant K_D according to the equation (Dwek, 1973):

$$[L]_0 = \frac{\Delta\delta_M [P]_0}{\Delta\delta} - K_D \qquad (1)$$

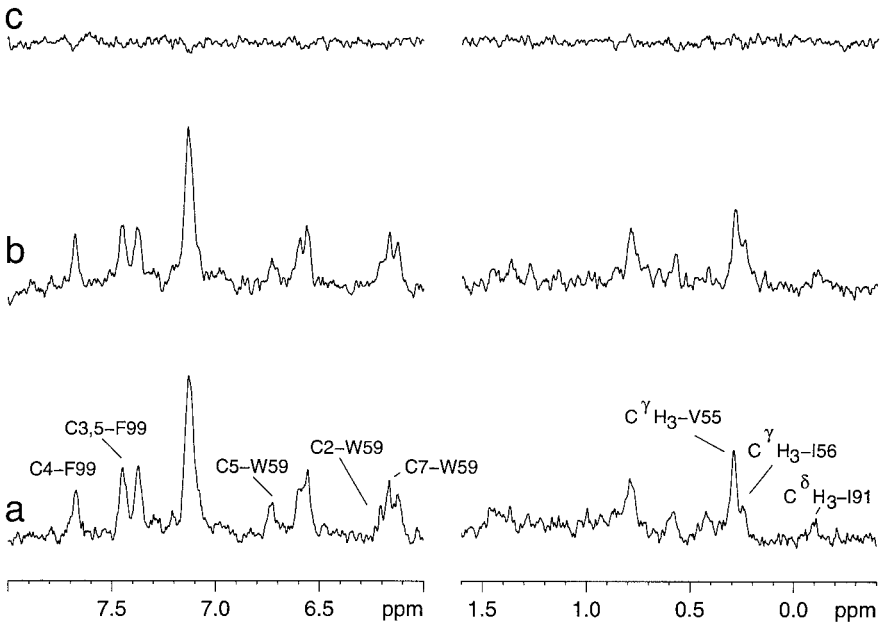


Figure 3. One-dimensional ePHOGSY spectra with multiple solvent suppression recorded for the FKBP12/DMSO complex. The protein and DMSO concentrations were 0.3 mM and 1.54 M (9.24 M in protons), respectively. The selective excitation achieved with a 50 ms long 180° Gaussian pulse was set at the DMSO frequency. The doubly selective rectangular 180° pulses for H_2O and DMSO suppression were 3 ms long. A total of 8192 scans were recorded with a repetition time of 2.8 s. The length of the mixing time was 301 ms (300 ms with a 0.12 G/cm rectangular gradient + 1 ms of gradient recovery time). The length of the first two PFGs and of the last four PFGs was 3 and 0.6 ms, respectively. The gradient recovery time for the first two PFGs was 5 ms (a,c) and 25 ms (b). The gradient recovery time for the last four PFGs was 200 μs. The strength of the first two sine-shaped z-PFGs was 4.8 G/cm (a,b) or 40.8 G/cm (c). Some intermolecular NOEs are labelled in (a).

where $[L]_0$ and $[P]_0$ are the total ligand and protein concentrations, respectively and $\Delta\delta_M$ is the chemical shift difference of the fully bound form with respect to its position in the unbound form. For DMSO the calculated K_D according to Equation 1 was 275 mM (see Table 1). The volume changes upon DMSO addition were taken into account in calculating the binding constant. In order to identify the binding site we have used the recently developed ePHOGSY experiment (Dalvit and Hommel, 1995) with multiple solvent suppression (Dalvit, 1998). The selective excitation was set at the DMSO frequency and the signals of H_2O, DMSO and its ^{13}C satellite signals were suppressed before acquisition. The resulting spectra (see Figure 3) contain only the observed NOEs between DMSO and some specific proton resonances of the protein. The protein concentration in these experiments was only 0.3 mM. Two different control experiments, depicted in Figures 3b and 3c, were recorded in order to exclude the possibility that some of the observed NOEs in Figure 3a originated from protein signals resonating at the DMSO proton frequency. The spectrum of Figure 3b was recorded with a long gradient recovery time for the first two PFGs of the ePHOGSY experiment. In this case all the protein signals have completely decayed at the end of the spin echo due to their short T_2 relaxation. The spectra of Figures 3a and 3b are identical indicating that all observed NOEs originate from DMSO. The spectrum in Figure 3c was instead recorded with strong power for the first two PFGs. The rapid exchange between bulk and bound DMSO results in a large diffusion coefficient *D* for DMSO. Therefore, in the presence of strong gradients, significant loss of DMSO signal intensity results at the end of the spin-echo period. In contrast, the intensities of the protein resonances are not affected by the gradient strength used in Figure 3c, owing to the small diffusion coefficient of the protein. The complete absence of signals in Figure 3c is again an experimental proof that all the NOEs observed in Figure 3a stem from DMSO. Despite the low K_D several intermolecular NOEs are observed, indicating that molecules with very low binding affinity can be studied and the binding interface characterized.

Two-dimensional NOESY and TOCSY with multiple solvent suppression (Dalvit, 1998; Dalvit et al., 1999) and 2D Magic Angle Gradient (MAG) DQ experiments (Dalvit and Böhlen, 1996) were performed

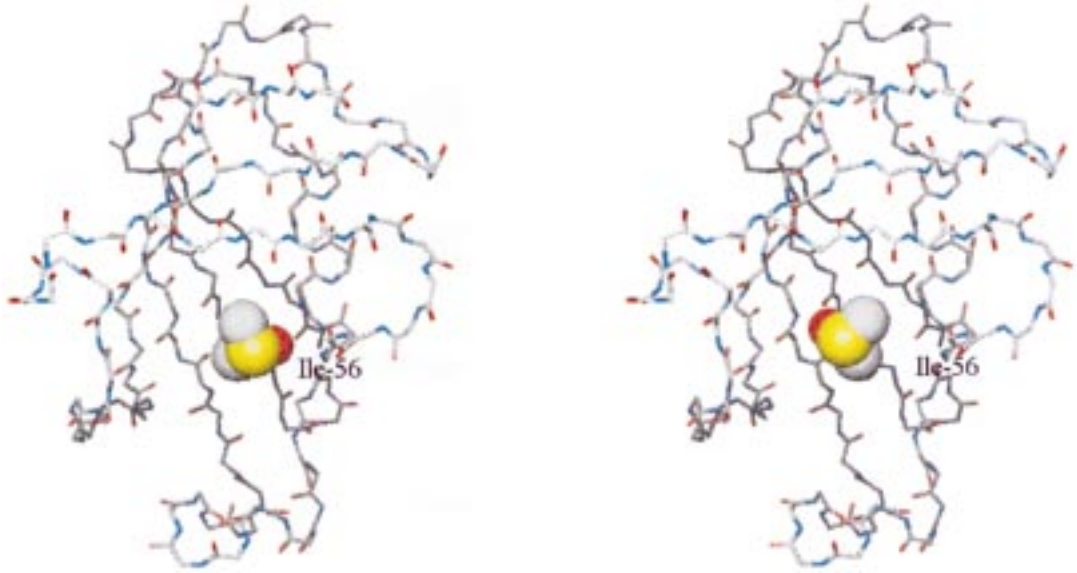


Figure 4. The 3D backbone structure of FKBP12 in complex with the DMSO molecule. The CPK representation for DMSO is displayed. Two possible structures for DMSO are found which are compatible with the experimentally observed NOEs.

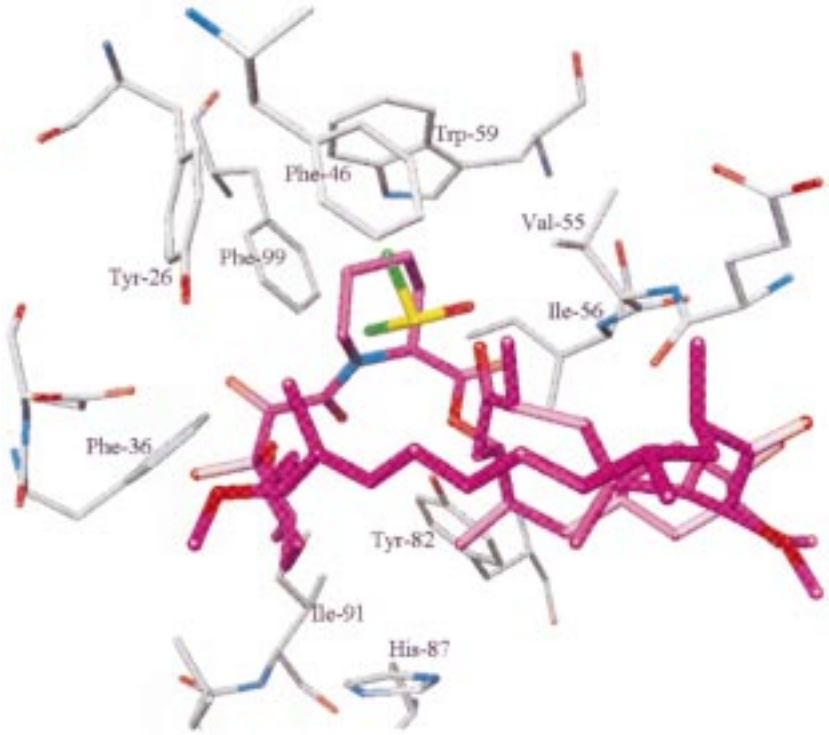


Figure 5. Superposition of the 3D NMR structure of bound DMSO (green) with the X-ray structure of Rapamycin (magenta) complexed with FKBP12. All residues of the protein contained in a radius of 5 Å around the ligand are displayed.

for the assignment procedure of the protein resonances observed in Figure 3. The intermolecular NOEs in Figure 3 were then used as distance constraints in X-PLOR for the identification of the DMSO binding site. A limitation of the DMSO molecule is represented by the chemical equivalence of the two methyl groups. No splitting of the DMSO signal is observed in the NMR spectrum and therefore one is unable to distinguish between the different NOEs originating from each DMSO methyl group. Nevertheless, the significant number of distance constraints (14) allows the identification of the binding site. Two possible structures for bound DMSO were found which are compatible with the observed NOEs as shown in Figure 4. The most important difference between the two structures is the location of the oxygen. Note that the two methyl groups, although exchanged, occupy the same spatial location. In the structure on the left the oxygen atom of DMSO is close to the N of Ile^{56} (distance 3.5 Å) and could form a weak hydrogen bond. In the structure on the right the distance between the two atoms is 7.9 Å. Recent X-ray studies of the complex suggest the presence of a hydrogen bond between the NH of Ile^{56} and the sulfoxide (Burkhard, 1995). At the maximum ligand concentration used in these studies (1.54 M) only one molecule of DMSO bound to FKBP12 could be identified. Neutron diffraction studies of the complex lysozyme/DMSO (Lehmann and Stansfield, 1989) have revealed the presence of six molecules of DMSO bound to the protein. However, four of these molecules are located at the contact regions between two or more lysozyme molecules and are not detected in NMR experiments in solution (Liepinsh and Otting, 1997).

Figure 5 shows the bound structure of DMSO derived from NMR superimposed to the X-ray structure of the immunosuppressive drug Rapamycin complexed with FKBP12 (Van Duyne et al., 1991). Several intermolecular contacts between FKBP12/Rapamycin are also observed in the FKBP12/DMSO complex. In particular, the small DMSO molecule mimics the pipecolidine ring of the drug. Recently the combined use of docking programs and NMR studies has led to the discovery that also steroids can bind in this protein cleft (Burkhard et al., 1999).

DMSO derivatives

Based on these findings, small molecules similar to DMSO were searched. One of the two methyl groups of DMSO was substituted with different moieties as described above. The molecules analyzed with NMR

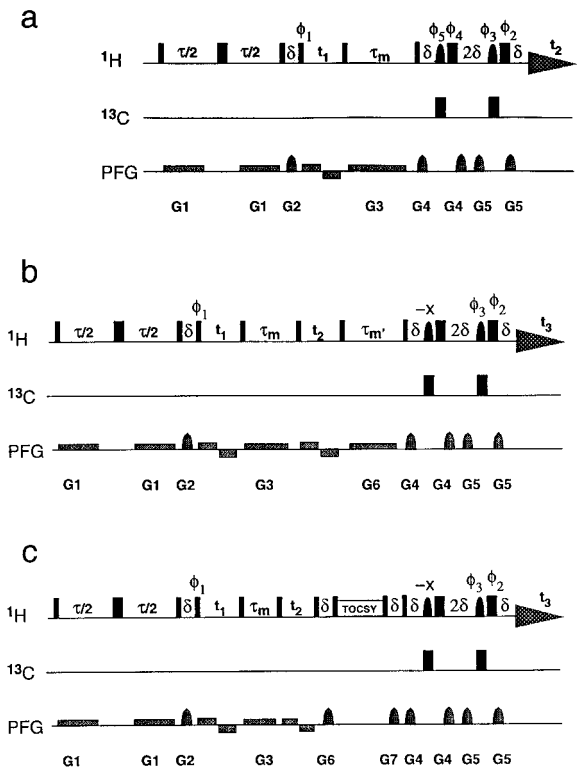
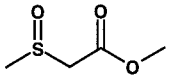
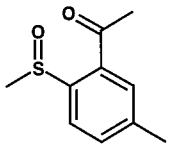
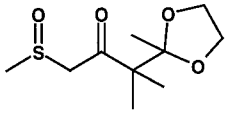


Figure 6. Pulse sequences for the 2D T_2 filter NOESY (a), 3D T_2 filter NOESY-NOESY (b) and 3D T_2 filter NOESY-TOCSY (c) experiments with multiple solvent suppression. The narrow and broad bars represent hard 90° and 180° pulses, respectively. The two selective 180° pulses of the excitation sculpting scheme are frequency shifted laminar pulses with excitation at the different solvent chemical shifts. These pulses are either soft rectangular pulses or strongly truncated Gaussian pulses in order to allow good selectivity (Dalvit et al., 1999). Suppression of the solvent ^{13}C satellite signals is achieved with ^{13}C decoupling applied during the SLP pulses. The phases in (a) are: ϕ_1 = x, −x; ϕ_2 = 2(x), 2(−y), 2(−x), 2(y); ϕ_3 = 2(−x), 2(y), 2(x), 2(−y); ϕ_4 = 8(x), 8(−y), 8(−x), 8(y); ϕ_5 = 8(−x), 8(y), 8(x), 8(−y); and ϕ_{rec} = 2[2(x, −x, −x, x), 2(−x, x, x, −x)]. The phases in (b,c) are: ϕ_1 = x, −x; ϕ_2 = 2(x), 2(−y), 2(−x), 2(y); ϕ_3 = 2(−x), 2(y), 2(x), 2(−y); and ϕ_{rec} = (x, −x, −x, x). All other pulses have phase x unless otherwise indicated. The delay δ is equal to the length of the gradient plus the gradient recovery time. The delay τ is sufficiently long to destroy the rapid T_2 relaxing signals of the protein and it should be properly tuned in the presence of scalar coupled multiplet resonances as described in the text. The delay τ_m is the mixing time needed for intermolecular magnetization transfer whereas the delay $\tau_{m'}$ in (b) is used for relaying this magnetization to other protons of the protein. In (c) this relay is performed via a TOCSY step. The gradient G1 is a weak gradient applied for a finite length to suppress solvent radiation damping during the period τ. G2, G6 and G7 (c) are short *crushers* which destroy all the transverse magnetization. The gradients G3 and G6 (b) are weak gradients applied for the entire length of the mixing times. Radiation damping is suppressed during the evolution periods t_1 and t_2 with weak bipolar gradients (Sklenár, 1995). Quadrature detection in t_1 (a–c) and in t_2 (b,c) is achieved with TPPI (Drobny et al., 1979; Marion and Wüthrich, 1983).

Table 1. Chemical structures, theoretical interaction energies and experimental K_D for the analysed compounds

Sulfoxide		MF	MW	E(inter) [kcal/mol]	K_D [mM]
	**1**	C2H6OS	78	-14.5	275 +/- 10
	**2**	C4H8O3S	136	-20.8	93 +/- 12
	**3**	C10H12O2S	196	-24.7	10 +/- 3
	**4**	C10H18O4S	234	-26.7	5 +/- 1

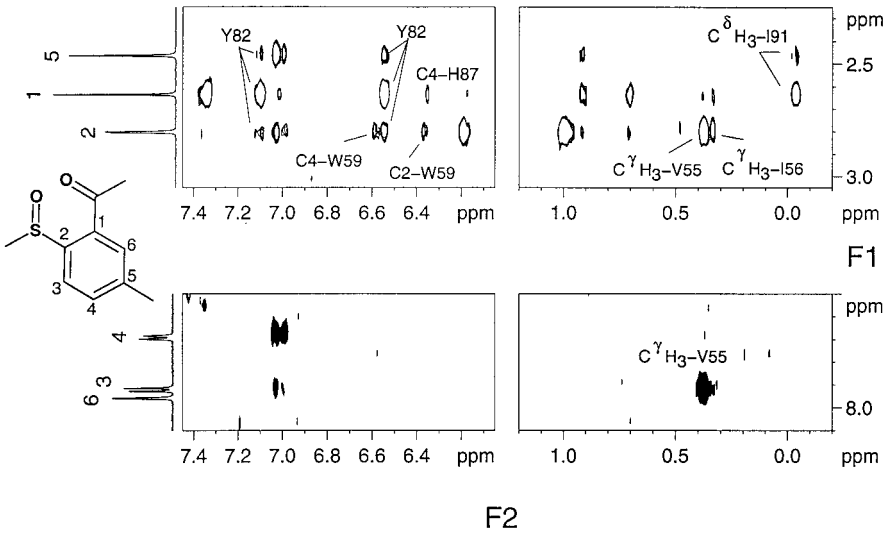


Figure 7. Spectral regions containing the intermolecular NOEs between molecule **3** and the protein FKBP12. The spectrum was recorded with the pulse sequence of Figure 6a for a 0.5 mM solution of FKBP12 (50 mM phosphate buffer, pH 6.6) in H_2O in the presence of 330 mM of **3**. The ligand was dissolved in CD_3OD before adding it to the protein solution. A total of 128 scans with a repetition time of 2 s were acquired for each of the 200 t_1 increments. The delay τ was 115 ms long, which corresponds exactly to $1/^3J_{34}$. The length of the two 0.4 G/cm gradients G1 was 30 ms. The mixing time was 102.5 ms long (100 ms with a 0.2 G/cm rectangular gradient plus 2.5 ms gradient recovery time). The length of the two triple-selective 180° soft SLP and of the gradients G4 and G5 was 2.4 and 0.6 ms, respectively. The gradient recovery time for G4 and G5 was 200 μs. The 1D reference spectrum recorded for the complex is shown on the left. The small splitting observed for the $SOCH_3$ resonance is due to the slight difference in the environment for the R- and S-configurations when bound to the protein. The cross peaks represent intermolecular magnetization which originates from the ligand (F_1) and is detected at the frequencies of the protons of the protein (F_2). The negative cross peaks have been filled for clarity. Several of the unambiguous intermolecular NOEs are labelled.

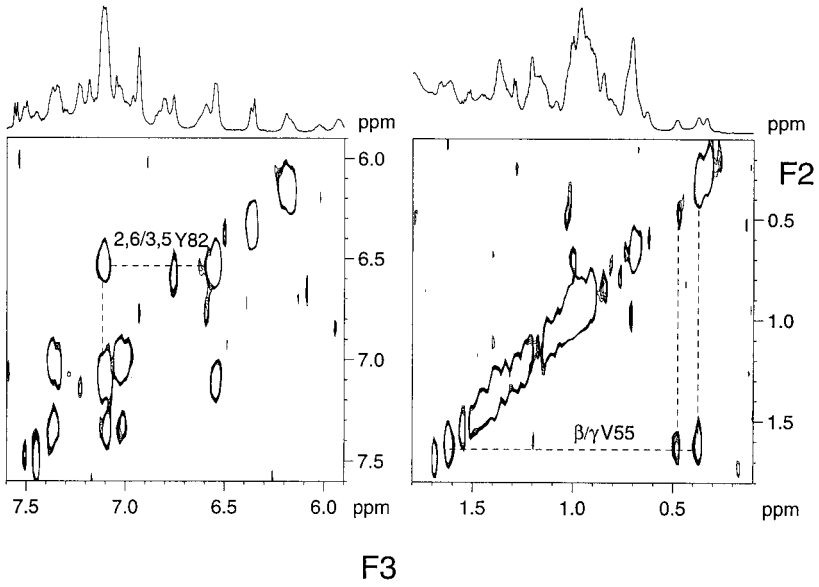


Figure 8. An F_2F_3 plane extracted from the 3D T_2 filter NOESY-TOCSY spectrum recorded with the pulse sequence of Figure 6c. The complex is the same as that used for Figure 7. The plane was taken at the F_1 frequency of the CH_3SO signal. The spectrum on the left contains a small section of the aromatic region and on the right the upfield section. The one-dimensional spectrum obtained with multiple-solvent suppression is displayed above the contourplots. A total of $64\times120\times1024$ points were detected in the three dimensions and the matrix data were zero-filled to $256\times256\times1024$ points prior to Fourier transformation. Sixteen scans with 1.7 s repetition time were acquired for each of the t_1 and t_2 increments. The length of the TOCSY step was 32 ms. All other parameters are the same as described in Figure 7. The cross peaks in these spectra are used to obtain unambiguous protein resonance assignments. Two such connectivities are drawn and labelled.

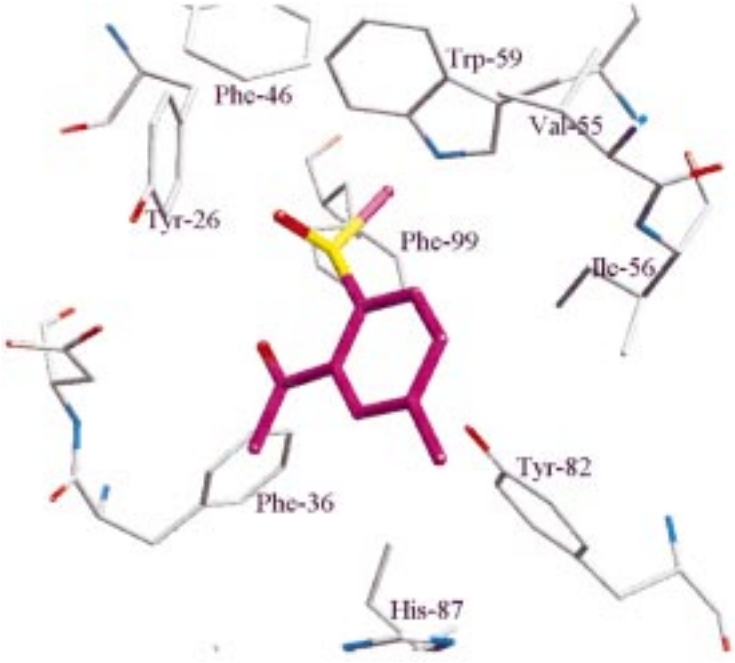


Figure 9. The 3D NMR structure of molecule **3** complexed with FKBP12. All residues of the protein contained in a radius of 5 Å around the ligand are displayed.

are displayed in Table 1. The binding constants were determined by NMR. All these molecules bind in the same protein cleft as DMSO and all with an improved binding constant compared to DMSO (see Table 1). Molecule **3** was chosen for additional NMR work. The spectrum of FKBP12 complexed with **3** is complicated by the presence of seven strong signals, i.e. six signals of the compound and the H_2O signal. The intermolecular NOEs between the weakly binding ligands and the protein can be studied in a simple way with the scheme of Figure 6a. A T_2 filter for suppression of the protein resonance is applied before the evolution period of a 2D NOESY experiment. All the signals of the ligand and H_2O are then suppressed before the acquisition period using a modified version of the excitation sculpting sequence (Hwang and Shaka, 1995) for multiple solvent suppression. Although the scheme uses a T_2 filter (Mori et al., 1996) as used in the experiments proposed by Otting and co-workers (Ponstingl and Otting, 1997), it differs in the scheme of multiple solvent suppression (Dalvit, 1998). All in-phase magnetization of the singlet resonances present immediately after the first 90° pulse will exist at the end of the spin-echo period, apart from some attenuation due to T_2 relaxation. On the contrary, the magnetization of multiplets will oscillate between in-phase and anti-phase magnetization (Ernst et al., 1987). Therefore it is important to choose a delay τ for the spin echo to maximize the content of in-phase magnetization at the end of the T_2 filter. Molecule **3** contains four singlets (3 CH_3 and 1 CH) and two doublets (C3-H and C4-H) with a coupling constant of 8.7 Hz. The choice of a spin-echo period of 115 ms results in complete in-phase magnetization for the two doublets at the beginning of the evolution period. The sign of this magnetization will be opposite to the sign of the magnetization of the singlets. When the chemical shift separation between the ligand resonances is large, the 2D experiment of Figure 6a can be recorded with only a very limited number of t_1 increments. Figure 7 shows the 2D spectrum recorded with the pulse sequence of Figure 6a for FKBP12 in the presence of 0.33 M of **3**. Different NOEs are observed from the different protons of the ligand. Note also that the cross peaks originating from the two ligand doublets have opposite sign with respect to the other cross peaks, as explained above.

The severe resonance overlap in the ^1H NMR spectrum of the protein does not allow complete unambiguous assignments of the NOE cross peaks in Figure 7. The ambiguity can be removed by recording the three-dimensional versions of the experiment of Figure 6a, as depicted in Figures 6b and 6c. These schemes represent the 3D ^1H T_2 filter NOESY-NOESY (b) and NOESY-TOCSY (c) experiments with multiple solvent suppression. The magnetization of the ligand transferred to protons of the protein is then relayed via coherent or incoherent mixing to other protons of the proteins. This additional relay is important for the unambiguous assignment of the intermolecular NOEs. A typical F_2F_3 plane extracted from the 3D spectrum recorded with the pulse sequence of Figure 6c is shown in Figure 8. The plane was taken at the F_1 frequency of the CH_3SO resonance. The observation of the cross peaks ($F_1 \neq F_2 \neq F_3$) permits the resonance assignments. These cross peaks are usually asymmetric with respect to the F_2F_3 cross-diagonal. The experiments of Figure 6 can also be used to study possible intermolecular interactions between a ligand tightly bound to a protein and a weakly interacting organic solvent or small molecule. This is important in the design of ligands with high selectivity for the target protein.

The NMR structure of molecule **3** bound to FKBP12 calculated with the distance constraints derived from the spectra of Figure 7 is shown in Figure 9. The molecule binds in the same protein cleft as DMSO. Owing to the larger size of **3** more intermolecular contacts are observed compared to DMSO. For example, the intermolecular contact with His^{87} observed in Figure 7 is absent in the FKBP12/DMSO complex. The additional intermolecular contacts are probably the reason for the $\sim$27 times improvement in the binding constant of this compound compared to DMSO. The only proton of **3** which does not show any intermolecular NOEs with the protein is C6-H. This proton points toward the solvent in the 3D derived structure and makes no contact with the protein. Therefore a possible way for improvement of the binding affinity of **3** is the replacement of this proton with a polar group.

Conclusions

In conclusion, it has been shown that the novel NMR experiments represent a powerful tool for studying the weak interactions between organic solvents and proteins in solution. Their application to an H_2O/DMSO solution of FKBP12 reveals the presence of one DMSO molecule bound in the substrate binding site. This molecule occupies the same spa-

tial region occupied by the pipecolidine moiety of the immunosuppressive drugs FK506 and Rapamycin complexed to FKBP12. Simple modifications of the DMSO molecule resulted in ligands with improved binding affinity.

Acknowledgements

We thank S. Raccuglia for help with the preparation of the protein. We are grateful to Dr. U. Hommel for a preprint of Burkhard et al. (1999) and for helpful discussions.

References

Allen, K.N., Bellamacina, C.R., Ding, X., Jeffery, C.J., Mattos, C., Petsko, G.A. and Ringe, D. (1996) *J. Phys. Chem.*, **100**, 2605–2611.

Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer, Jr., E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) *J. Mol. Biol.*, **112**, 535–542.

Boyd, J. and Soffe, N. (1989) *J. Magn. Reson.*, **85**, 406–413.

Brünger, A.T. (1992) *X-PLOR (Version 3.1) Manual*, Howard Hughes Medical Institute and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT.

Burkhard, P. (1995) Ph.D. Thesis, University of Basel, Switzerland.

Burkhard, P., Hommel, U., Sanner, M. and Walkinshaw, M. (1999) *J. Mol. Biol.*, in press.

Clark, M., Cramer III, R.D. and Van Opdenbosch, N. (1989) *J. Comput. Chem.*, **10**, 982–1012.

Dalvit, C. and Hommel, U. (1995) *J. Magn. Reson.*, **B109**, 334–338.

Dalvit, C. and Böhlen, J.-M. (1996) *J. Magn. Reson.*, **B111**, 76–80.

Dalvit, C. (1998) *J. Biomol. NMR*, **11**, 437–444.

Dalvit, C., Shapiro, G., Böhlen, J.-M. and Parella, T. (1999) *Magn. Reson. Chem.*, **37**, 7–14.

Douzou, P. and Petsko, G.A. (1984) *Adv. Protein Chem.*, **36**, 245–361.

Drobny, G., Pines, A., Sinton, S., Weitekamp, D.P. and Wemmer, D. (1979) *Symp. Faraday Soc.*, **13**, 49–55.

Dwek, R.A. (1973) *Nuclear Magnetic Resonance (N.M.R.) in Biochemistry*, Clarendon Press, Oxford.

Ernst, R.R., Bodenhausen, G. and Wokaun, A. (1987) *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Clarendon Press, Oxford.

Hwang, T.-L. and Shaka, A.J. (1995) *J. Magn. Reson.*, **A112**, 275–279.

Lehmann, M.S., Mason, S.A. and McIntyre, G.J. (1985) *Biochemistry*, **24**, 5862–5869.

Lehmann, M.S. and Stansfield, R.F.D. (1989) *Biochemistry*, **28**, 7028–7033.

Liepinsh, E. and Otting, G. (1997) *Nat. Biotechnol.*, **15**, 264–268.

Marion, D. and Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun.*, **113**, 967–974.

Mattos, C. and Ringe, D. (1996) *Nat. Biotechnol.*, **14**, 595–599.

Momany, F.A. and Rone, R. (1992) *J. Comput. Chem.*, **13**, 883–900.

Mori, S., Berg, J.M. and Van Zijl, P.C.M. (1996) *J. Biomol. NMR*, **7**, 77–82.

Otting, G. (1997) *Prog. NMR Spectrosc.*, **31**, 259–285.

Patt, S.L. (1992) *J. Magn. Reson.*, **96**, 94–102.

Pearlman, R.S. (1998) *CONCORD/Stereoplex User's Manual*, distributed by Tripos Inc., St. Louis, MO, U.S.A.

Ponstingl, H. and Otting, G. (1997) *J. Biomol. NMR*, **9**, 441–444.

Rosen, M.K., Michnick, S.W., Karplus, M. and Schreiber, S.L. (1991) *Biochemistry*, **30**, 4774–4789.

Shuker, S.B., Hajduk, P.J., Meadows, R.P. and Fesik, S.W. (1996) *Science*, **274**, 1531–1534.

Sklenár, V. (1995) *J. Magn. Reson.*, **A114**, 132–135.

Van Duyne, G.D., Standaert, R.F., Schreiber, S.L. and Clardy, J. (1991) *J. Am. Chem. Soc.*, **113**, 7433–7434.