Acc. Chem. Res. **2006,** *39,* 216−220

Dynamical Structure of Peptide Molecules

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Received June 2, 2005

ABSTRACT

In view of the importance of the peptide linkage in structural biology, we have carried out intensive investigations on peptide molecules consisting of a peptide linkage with one or two substituents in the gas phase by Fourier transform microwave spectroscopy, paying special attention to the internal rotation of the substituents relative to the central linkage framework. We have found that, in sharp contrast with the stiff structure around the central C−N bond of the linkage, the internal rotations of the substituents are of low frequency and thus of large amplitude and are extremely susceptible to their local environment such as the presence of other substituents.

Introduction

The peptide linkage constitutes one of the central backbone units in biological systems.[1,2] In view of the important role of this linkage and its peripherals in many problems of current concern in structural biology, we have recently undertaken a systematic study of the structure and dynamics of a series of peptide molecules, XCONHY, shown in Figure 1, by using Fourier transform microwave spectroscopy. When our results are combined with results previously reported by other groups, we are able to derive characteristic features of the linkage system, which can be of considerable significance to structural problems in biology. Our results will be also of great interest from the viewpoint of molecular science, because the dynamical structure of the peptide linkage system is quite unique, much different in character from that of most other organic molecules.

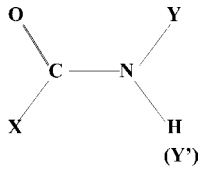

FIGURE 1. Peptide molecule.

To start with, we decided to focus our attention on the internal rotation of substituents X and Y directly bonded to the peptide linkage, because rotations about the C−X and N−Y bonds correspond to changes in the Ramachandran angles ψ and φ, respectively. We have also considered substitutions at the Y′ position, although such substitutions only occur in natural peptides containing

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Jon Hougen was born in Sheboygan, WI, in 1936. He received his B.S. in chemistry from the University of Wisconsin in 1956 and his Ph.D. in physical chemistry from Harvard in 1960. Dr. Hougen was a postdoctoral fellow and staff member in Prof. G. Herzberg's group at the National Research Council of Canada from 1960 to 1966. He returned to the U.S. to join Prof. David Lide's molecular spectroscopy group at the National Bureau of Standards (now called NIST) in 1967 and remained at NIST until his retirement in 2001. He is presently at NIST as a Scientist Emeritus. Dr. Hougen's research interests center on the derivation of quantum mechanical effective Hamiltonians for various cases of interaction between rotational, vibrational, and electronic motion.

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10.1021/ar040310c CCC: $33.50
Published on Web 01/19/2006

Table 1. Structure and Internal Motions of Peptide Molecules

molecule	X	Y	Y′	internal motions	reference
formamide	H	H	H	NH_2 out-of-plane (wagging) mode is anharmonic with a large quartic term.	17, 31, 32
acetamide	CH_3	H	H	CH_3 internal-rotation barrier is very low, 24 cm^{-1}.	11, 18, 33, 34, 35
N-methylformamide					19, 36, 37
trans	H	CH_3	H	CH_3 internal-rotation barrier is 56 cm^{-1}.	
cis	H	H	CH_3	CH_3 internal-rotation barrier is 288 cm^{-1}.	
N,*N*-dimethylformamide	H	CH_3	CH_3	CH_3 internal-rotation barrier is 366 and 772 cm^{-1} in the *trans* (Y) and *cis* (Y′) positions, respectively.	24
N-methylacetamide	CH_3	CH_3	H	CH_3 internal-rotation barrier is 73.5 and 79.1 cm^{-1} for CH_3 (X) and CH_3 (Y), respectively. The potential coupling term between the two CH_3 is 0.914 (cosine) and −2.9891 (sine) cm^{-1}, and the kinetic energy coupling term is 0.6397 cm^{-1}	21
N-ethylacetamide	CH_3	C_2H_5	H	CH_3 internal-rotation barrier is 75.4 cm^{-1} for (X), whereas that for CH_3 in the ethyl group (Y) is too high to determine (perhaps >950 cm^{-1})	38
propionamide	C_2H_5	H	H	Frequency of the torsion about the C−C bond between the ethyl and carbonyl groups is 45 cm^{-1}, and the C−C torsional potential is lowest when CH_3 in the ethyl group is *syn* to C═O and monotonically increases with the torsional angle to 500 cm^{-1} at an *anti* position.	22
N-methylpropionamide	C_2H_5	CH_3	H	CH_3 internal-rotation potential barrier is 796 and 80.1 cm^{-1} for CH_3 in the ethyl group (X) and CH_3 (Y), respectively. Potential coupling between the two CH_3 is not observable in the spectrum, whereas the kinetic coupling term is 0.227 cm^{-1}. Frequency of the torsion about the C−C bond between C_2H_5 and C═O is of the order of 10 cm^{-1}, and the potential function is similar to that in propionamide.	23
N-ethylformamide					20
trans	H	C_2H_5	H		
cis	H	H	C_2H_5		

the amino acid proline. The CH_3 group was chosen as the principal substituent, because its internal rotation is easy to analyze and the potential barriers derived can be compared quantitatively with one another. Readers who are interested in the structures characteristic of the peptide molecules but not in the details of the spectroscopic studies, described in the present paper, may go directly to the last section: Dynamical Structures Characteristic of Peptide Molecules.

Experimental: Fourier Transform Microwave Spectroscopy

Since its inception in the early 1980s by Flygare and coworkers,[3,4] pulsed molecular beam Fourier transform microwave spectroscopy has held great promise for the study of many molecular species that were heretofore impossible because of sensitivity issues associated with the, then current, typical source- or Stark-modulation-type spectrometers. The advantage of producing a molecular beam of the species of interest, cooled to approximately 2 K, forces the Boltzmann peak of even rather large species containing as many as 10−12 carbon atoms to lie in the frequency range (8−26.5 GHz) of the pulsed molecular beam Fourier transform microwave spectrometer. Over the years as this technique improved and evolved,[5−7] it has become the technique of choice for studying the rotational spectra of molecular species in the gas phase. Pertinent aspects of this type of spectrometer for this work are (a) the nearly textbook-like spectra (low *J* and low *K*) that result from the 2 K molecular beam,[8,9] (b) high sensitivity with parts per billion/vol detection limits,[10] (c) heatable nozzles, which contain a reservoir of the sample,[11,12] because all of the compounds of interest here are either liquids or solids at room temperature with very low vapor pressures, and (d) automated broadband scanning capabilities.

The ability of being able to cover large spectral regions quickly to obtain a survey spectrum is a requisite for obtaining internal-rotation E-state assignments because in some instances these transitions can be widely split from the rigid-rotor-like internal-rotation A state.[11,13]

Rotational spectra of the molecules investigated by the present study were observed using one of the mini-Fourier transform microwave spectrometers at the National Institute of Standards and Technology[14] and at Kanagawa Institute of Technology.[15] Samples were diluted with a rare gas buffer, either Ne/He (80:20%) or Ar, with a backing pressure of typically 1 bar (10^2 kPa), before they were introduced into a vacuum chamber through a pulsed nozzle, which was sometimes heated to 200 °C, depending upon the vapor pressure of the sample. The end of the nozzle contains a small reservoir that holds ∼100−300 mg of either the liquid or solid material.[12] Most of the compounds studied in this Account are heat-sensitive to some extent; therefore, to minimize the decomposition, the inside of the nozzle was also coated with an inert material such as Silcosteel or Sulfinert.[12,16]

Some Basic Examples of Peptide Molecules

Table 1 lists the peptide molecules investigated thus far. Although the main results obtained on the internal motions are described in Table 1, we shall discuss a few

representative molecules briefly. We adopt a widely accepted form $V = (V_3/2)(1 - \cos 3\alpha) + (V_6/2)(1 - \cos 6\alpha) + \ldots$ for the potential function to CH_3 internal rotation, where α denotes the internal-rotation angle and $V_3 \gg V_6$ holds; i.e., the series is rapidly converging in most cases.

The parent (i.e., unsubstituted) peptide molecule is formamide.[17] The most unique feature of its dynamical structure lies in the NH_2 wagging vibration; this mode has a large quartic anharmonicity, although the potential function has no hump at the planar configuration, so that the molecule is completely planar at equilibrium. Substitution of a CH_3 group at the X position gives acetamide, which has a small V_3 potential constant of 25.04 cm^{-1} to CH_3 internal rotation, accompanied by a quite large V_6 constant of -10.04 cm^{-1}.[18] Substitution of a CH_3 group at the Y (Y′) position gives *trans* (*cis*) *N*-methylformamide. Kawashima et al.[19] succeeded in observing the spectra of both *trans* and *cis N*-methylformamide. Although the *cis* form is *ab initio* calculated at the MP2/6-31G** level to be higher in energy than *trans* by 466 cm^{-1}, the observed *cis* spectra were quite strong compared with the *trans* spectra, much stronger than expected from the energy difference. This observation suggests that the barrier to the internal rotation about the central C−N bond is quite high, so that molecules in the *cis* potential minimum are effectively trapped there during the supersonic-jet cooling process; i.e., they are not further relaxed to the lowest minimum at *trans*. It is remarkable that the barrier height for *trans* CH_3, which corresponds to the naturally occurring C_α position, is 5 times lower than that for *cis* CH_3. *N*-Ethylformamide is another example, for which we have detected both *trans* and *cis* forms.[20] The energy difference between the two forms was calculated by an *ab initio* method at the MP2/6-31G** level to be 179 cm^{-1}, much smaller than that of *N*-methylformamide, and in fact, the spectra of *cis N*-ethylformamide were observed almost as strongly as those of *trans N*-ethylformamide. *N*-Methylacetamide[21] represents the most basic system containing both Ramachandran angles, and we are naturally interested in how the two CH_3 groups interact with each other and contribute to signal transfer through the interactions between the two tops. The internal-rotation potential barrier is quite similar for the two tops, as shown in Table 1, and the potential coupling term is 0.914 (cosine coupling, i.e., the coefficient of $\cos 3\alpha_1 \cos 3\alpha_2$) and -2.9891 (sine coupling, i.e., the coefficient of $\sin 3\alpha_1 \sin 3\alpha_2$) in cm^{-1}, to be compared with the kinetic energy coupling term of 0.6397 cm^{-1} (the coefficient of p_1p_2, with p_i denoting the angular momentum conjugate to α_i). All of these CH_3-CH_3 interaction terms turn out to be much smaller than the thermal energy at room temperature of about 200 cm^{-1}, an environment where most biological systems live.

Dynamical Structures Characteristic of Peptide Molecules

The results thus far obtained on the dynamical structures of peptide molecules are summarized as follows:

(1) The internal-rotation potential barrier of a single CH_3 about either of the Ramachandran angles ψ (X position) or φ (Y position) is lower than 100 cm^{-1}. The torsional frequency of a CH_3 group in the internal-rotation A level is calculated to be 64 cm^{-1}, when the potential barrier V_3 is 100 cm^{-1} and the kinetic energy term is 5.5 cm^{-1}. The internal rotation of an asymmetric substituent like the ethyl group has, in most cases, minima smaller in number than three, and thus, the substituent will execute an oscillatory motion even lower in frequency than that of CH_3. In fact, the torsional frequency of the ethyl group around the single minimum was estimated to be 45 and 10 cm^{-1} for propionamide[22] and *N*-methylpropionamide,[23] respectively.

(2) It is difficult to assign a typical value for the CH_3 internal-rotation potential barrier in the X and Y positions; the observed barrier heights differ considerably for different molecules. The CH_3 internal rotation is coupled with other internal motions. We have found[19] by an *ab initio* calculation at the MP2/6-31G** level that the CH_3 internal rotation in *trans N*-methylformamide is accompanied by the N−H out-of-plane angle bending by as large as 13° and by even larger 24° in *cis*, as shown in Figure 2. The CH_3 internal-rotation potential barrier is affected greatly by the presence of other substituents; the two CH_3 internal rotations in *N*,*N*-dimethylformamide are good examples,[24] in comparison with those in *trans* and *cis N*-methylformamide, although much of the increase in *N*,*N*-dimethylformamide may be explainable by simple steric hindrance considerations.

(3) The torsion about the central C−N bond of the peptide linkage is hindered by a potential barrier as high as 7000 cm^{-1},[25] which, as widely accepted, is ascribed to the resonance structure $O^-C{=}N^+H$, namely, to the partial double character of the C−N bond. The resonance maintains the linkage planar, as we found to be the case for the molecules listed in Table 1. The high barrier to torsion about the C−N bond hinders the higher energy *cis* form from being relaxed to the more stable *trans* minimum during adiabatic expansion of the molecular beam, as mentioned above.

We thus arrive at the following model for the structure and dynamics of the peptide linkage and its peripherals. The skeleton O=C−NH of this system is planar and stiff to twisting about the central C−N bond, whereas torsional motions about the C−X and N−Y bonds are inhibited by barriers that range from $^1/_{10}$ to $^1/_2$ of thermal energy at room temperature. The torsional motion of Y is correlated with the N−H (Y′) out-of-plane bending, and its potential barrier increases drastically when the H atom at Y′ is substituted by another heavier group. These low barriers to rotation about the Ramachandran angles indicate that protein folding can be carried out at energy costs that can be easily compensated for by the formation of intramolecular hydrogen and/or van der Waals bonds. Such bonds are lacking in the very small, one-peptide-linkage molecules listed in Table 1.

Usami et al.[26] have shown that malonamide $H_2NCOCH_2CONH_2$ takes an unsymmetrical, nonplanar conformation.

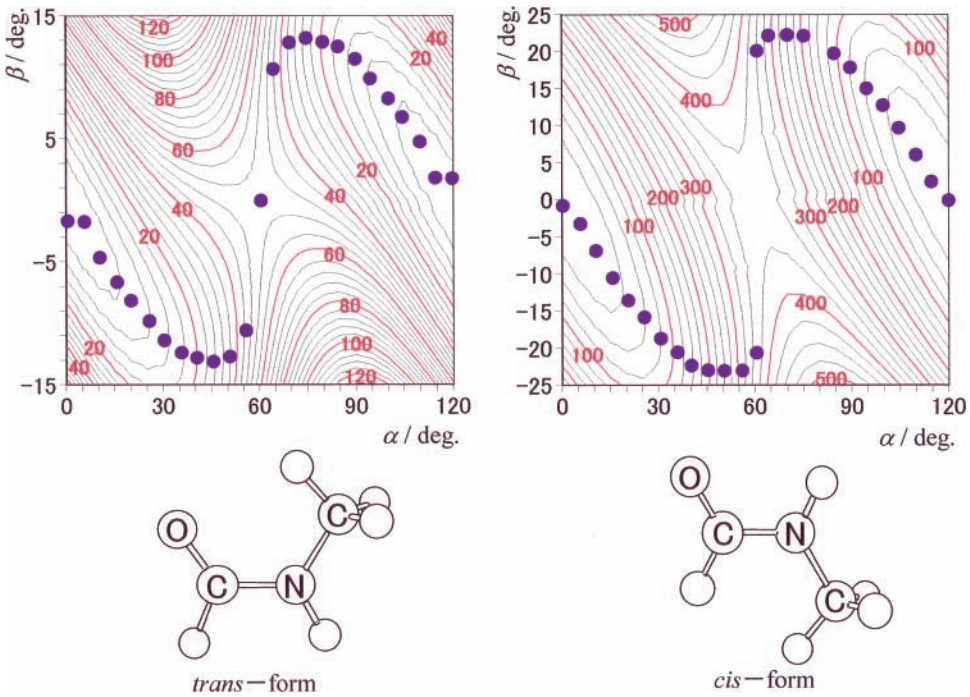

FIGURE 2. Potential energy surface calculated by an *ab initio* method at the MP2/6-31G** level as a function of the CH_3 internal rotation (α) and N−H out-of-plane bending (β) coordinates of *N*-methylformamide *trans* and *cis* form. Values in red in the figure denote energy in cm^{-1}, and the minimum energy path is indicated by blue dots.

From our findings described above, we anticipate that the torsions about the two OC−CH_2 bonds are of large amplitude and that the molecule can take almost any conformation with respect to these two internal-rotation angles, unless the two $CONH_2$ come close to and repel each other. Thus, a hydrogen bond is likely to be formed between an N−H of one $CONH_2$ group and C=O of the other, which fixes the otherwise almost freely rotating two groups at one preferred conformation. In fact, the observed rotational constants, combined with an *ab initio* MP2/6-31G** calculation, led to the two CCCO dihedral angles of 55.6° and 131.0°, a conformation that is most suitable for the formation of a hydrogen bond.

Turning next to the problem of determining precise folding conformations in the gas phase, we note that recently Lavrich et al.[27] succeeded in observing and analyzing the rotational spectra of *N*-acetyl-alanine *N*′-methylamide (abbreviated as AAMA): $CH_3CONH-(\varphi)-CH(CH_3)-(\psi)-CONHCH_3$, where φ and ψ denote the usual Ramachandran internal-rotation angles. AAMA serves as a model for the studies of protein conformations. We may regard the top part of the molecule: $CH_3CONH-CH(CH_3)-$ as a derivative of acetamide (X = CH_3) with the substituent Y = $CH(CH_3)\cdots$ in the amide group, of which the internal-rotation angle is φ, and the tail part: $-CH(CH_3)$-$CONHCH_3$ as an *N*-methylacetamide derivative, X = $\cdots CH(CH_3)$ with the internal-rotation angle ψ and Y = CH_3. We again expect that the molecule can take almost any conformation for the two Ramachandran angles, if we may disregard additional factors such as hydrogen bonding. From the analysis of the observed spectra, Lavrich et al.[27] concluded that the Ramachandran angles took the following values: $\varphi = -80.1°$ and $\psi = 71.3°$, by analyzing the internal-rotation axes of the two CH_3 groups, one in the acetyl of the top part and the other in the amide of the tail part. This conformation corresponds well to that which the molecule would take when stabilized by the hydrogen bond between the CO group of the top part and the NH group of the tail part. The internal-rotation barriers derived from the analysis of the spectra are 98.4 (2) and 84.0 (3) cm^{-1} for CH_3 in the acetyl group of the top part and in the *N*-methylamino group of the tail part, respectively, with both being in conformity with our observations summarized above.

The results listed in Table 1 combined with those of Usami et al.[26] and Lavrich et al.[27] were all obtained by spectroscopic studies in the gas phase. On the other hand, biological molecules are in most cases dissolved in various kinds of liquids in living systems, and their conformations will differ to a considerable extent for different environments. In these circumstances, we certainly require reliable standards that are not affected by solvation, and the results presented in Table 1 and elsewhere will serve as such standards. Nevertheless, we should extend our study to explore the effects of hydration. Lovas et al.[28] reported the rotational spectra of the formamide−water complex; Suenram et al.[29] studied formamide complexed with two water molecules; and Lavrich and Tubergen[30] detected one conformer for the alaninamide−water complex. It should be possible to observe the spectra of other species listed in Table 1 combined with one or more water molecules. Studies such as these will provide us with invaluable information on the effects of early solvation on the peptide linkage systems.

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AR040310C