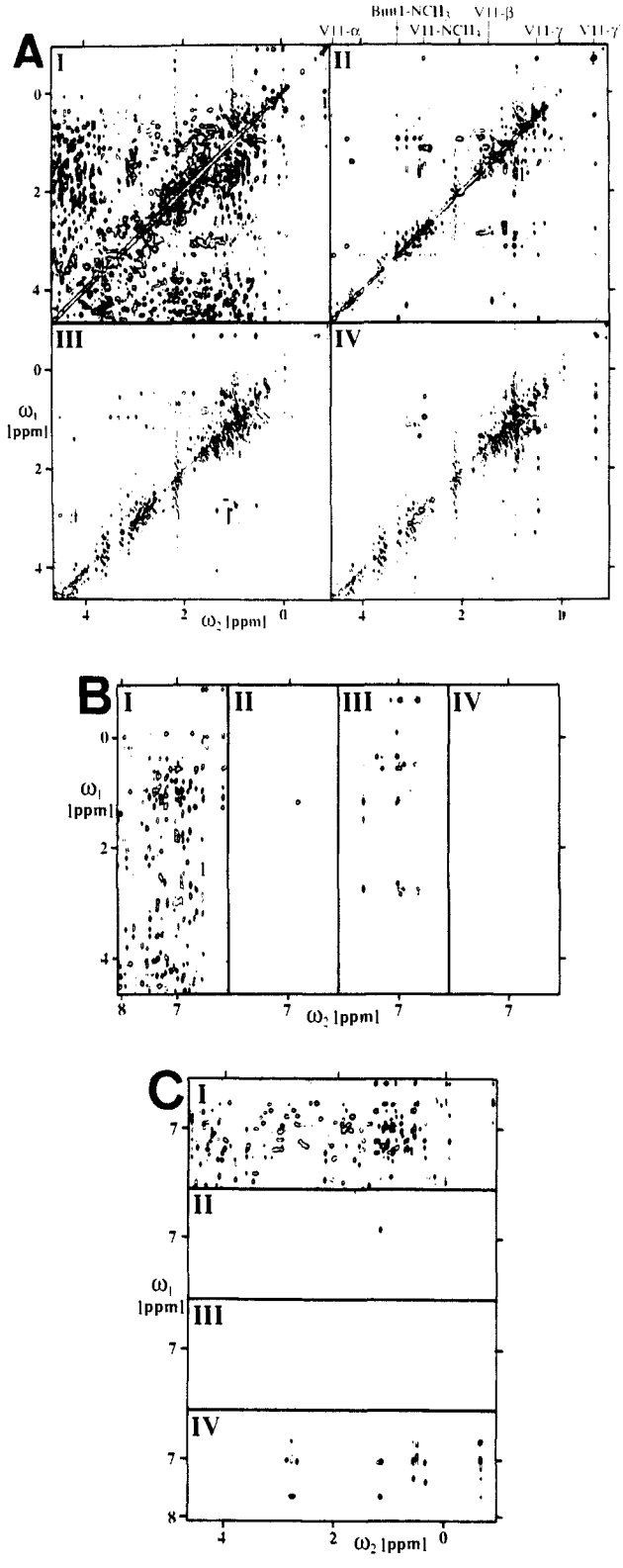

Figure 2. Three spectral regions from a ^1H NOESY spectrum of the cyclophilin complex with ^{13}C-labeled CsA recorded at 500 MHz with a mixing time of 80 ms using the $^{13}C(\omega_1,\omega_2)$-double-half-filter experiment of Figure 1 (complex concentration 0.7 mM, solvent D_2O, pD = 6.0, T = 26 °C, τ = 3.6 ms, t_{1max} = 29 ms, t_{2max} = 127 ms). The following subspectra are shown (see also Table I): (I) $^{13}C(\omega_1)$-$^{13}C(\omega_2)$ doubly filtered; (II) $^{13}C(\omega_1)$-$^{13}C(\omega_2)$ doubly selected; (III) $^{13}C(\omega_1)$-selected/$^{13}C(\omega_2)$-filtered; (IV) $^{13}C(\omega_1)$-filtered/$^{13}C(\omega_2)$-selected. (A) Aliphatic region. In II, the chemical shift of Val 11 $C^{\gamma}H_3$ of CsA is indicated and its NOEs are identified, where Bmt1-NCH_3 stands for the *N*-methyl group of butenylmethylthreonine 1. (B) Region containing the cross peaks between the aromatic region along ω_2 and the aliphatic region along ω_1. (C) The same as B, except that the aromatic region is along ω_1.

of selected resonances in Figure 2A are indicated and some cross peaks with these resonance lines are identified in the spectrum. This subspectrum can be used to study the NOE buildup[4] in ^{13}C-labeled cyclophilin-bound CsA (the proton spin relaxation is significantly influenced by the presence of the ^{13}C spins) and to collect a set of NOE distance constraints as the experimental basis for establishing sequence-specific ^1H resonance assignments and preparing the input for a three-dimensional structure calculation.[4]

Subspectra I, III, and IV in Figure 2 represent a source of information for further characterization of the receptor-ligand complex. In the subspectrum I, all the diagonal and cross peaks originate exclusively from the unlabeled cyclophilin. It corresponds to a conventional ^1H NOESY spectrum of a complex formed between cyclophilin and perdeuterated CsA and, thus, contains all the information needed for a structure determination of liganded cyclophilin. Comparison of this spectrum with that of free cyclophilin can be used to identify the ^1H resonance lines with significant chemical shift changes upon CsA binding. Subspectra III and IV in Figure 2 contain only intermolecular ^1H-^1H NOE cross peaks between cyclophilin and CsA. Once sequence-specific assignments for the receptor-bound ligand are available from spectrum II (Figure 2), these spectra provide the basis for identification of the sites on the ligand that are in contact with the receptor. If in addition sequence-specific ^1H NMR assignments are available also for the receptor protein (subspectrum I in Figure 2A), subspectra III and IV provide direct information on the intermolecular contacts in the receptor-ligand complex.

It is an additional advantage of subspectra III and IV that the diagonal peaks are suppressed.[2,3] As a consequence, the spectra contain only few perturbations from t_1 artifacts and have a flat base plane. Ideally, the diagonal in these subspectra should be completely absent. Residual diagonal peak intensities, such as those seen in Figure 2A, may arise from imperfections in the pulse sequence[2] and from instrumental instabilities. At the same time, strong cross peaks from subspectrum II may also appear as weak signals in subspectra III and IV (in the experiment of Figure 2, we estimated that such leakage occurs to the extent of ca. 5% of the peak intensity in subspectrum II). With the necessary care, such spurious peaks can be identified on the grounds that while subspectrum II is symmetric, subspectra III and IV are asymmetric with respect to the diagonal.

In conclusion, this paper illustrates the potentialities of heteronuclear double-half-filters as a technique enabling conformational studies of receptor-bound ligands in systems that would otherwise be too complex for detailed investigation by ^1H NMR. The experiments provide supplementary data that should eventually be sufficient to characterize complete systems of receptor protein and bound ligand.

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Vibrational Dynamics of the Cis Peptide Group

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While the trans peptide group predominates in the stable polypeptide chain of proteins, some non-proline cis peptide groups are found[1] and the less stable cis isomer is observed in aqueous

(1) Stewart, D. E.; Sarkar, A.; Wampler, J. E. *J. Mol. Biol.* **1990**, *214*, 253-260.

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N-methylacetamide (NMA) to the extent of ~1.5%.[2] The latter value is consistent with the energy difference between NMA isomers obtained from temperature-dependent matrix-isolation studies[3] (~2.3 kcal/mol) and from ab initio calculations[2,4] (~2.5 kcal/mol). Recent resonance Raman studies under conditions of high laser pulse energies[5,6] indicate that *trans*-NMA can be photoisomerized to *cis*-NMA, the argument being based in part on a proposed assignment of the amide II mode of the cis peptide group. It is therefore important to have a detailed understanding of the vibrational dynamics of this group. A previous normal-mode analysis of *cis*-NMA[3] was based on the assumption that its force field and internal geometry are the same as those of *trans*-NMA, which may not be warranted. We have computed the ab initio geometry and force field of *cis*-NMA, both the isolated molecule and with two H_2O molecules hydrogen bonded to it, and have obtained its normal modes. These confirm the assignment made in the resonance Raman studies,[5,6] but give significantly different normal modes than predicted by the previous analysis.[3]

The geometry of isolated *cis*-NMA was obtained at the Hartree-Fock level with five different basis sets: 3-21G*, 4-31G, 4-31G*, 6-31G, and 6-31G*. The four possible conformations with respect to CH_3 group rotations were completely optimized, and it was found that the order of stability is the same for all the basis sets. The most stable structure has a *C*-methyl H eclipsing the O and an *N*-methyl H eclipsing the (N)H, which differs from that assumed in the earlier normal-mode calculation[3] but is the same as that found in another ab initio study.[4] The bond lengths are very close to those of the most stable form of *trans*-NMA, for which we also optimized the four conformations[7] (the most stable trans structure has the same local eclipsed conformations as the cis). The bond angles, however, are more variable, some differing by 5-6° between the two structures.

Force fields were calculated with the 4-31G* and 6-31G* basis sets, and normal modes obtained from each set were compared. The frequencies and modes are essentially the same, and we therefore based all our results on the 4-31G* calculations. Since reliable eigenvectors depend on having accurate force constants, and since the ab initio values are generally high by 10-20% for such basis sets,[8] we chose to scale the force constants to experimental frequencies. Scale factors were obtained by optimizing the force constants for *trans*-NMA to its matrix-isolated frequencies[7] and then transferring these 10 scale factors to the ab initio force field of *cis*-NMA. Good agreement with experiment[3] required the modification of only one scale factor, that for CN stretch (s) being changed from 0.74 to 0.84. These scale factors were then transferred unchanged to the hydrogen-bonded *cis*-NMA system.

The effects of aqueous hydrogen bonds in modifying the normal modes of a *cis*-NMA molecule should be satisfactorily modeled by a complex that involves only the C=O and NH groups in interactions with water molecules. Although some ab initio energy studies[9-11] have examined formamide complexed with several such H_2O molecules, we feel that the cluster number and geometry are not critical and that the predominant effects on the normal modes are revealed by the presence of two H_2O molecules, one bonded to each group. We have therefore obtained the geometry and force field by the total optimization of such a *cis*-NMA-$(H_2O)_2$ complex.

The results of the calculations on isolated and hydrogen-bonded NMA are shown in Table I for the main amide and skeletal modes and are compared with experimental data for matrix-isolated[3] and aqueous[6] *cis*-NMA, respectively. We see that the amide II mode of the hydrogen-bonded molecule is predicted near the observed value of 1496 cm^{-1} [5,6] and is mainly CN s with no NH in-plane bend (ib) (≥10), consistent with experimental evidence.[6] This mode is significantly different in the isolated molecule, although it still contains no NH ib, in distinction to this coordinate being the largest contributor in an earlier calculation.[3] Other modes are also affected by hydrogen bonding, but to different degrees. Amide I, near 1650 cm^{-1}, can involve an HOH bend, although the extent is very sensitive to the scale factor for the latter force constant. These differences in modes, as well as in frequencies, are undoubtedly due to the significant changes in force constants that occur on hydrogen bonding; e.g., CN s, 8.1%; CO s, -8.3%; CO ib, 13.5%; NH ib, 46.7%; NH out-of-plane bend, 54.1%; CN s, NH ib, 94.8%. These changes are evaluated elsewhere in greater detail,[12] but it is clear that isolated molecules may not be adequate models for the normal modes of molecules that interact strongly with solvent.

Table I. Amide and Skeletal Modes of Isolated and Aqueous Hydrogen-Bonded *cis*-NMA

ν(obsd)[a]	ν(calcd)	potential energy distribution[b]
1707	1717	CO s (78), CCN d (12)
~1650	1645	CO s (28), HOH sb (50)
1485	1481	NCH_3 ab (54), CN s (16), NCH_3 r (14)
1496	1499	CN s (33), CC s (11), NCH_3 ab (10)
1325	1331	CCH_3 sb (51), CN s (17), NH ib (12), CO ib (12)
1316	1354	CCH_3 sb (63), CN s (11)
	798	CC s (52), CN s (20), NC s (10)
821	808	CC s (58), CN s (20), NC s (11)
	516	CN t (36), CO ob (26), NH ob (17), CCH_3 r (13)
	678	CN t (33), CO ob (29), NH ob (21), CCH_3 r (13)

[a] Top line: matrix isolated.[3] Bottom line: aqueous solution.[6] [b] s = stretch, d = deformation, t = torsion, ab = antisymmetric bend, sb = symmetric bend, ib = in-plane bend, ob = out-of-plane bend. Contributions ≥10.

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(12) Mirkin, N. G.; Krimm, S., to be published.

Dipolar NMR Spectroscopy of Nonoxidic Glasses. Structural Characterization of the System Phosphorus-Selenium by ^{31}P-^{77}Se Spin Echo Double Resonance NMR

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In spite of the long-standing importance of non-oxide chalcogenide glasses in infrared optics and semiconductor technology, quantitative concepts describing the structural principles in these systems are just emerging. Recently, unique insights have been obtained via dipolar solid-state NMR techniques.[1]

The binary phosphorus-selenium system forms glasses over a wide compositional region (0-52 atom % P)[2] and is an ideal model system for studies directed at the development of structural concepts for chalcogenide glasses. Previous ^{31}P spin-echo experiments have served to establish the statistics of P-Se vs P-P

(2) Radzicka, A.; Pedersen, L.; Wolfenden, R. *Biochemistry* **1988**, *27*, 4538-4541.
(3) Ataka, S.; Takeuchi, H.; Tasumi, M. *J. Mol. Struct.* **1984**, *113*, 147-160.
(4) Jorgensen, W. L.; Gao, J. *J. Am. Chem. Soc.* **1988**, *110*, 4212-4216.
(5) Wang, Y.; Purrello, R.; Spiro, T. G. *J. Am. Chem. Soc.* **1989**, *111*, 8274-8276.
(6) Song, S.; Asher, S. A.; Krimm, S.; Shaw, K. D. *J. Am. Chem. Soc.*, in press.
(7) Mirkin, N. G.; Krimm, S. *J. Mol. Struct.*, in press.
(8) Fogarasi, G.; Pulay, P. *Vibrational Spectra and Structure*; Durig, J. R., Ed.; Elsevier: Amsterdam, 1985; pp 125-219.
(9) Johansson, A.; Kollman, P.; Rothenberg, S.; McKelvey, J. *J. Am. Chem. Soc.* **1974**, *96*, 3794-3800.
(10) Del Bene, J. E. *J. Chem. Phys.* **1975**, *62*, 1961-1970.
(11) Hinton, J. F.; Harpool, R. D. *J. Am. Chem. Soc.* **1977**, *99*, 349-353.

(1) Eckert, H. *Angew. Chem.* **1989**, *101*, 1723.
(2) Borisova, Z. U.; Kasatkin, B. E.; Kim, E. I. *Izv. Akad. Nauk SSSR, Neorg. Mater.* **1973**, *9*, 822. Heyder, F.; Linke, D. *Z. Chem.* **1973**, *13*, 480. Blachnik, R.; Hoppe, A. *J. Noncryst. Solids* **1979**, *34*, 191.

0002-7863/90/1512-9017$02.50/0