N-ethylformamide dimer. A β -turn model peptide in the gas phase.

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Abstract.

Reported here are CP-FTMW experiments on N-ethylformamide (NEF), previously studied by pure rotational spectroscopy in the gas phase by Hirota and co-workers [J. Mol. Struct. 744 (2005) 815-819]. Two conformers of the monomer (*trans* *sc* and *cis* *ac*), the *trans-trans* *sc* dimer, and several NEF-water complexes have been detected. (Only *trans* *sc* was observed in the earlier experiments.) Of these species, the *trans-trans* *sc* dimer is the most interesting since its three-dimensional structure bears a striking resemblance to β -turns in folded peptides. The well-known *anti*-parallel β -sheet structures would be expected to have near-zero dipole moments and are therefore discriminated against by the CP-FTMW method.

Introduction.

The several levels of protein structure (primary, secondary, tertiary, and quaternary) are influenced by a variety of intermolecular forces, both attractive and repulsive. Included among them are hydrogen bonds, dipole-dipole interactions, and London dispersion forces. Normally, a combination of these forces is responsible for the uniqueness of a structure, but in some cases one particular interaction plays the dominant role. Thus, in α -helices, adjacent turns are stabilized by the formation of hydrogen bonds between amino acids, whereas β -sheet moieties are stabilized by hydrogen bonds involving NH and CO groups on adjacent strands oriented in a parallel or anti-parallel fashion. But more generally, all of these forces are likely to be responsible for the three-dimensional structure of a protein, whether it be a functioning protein in a healthy human being or a misfolded one in a diseased person suffering from Alzheimer's disease, Parkinson's disease, or Type 2 diabetes. Significant progress is being made in understanding the molecular basis of these disorders.¹ The timing of the different intermolecular encounters, as well as their energies, is proving to play a major role.

Previous studies of isolated molecules using a variety of experimental techniques have revealed that small peptides consisting of up to six linked amino acids exhibit structural features that mimic the β -sheets, helices, and turns which are found in real proteins.² But, even in these cases, the studied molecules were too large to obtain detailed information about the forces that control their structure. A more promising approach is to use rotationally resolved spectroscopy, which has already been applied in several pioneering studies of a large number of homo-dimers of relatively small molecules. These include microwave studies of hydrogen-bonded complexes like (HF)₂

and (H₂O)₂, for which accurate measurements of the heavy-atom distances, electron distributions, and (in some cases) intramolecular dynamics have been obtained,³ as well as high resolution UV studies of the *cis*-peptide-like 2-pyridone dimer and related base pair mimics.⁴

We focus in this report on the hydrogen-bonded dimer of N-ethylformamide (NEF), a simple molecule containing a peptide bond. Formamide itself has previously been used to study the properties of the hydrogen bonds in formamide-water and formamide-methanol,⁵ but its dimer has not been detected. NEF was first studied with microwave spectroscopy by Hirota and co-workers.⁶ They reported the observation of the most stable conformation of the molecule, called *trans-sc*, and determined its heavy-atom structure by isotopic substitution. Two other predicted monomer structures, *cis-ac* and *trans-ap*, were not detected. Our further work on this system has revealed several transitions of the *cis-ac* monomer, single water complexes of *trans-sc* and *cis-ac*, and the *trans-sc* dimer. The dimer structure was confirmed from N-14 quadrupole splittings that appear at high resolution. Not surprisingly, the more stable symmetric *cis-ac* dimer was not observed, presumably because of its expected near-zero dipole moment.

Theoretical and Experimental Methods.

The structures of the different possible conformations of monomeric NEF were first optimized using MP2/6-311++G(d,p).⁷ Three minima were observed and are shown in Fig. 1; they are designated *trans-sc* (the global minimum), *trans-ap* ($\Delta E=137$ cm⁻¹), and *cis-ac* ($\Delta E=482$ cm⁻¹), according to the notation of Ref. 6. (The *trans-ac* structure identified there has a dihedral angle of 78°; hence, we re-designate this as *trans-sc*.) Here, *trans*

and *cis* represent the relative positions of the NH and CO groups with respect to the peptide bond, and *sc*, *ac*, and *ap* represent the angular position of the -CH₃ group relative to the plane defined by the N-C-O backbone; *synclinal* or *sc* (+/-30 to +/-90°), *anticlinal* or *ac* (+/-90 to +/-150°), and *antiperiplanar* or *ap*, (+/-150 to 180°). Notably, the *trans-sc* structure features an intramolecular hydrogen bond, which is no doubt responsible for its lower energy.

Figure 1. Structures of the three lowest energy NEF monomers and their relative energies in cm⁻¹.

Similar calculations were performed on several possible homodimers of NEF, shown in Figure 2 along with their relative energies, their dissociation energies calculated using the BSSE method given by Xantheas⁸ (both in cm⁻¹), and their dipole moments. According to theory, the most stable dimers are *cis-cis (ac)* structures with near-zero dipole moments; these exhibit two N-H...O *intermolecular* hydrogen bonds much like those found in β -sheet structures. Next in energy are two pairs of complexes with relatively large dipole moments and non-negligible dissociation energies.

Figure 2. Six different possible homodimers of NEF, ranked according to their relative energies, in cm^{-1} (In parentheses are shown the dissociation energy of the complex in the same units). Also shown are the predicted values of their dipole moments.

Calculations at the MP2 level using the 6-311++G(d,p) basis set show a slight change of the dihedral angle of the methyl group of the monomer (78.05°) upon dimerization. This change is slightly different for each subunit of the complex, ranging from 2 - 12° , to accommodate the possible dispersion forces that further stabilize the global

structures. The difference in energy between the structures having the monomer dihedral angles and the others having optimized ones are a few tens of cm^{-1} (see Fig. 2).

The sample of NEF was acquired from Pfaltz-Bauer (98%) and used without further purification. Preliminary studies were carried out at the University of Pittsburgh using a miniaturized CP-FTMW spectrometer working in the 8-18 GHz range.⁹ Subsequent studies utilized the 2-8 GHz CP-FTMW spectrometer at the University of Virginia.¹⁰ In this case, two independent nozzles were used; each nozzle was heated to 90° C and the vaporized sample was seeded in a stream of Ne gas flowing at a backing pressure of 4 atm. (Experiments using other backing gases were not attempted.) Then, a 2 μs length chirped pulse covering the 2-8 GHz range was generated by a 24 GS/s arbitrary wave generator and amplified up to 300 Watts before it was broadcast inside the chamber through a first microwave horn antenna. After the interaction with the expanding molecules and the subsequent relaxation, 20 μsec FID's were collected with a second horn, amplified and sent to a 25 GS/s digital oscilloscope. The signal was accumulated and averaged over the course of six hours (88000 cycles of acquisition). The resolution of the final spectrum was better than 10 kHz.

Results.

Figure 3 shows a schematic diagram of the pure rotational spectrum of NEF over the frequency range 3.5-7.5 GHz, where most of the dimer signals were observed. Lines marked with "M" ("D") are monomer (dimer) transitions; those from the *trans-sc* and *cis-ac* forms of the monomer were the first ones assigned. As shown in the figure, signals from the dimer would easily escape and observer's attention at first sight.

Figure 3. Pure rotational spectrum of N-ethylformamide.

Transitions belonging to the *trans-sc* conformer of the monomer were readily recognized from JB95¹¹ simulations of the spectrum using the experimental rotational constants measured by Ohba *et al.*⁶ in their original study of this molecule. Transitions belonging to the new *cis-ac* conformer (much weaker than those shown in Fig. 2) were discovered by comparing simulated spectra using theoretical rotational constants with the experimental ones; the new conformation was found to exhibit relatively strong ^aR-type transitions in this region. The $1_{01}-0_{00}$ transition of *cis-ac* was located and easily assigned through its characteristic quadrupole splitting pattern (only three quadrupole components and a quadrupole splitting similar to the one predicted). The observation of the following

transitions in the series in J and the absence of *b*- and *c*-type transitions allowed us to confirm the assignment of the new species to the third most stable structure of NEF. This and the following spectra were fit using Pickett's programs.¹²

Comparison of the observed rotational constants, distortion constants, and quadrupole parameters of *trans-sc* NEF with those previously measured by Hirota and co-workers⁶ shows excellent agreement; we detected 73 lines compared to their 97. The comparison with theory is less favorable (see Table 1); the predicted values of A, B, and C differ from the observed ones by 2.3, 1.6, and 0.9%, respectively. Despite this fact, the predicted values of the rotational constants of *cis-ac* NEF are in much better agreement with the experimental values of the second conformer than are the predicted values for *trans-ap*, despite its higher relative energy. (The predicted value of the dipole moment of *trans-ap* is 4 D, but further searches for this conformer were not successful.)

The theoretical values of the quadrupole parameters of both observed monomer structures are in excellent agreement with experiment. The different behavior of the quadrupole constants compared to the rotational constants is due to the different frames of coordinates in which they are expressed. The rotational constants are given in a frame centered on the center of mass of the molecule, whereas the quadrupole constants are given in a frame centered on the N atom. Thus, even relatively small changes in the geometry will produce larger deviations in the rotational constants than in the quadrupole constants of the molecule.

Table 1. Comparison of the predicted and observed rotational constants of three possible conformations of N-ethylformamide.

MP2/6-311+G(d,p)	trans-sc	Expt.	trans-ap	cis-ac	Expt.
A (MHz) ^a	9672	9904.867(3) ^h	16174	19221	[19221] ⁱ
B (MHz)	3578	3521.090(1)	2716	2340	2378.124(1)
C (MHz)	3013	2985.010(1)	2451	2287	2320.822(9)
Δ_J (kHz) ^b		9.3(1)			2.47(11)
Δ_{JK} (kHz)		-61.76(5)			-97.31(35)
Δ_K (kHz)		165(2)			
δ_J (kHz)		3.2(1)			
δ_K (kHz)		0.24(7)			
μ_a (D) ^c	2.2		-3.1	-4.3	
μ_b (D)	-2.9		-2.4	-0.9	
μ_c (D)	1.0		-0.5	0.0	
μ_T (D)	3.8		4.0	4.3	
χ_{aa} (MHz) ^d	1.42	1.379(2)	1.72	1.93	1.843(3)
χ_{bb} (MHz)	0.66	0.816(13)	2.27	-2.62	-3.368(12)
χ_{cc} (MHz)	-2.09	-2.195(13)	-3.99	0.68	1.525(11)
$\Delta E_{MP2+ZPC}$ (cm ⁻¹) ^e	0		137	482	
n ^f		73			12
σ ^g / kHz		2.7			2.0

^a A, B and C are the rotational constants. ^b Centrifugal distortion constants. ^c μ_a , μ_b , μ_c and μ_T are the absolute values for the electric dipole moment components and the total dipole moment. ^d χ_{aa} , χ_{bb} and χ_{cc} are the diagonal elements of the ¹⁴N nuclear quadrupole coupling tensor. ^e Relative energies. ^f Number of fitted lines. ^g Standard deviation of the fit. ^h Errors in parentheses are expressed in units of the last digit. ⁱ Value kept fixed in the fit.

A population ratio of *trans-sc*:*cis-ac* = 60:1 was estimated from a comparison of the observed intensities of their respective 1_{01} - 0_{00} transitions, yielding an energy difference of 850 cm^{-1} , two times larger than the predicted value (482 cm^{-1} , see Fig. 1). MP2 calculations show a low interconversion barrier from the *trans-sc* to the less stable *trans-ac* conformer, around the C-C-N-C coordinate, of just 240 cm^{-1} . This suggests relatively facile interconversion into the more stable *trans-sc* form during the free-jet expansion. Typically, such interconversions occur when barriers separating different conformers are 300 cm^{-1} or less.¹³

Examination of the data in Fig. 3 also revealed several transitions that could be assigned to water complexes of NEF, formed adventitiously in the free-jet expansion. Three such complexes were discovered; their properties are summarized in Figure 4

Figure 4. Water complexes of NEF and their relative energies in cm^{-1} .

and Table 2. Lowest in predicted relative energy is *(cis-ac)*-water for which 12 transitions were observed; next are the *trans-sc* complexes, *(trans-sc)*-water1 (17 transitions) and *(trans-sc)*-water2 (13 transitions). [(*Trans-ap*)-water was not detected.] In all cases, single acceptor hydrogen-bonded structures are formed that involve the carbonyl oxygen of NEF and a proton of the attached water molecule. Despite the relatively good agreement between the observed and predicted rotational constants, assignments of

specific transitions to specific complexes were made possible in each case only after comparison of the observed quadrupole splittings with the predicted ones. Dynamical splittings that might be associated with motion along one or more intermolecular coordinates were not observed in any of the water complex spectra, either because of high barriers, or because equivalent structures of the observed conformers do not exist.

Table 2. Water complexes of N-ethylformamide.

MP2/6-311+G(d,p)	<i>(cis-ac)</i> -water		<i>(trans-sc)</i> -water		<i>(trans-ap)</i> -water	<i>(trans-sc)</i> -water2	
A (MHz) ^a	4431	4530.49(38) ^h	3310	3309.4389(57)	3498	8052	[8052] ⁱ
B (MHz)	1785	1754.944(2)	2559	2545.7663(13)	2136	1257	1224.6088(9)
C (MHz)	1371	1368.005(2)	1637	1599.1077(6)	1373	1176	1148.6454(9)
Δ_J (kHz) ^b				-			0.309(42)
Δ_{JK} (kHz)				19.22(96)			26.25(38)
μ_a (D) ^c	2.7		3.5		3.0	3.6	
μ_b (D)	-0.3		-0.2		-1.6	-1.7	
μ_c (D)	-1.4		0.4		0.8	-0.9	
μ_T (D)	3.0		3.5		3.5	4.0	
χ_{aa} (MHz) ^d	2.11	1.949(9)	1.99	1.7859(42)	1.31	1.88	1.737(8)
χ_{bb} (MHz)	0.97	1.32(16)	1.36	1.3294(62)	1.81	-0.41	0.158(22)
χ_{cc} (MHz)	-3.37	-3.27(16)	-3.36	-3.1153(62)	-3.12	-1.47	-1.895(22)
ΔE_{MP2} (cm ⁻¹) ^e	0		288		506	551	
N ^f	12		17			13	
σ^g / kHz	8.3		3.6			2.8	

^a A, B and C are the rotational constants. ^b Centrifugal distortion constants. ^c μ_a , μ_b , μ_c and μ_T are the absolute values of the electric dipole moment components and the total dipole moment. ^d χ_{aa} , χ_{bb} and χ_{cc} are the diagonal elements of the ¹⁴N nuclear quadrupole coupling tensor. ^e Relative energies. ^f Number of fitted lines. ^g Standard deviation of the fit. ^h Errors in parentheses are expressed in units of the last digit. ⁱ Value kept fixed in the fit.

After discarding all of the observed rotational transitions due to assigned species in the spectrum (NEF monomers, their water complexes and also the water dimer), attempts were next made to identify possible lines for the homodimer of NEF, (NEF)₂. Among the possibilities, the globally most-stable complexes formed from two units of the *cis-ac* monomer were neglected because of their predicted near-zero dipole moments (*cf.* Fig. 2). So, initially we focused our attention on the dimers formed from two units of the *trans-sc* monomer, the most abundant conformer in our experiment. Two such structures (A and B) are possible (*cf.* Fig. 2), both featuring only a single hydrogen bond and having very similar structures. As shown in Table 3, their predicted rotational constants and dipole moments are very similar, so our only hope to distinguish them was *via* their quadrupole splittings, which were expected to be different (*vide supra*).

A transition with multiple quadrupole components was first observed at 6664 MHz and assigned to the $3_{21}-2_{11}$ transition of a dimer. Subsequently, other similar transitions were identified with the help of JB95, used to obtain preliminary values of the rotational constants. This led to assignments of all the main ^aR-type transitions in the 2-8 GHz range. (The μ_a component is predicted to be the largest component of the permanent dipole moment of both dimers.) Then, one ^cR-type line was assigned, which made it possible to obtain a good value for the A rotational constant. All observed transitions showed a distinctive and complex quadrupole splitting, since the dimer has two ¹⁴N nuclei surrounded by asymmetric distributions of electronic charge. An example of this behavior is shown in Figure 5. Comparison of the observed splittings in this spectrum with the predicted ones leads to a clear assignment of all dimer transitions to structure A.

Table 3. Predicted rotational parameters of two possible NEF dimers, compared to experiment.

MP2/6-311+G(d,p)	Dimer A (trans-sc)-(trans-sc)	Dimer B (trans-sc)-(trans-sc)	Expt.
A (MHz) ^a	1610	1665	1646.0289 (9) ^h
B (MHz)	680	607	632.0714 (7)
C (MHz)	597	591	571.0359 (6)
Δ_J (kHz) ^b			0.470(5)
Δ_{JK} (kHz)			-0.07(3)
$\square_J$ (kHz)			0.088(7)
μ_a (D) ^c	5.9	5.9	
μ_b (D)	1.4	0.8	
μ_c (D)	-2.1	-2.0	
N1 χ_{aa} (MHz) ^d	0.65	-0.46	1.065(19)
χ_{bb} (MHz)	1.38	0.02	1.679(36)
χ_{cc} (MHz)	-2.03	0.44	-2.744(36)
N2 χ_{aa} (MHz)	0.22	1.03	0.903(21)
χ_{bb} (MHz)	1.84	1.79	1.316(40)
χ_{cc} (MHz)	-2.06	-2.82	-2.219(40)
ΔE_{MP2} (cm ⁻¹) ^e	0	29	
n ^f			76
σ ^g / kHz			5

^a A, B and C are the rotational constants. ^b Centrifugal distortion constants. ^c μ_a , μ_b , μ_c and μ_T are the absolute values for the electric dipole moment components and the total dipole moment. ^d χ_{aa} , χ_{bb} and χ_{cc} are the diagonal elements of the ¹⁴N nuclear quadrupole coupling tensor. ^e Relative energies. ^f Number of fitted lines. ^g Standard deviation of the fit. ^h Errors in parentheses are expressed in units of the last digit.

Figure 5. (Top) Quadrupole fine structure of the $3_{21}-2_{11}$ rotational transition of NEF dimer, and (bottom) its comparison with the predicted splittings of the two different dimers of the molecule formed with two trans-sc monomers, A and B.

Following this exhaustive analysis of the spectrum of NEF, a few lines remained unassigned. Most of these exhibit quadrupole splitting patterns characteristic of a single nitrogen nucleus, and therefore are likely due to rare gas complexes.

Discussion.

Previous IR studies of formamide, acetamide and its methyl derivatives¹⁴ show that non-N-methylated amides form symmetric dimers, whereas singly N-methylated amides conserve their preferred conformation and form singly hydrogen-bonded dimers with separate donor and acceptor roles. Our results for NEF are consistent with these conclusions; the structures of the two trans-sc monomers in the observed (NEF)₂ dimer are very similar to the structure of the isolated monomer units. As the individual monomer units are “asymmetric”, only a single hydrogen bond connects them in the dimer structure.

Figure 6 shows a comparison of the observed dimer structure to two biologically canonical structures, a β -sheet and a β -turn formed with two and three NEF units, respectively. Intriguingly, the hydrogen bond distances and angles in the observed dimer lie midway between the values predicted for the β -sheet and β -turn structures, but a closer examination reveals some important differences.

Figure 6. (a) β -sheet and (c) β -turn formed with two and three units of NEF, respectively, calculated at MP2/6-311++G(d,p); (b) structure of the observed dimer. Below each structure are given the theoretical distances in angstroms and angles in degrees of the hydrogen bonds marked with discontinuous red lines.

There are two main types of β -sheets in biology; these differ in the relative orientation of the amino acids in the two interacting strands, parallel and antiparallel. In both cases, the N-H bonds in one strand form hydrogen bonds with the C=O bonds of the adjacent strand. In the parallel arrangement, the successive strands all point in the same direction, whereas in the antiparallel arrangement, the successive strands point in opposite directions. As a result, the backbone dihedral angles in the two configurations are different, being $(\phi, \psi) \sim (-120^\circ, 115^\circ)$ in parallel sheets and $\sim (-140^\circ, 135^\circ)$ in antiparallel sheets. The single peptide bond in the observed NEF dimer has a (first) dihedral angle of $|\phi| = 139^\circ$, closer to the observed value in antiparallel sheets. This is a reasonable result, since the remaining dihedral angle is not constrained in $(\text{NEF})_2$, and the inter-strand hydrogen bonds between amines and carbonyls in antiparallel sheets are (also) planar.

The essence of the hairpin turn in biology are two hydrogen bonds involving the CO and NH groups of residues n and $n+3$ in a polypeptide chain, causing it to reverse its direction, and leading to the compact, globular shapes that are so essential to life. The present work shows that a similar reversal can occur *via* van der Waals interactions involving attached alkyl groups, in cases where peptide linkages are not available for hydrogen bonding. Obviously, the structure of the NEF dimer differs from both the sheet and the turn owing to its higher flexibility, which drives both components in the dimer to optimize their interactions. Comparing the hydrogen bond distances and angles in the figure, the hydrogen bond in the proto- β -sheet structure seems to be stronger, but the corresponding bond in the turn is not negligible, given the decrease of frequency of the N-H stretch in this kind of H bonding.¹⁵ Clearly, the additional attractive van der Waals

interactions “tip the balance” towards the folded structure, a precursor to different kinds of globular structures in biology.

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