Transition State Infrared Spectra for the Trans→Cis Isomerization of a Simple Peptide Model

Michelle A. Sahai,*,† Milán Szöri,‡ Bela Viskolcz,‡ Emil F. Pai,†,§ and Imre G. Csizmadia*,‡,⊥

Department of Medical Biophysics, University of Toronto, Toronto Medical Discovery Tower, 101 College Street, Room 5-359, Toronto, Ontario, Canada M5G 1L7, Department of Chemistry and Chemical Informatics, Faculty of Education, University of Szeged, Boldogasszony sgt. 6, Szeged, Hungary, 6725, Department of Biochemistry and Molecular and Medical Genetics, University of Toronto, Toronto, Ontario Canada, M5S 1A8, and Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Ontario, Canada, M5S 3H6

Received: June 26, 2007

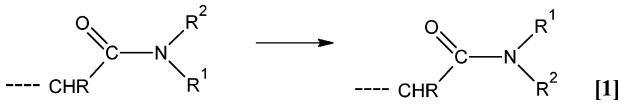
Trans→cis isomerization of *N*-methylacetylamide (MeCO-NHMe) has been studied at the G3MP2B3 level of theory and the vibration spectrum has been calculated as a function of the torsional mode of motion along the peptide bond. Noticeable spectral differences have been observed for the transition state interconnecting the cis and trans isomers.

Introduction

Peptide bonds in general prefer a trans conformation by about 2.5 kcal/mol over the cis isomeric form.[1] As amino acids are polymerized into a polypeptide chain on ribosomes in the cell the more stable trans peptide bond is formed. However, during the subsequent folding process the biologically active folded protein may require a cis peptide bond in certain positions. As long as the forces that govern protein folding and stability are not completely understood there can be no exact explanation for this observation.

The occasional occurrence of cis-type arrangements mainly involves proline amino acids in the −X-Pro− sequences.[2] This can be explained structurally since the peptide bond involving proline has a dialkyl-substituted nitrogen, therefore, the cis and trans forms are not that different. Additionally, the occurrence of non-Pro cis peptide bonds in proteins has been associated with steric strain. Their peculiar location near the active sites or binding pockets or at dimerization interfaces could imply that during the course of evolution there is a natural tendency to conserve non-Pro cis peptide bonds emphasizing their importance in protein structure and function.[2]

Trans−cis isomerization of the peptide [1] may occur from



[1]

the more stable trans to the less stable cis form in a kinetically feasible way.[1,2] Such processes have been studied extensively for secondary[3−7] (R^1 = H and R^2 = alkyl) as well as tertiary[5−9] amides (R^1 and R^2 = alkyl).

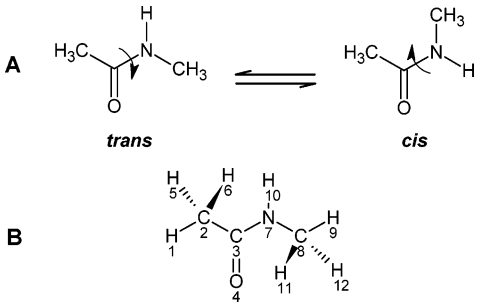


Figure 1. (A) Trans→cis isomerizations of the peptide bond in the simple peptide model MeCO-NHMe. (B) Schematic illustration of the numbering system applied to MeCO-NHMe.

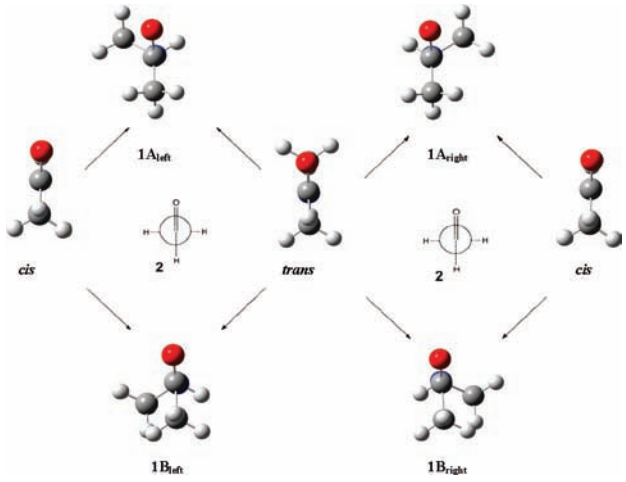


Figure 2. Chemical representations for the topology of the MeCO-NHMe PES.

For this trans→cis isomerization (Figure 1A), the transition state (TS) is of great importance. Historically, spectroscopy was used to study only stable structures, such as cis and trans isomers. However, with the invention of transition state spectroscopy[10] the investigation of IR spectra associated with trans→cis isomerization of peptide bonds became more than just theoretical interest.

* Address correspondence to these authors. M.A.S.: phone (01) 416-581-7548, fax (01) 416-581-7546, e-mail michelle.sahai@utoronto.ca. I.G.C.: phone (01) 416-581-7548, fax (01) 416-581-7546, e-mail imre.csizmadia@utoronto.ca.

† Department of Medical Biophysics, University of Toronto.

‡ University of Szeged.

§ Department of Biochemistry and Molecular and Medical Genetics, University of Toronto.

⊥ Department of Chemistry, University of Toronto.

10.1021/jp074991f CCC: $37.00
Published on Web 08/09/2007

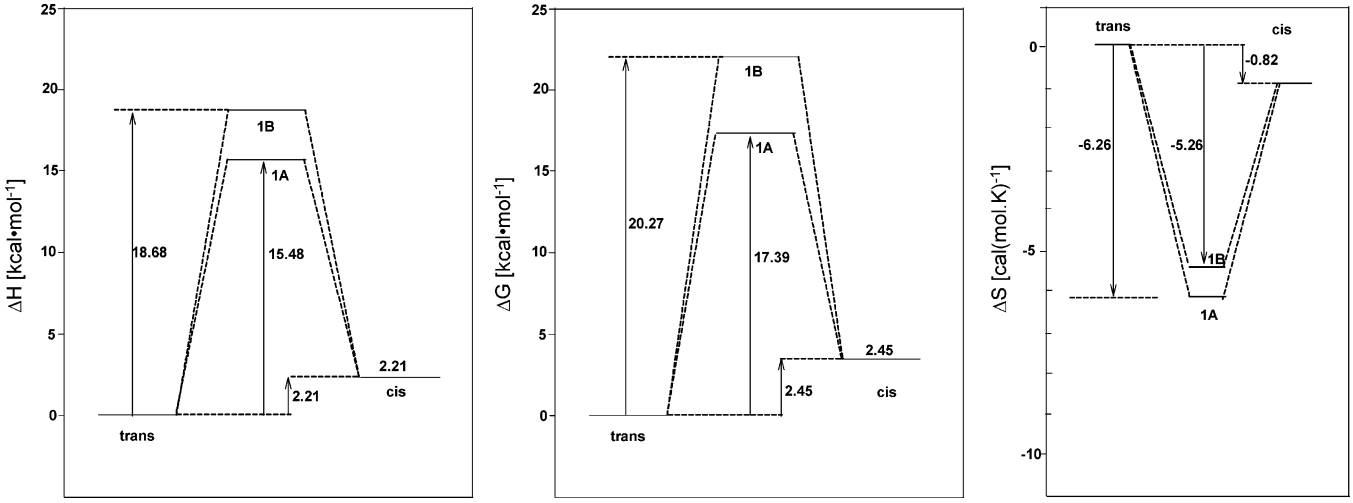


Figure 3. A schematic representation of the thermodynamic functions (ΔH, ΔG, ΔS) for the trans→cis isomerization of the model peptide MeCO-NHMe, computed at the G3MP2B3 level of theory.

According to TS theory[11,12] at the first-order critical point (i.e., transition state) the frequency associated with the reaction coordinate becomes imaginary. This frequency usually corresponds to the lowest of all the frequencies associated with positive force constants for the trans and cis isomers. Along the reaction coordinate, at about the inflection point of the curve, the force constant becomes negative and hence the frequency becomes imaginary.[13] Other spectral shifts may also occur in the vicinity of the TS. For this reason the isomerization process of the system could perhaps be followed by vibrational spectroscopy.

Methods

Molecular Structures. All computations were carried out with the Gaussian 03 program package (G03).[14] Each structure was initially optimized by using the ab initio[15] Restricted Hartree−Fock (RHF)[16] method with the split valence 3-21G basis set.[17−19] MultiDimensional Conformational Analysis (MDCA)[20] was used to define the topologically possible set of conformers represented by a grid-defined set of catchment regions. Presently, it is possible to accurately characterize the topologically probable set of stable conformers emerging from the larger set of topologically possible conformers.[21]

The RHF/3-21G geometry optimized structural parameters were then used as the input in a subsequent theoretical refinement step with the inclusion of electron correlation effects at the B3LYP/6-31G(d) level of theory to obtain more reliable geometry and stability data. Here, B3LYP[22] denotes the combination of Becke's three-parameter exchange functional with the Lee−Yang−Parr (LYP)[23] correlation functional and also employs the mathematically more complete 6-31G(d) basis set. To yield more accurate energies all stationary points were further refined by using the G3-based quantum chemistry method G3MP2B3.[24−26] Within the G3MP2B3 method, refinement of energy was employed by using the perturbation theory with the Møller−Plesset second order (MP2) method in combination with the 6-31G(d) basis set. Energies of this type are labeled as $E^{\text{uncorrected}}$. Total energies are given in hartrees,

TABLE 1: Selected Parameters for the Differing Species of the MeCO-NHMe Peptide Model Calculated at the G3MP2B3 Level of Theory[a]

parameters		species					
		trans	$1B_{left}$	$1B_{right}$	cis	$1A_{left}$	$1A_{right}$
stretch	C2−C3	1.52	1.52	1.52	1.52	1.51	1.51
	C3−O4	1.22	1.21	1.21	1.22	1.21	1.21
	C3−N7*	1.37	1.45	1.45	1.37	1.46	1.46
bend	N7−H10	1.01	1.02	1.02	1.01	1.02	1.02
	N7−C8	1.45	1.47	1.47	1.45	1.48	1.48
	C2−C3−O4	121.71	121.90	121.90	122.49	123.77	123.77
	C2−C3−N7	115.24	117.78	117.78	116.40	113.25	113.25
	C3−N7−C8*	122.57	114.79	114.77	127.23	111.32	111.32
	C3−N7−H10*	118.74	108.36	108.34	113.16	105.77	105.77
	O4−C3−N7	123.05	120.32	120.32	121.10	122.98	122.98
	H10−N7−C8*	118.69	109.11	109.09	118.18	107.22	107.22
torsion	C2−C3−N7−C8*	−179.98	58.10	−58.08	9.52	123.50	−123.50
energy	total	−248.141239	−248.111476	−248.111477	−248.137723	−248.116567	−248.116567
	relative	0.00	18.68	18.68	2.21	15.48	15.48
enthalpy	total	−248.140295	−248.110532	−248.110533	−248.136779	−248.115623	−248.115623
	relative	0.00	18.68	18.68	2.21	15.48	15.48
Gibbs free energy	total	−248.178441	−248.146136	−248.146133	−248.174531	−248.150728	−248.150728
	relative	0.00	20.27	20.27	2.45	17.39	17.39
entropy	total	79.66	74.40	74.39	78.84	73.35	73.35
	relative	0.00	−5.27	−5.28	−0.82	−6.31	−6.31
imaginary freq		N/A	−198.3039	−198.0880	N/A	−193.3393	−193.3392

[a] An asterisk indicates where significant differences in values are observed.

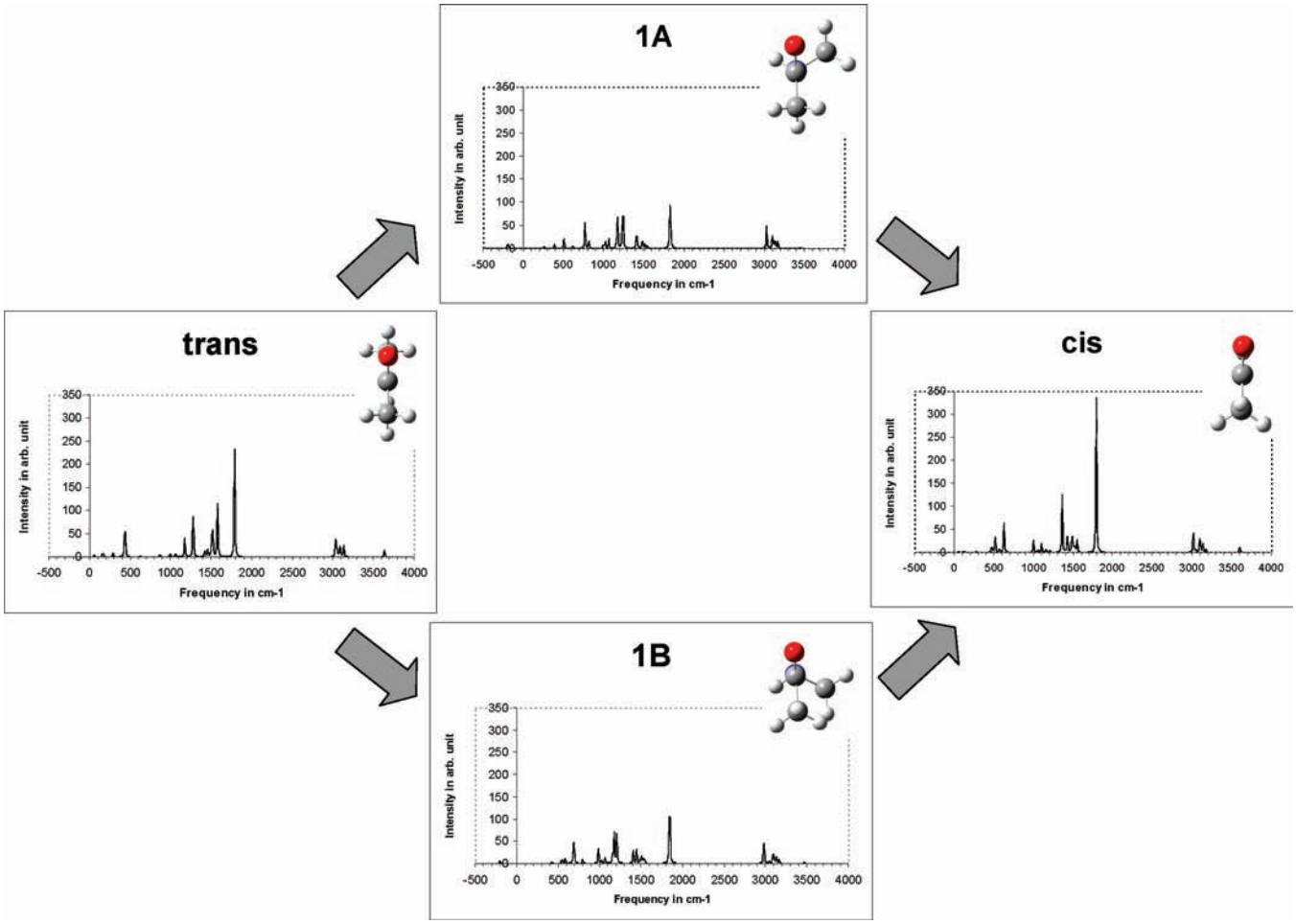


Figure 4. Infrared spectra for four differing species of the MeCO-NHMe peptide model: trans, TS (1A and 1B), and cis structures.

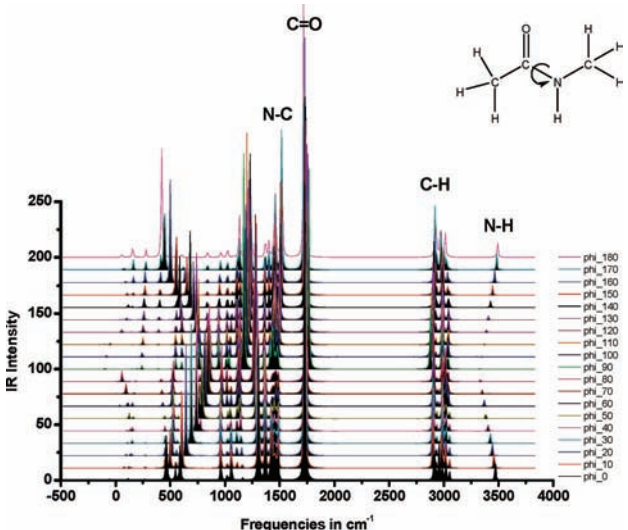


Figure 5. IR spectra as a function of C−C−N−C dihedral angle.

and the relative energies are given in kilocalories per mole (with the following conversion factor: 1 hartree = 627.5095 kcal·mol^{-1}).

Molecular Vibrations. Additionally, each stable conformer was subjected to frequency calculations at the B3LYP/6-31G(d) level of theory to confirm their identity as being true minima. The results also provided Zero Point Energy (ZPE) values, which were scaled by using a correction factor and added to the total energy of each conformer to provide more accurate energetic characterization of the conformers as well as the vibrational frequency of each of the normal modes. Corrected energies for these geometries are labeled as $E^{\text{corrected}}$.

Vibrational spectra were also plotted as a function of the C−C−N−C dihedral angle associated with the rotation about the C−N bond.

Thermodynamic Functions (ΔH, ΔG, and ΔS). Besides the relative energy ($\Delta E^{\text{uncorrected}}$ and $\Delta E^{\text{corrected}}$) values computed by the G3MP2B3 method, the thermodynamic functional changes of enthalpy (ΔH), Gibbs free energy (ΔG), and entropy (ΔS) were also calculated for the stable conformations. The values of total enthalpy (H) and Gibbs free energy (G) given in hartrees were converted to their respective relative values, ΔH and ΔG, in kcal·mol^{-1} as described above, while the values for entropy (S) and entropy change (ΔS) were given in cal·mol^{-1}·K^{-1}.

Results and Discussion

For the present study *N*-methylacetylamide (MeCO-NHMe) was used as a small peptide model, because it contains the characteristic CO-NH peptide bond. Its molecular structure, as prepared for computations, is illustrated in Figure 1B, while its isomerization scheme is shown in Figure 2. The trans and cis structures are interconnected by two pairs of transition states, $1A_{\text{left}}$, $1A_{\text{right}}$ and $1B_{\text{left}}$, $1B_{\text{right}}$, corresponding to two enantiomeric pairs. The structures labeled as "2" correspond to maxima on the potential energy surface (PES).

The TS structure in the present work was defined according to the C−C−N−C dihedral angle rather than according to the

TABLE 2: Computed Vibrational Frequencies[a] and IR Intensities for MeCO-NHMe Trans→Cis Isomerization

	trans		TS(1A)		TS(1B)		cis		
mode	frequency	IR intensity	frequency	IR intensity	frequency	IR intensity	frequency	IR intensity	mode
1	56.5	3.0	−185.6	12.7	−190.4	6.0	67.1	1.2	1
2	78.9	0.0	163.9	0.8	95.2	0.5	119.7	2.0	2
3	157.6	11.5	203.7	0.6	195.9	0.8	140.4	0.8	3
4	278.0	7.1	252.5	5.3	264.0	1.2	271.6	1.3	4
5	415.8	11.2	375.2	8.6	407.9	4.4	457.0	15.5	5
6	417.9	94.4	488.1	23.7	523.2	14.5	498.8	38.3	6
7	598.9	2.1	598.5	8.2	559.9	15.5	551.6	8.3	7
8	607.9	1.3	740.2	57.5	667.7	84.5	605.1	72.3	8
9	840.8	7.1	785.8	15.6	770.2	11.7	774.6	0.3	9
10	963.5	6.8	964.1	12.0	951.7	36.1	959.8	27.2	10
11	1023.2	9.9	986.7	17.3	986.3	9.5	1021.2	6.2	11
12	1072.7	0.9	1026.1	21.0	1030.1	16.3	1057.5	19.4	12
13	1117.7	0.4	1120.8	23.6	1112.5	16.2	1110.1	8.0	13
14	1132.0	40.9	1132.9	62.8	1131.1	77.3	1156.8	4.9	14
15	1231.3	109.9	1195.3	135.8	1164.0	81.6	1305.4	126.8	15
16	1368.6	20.9	1358.8	46.8	1354.1	28.9	1367.3	60.1	16
17	1399.5	16.7	1377.8	5.8	1390.2	35.6	1421.1	26.8	17
18	1441.3	5.7	1415.2	2.3	1421.4	1.6	1430.6	26.0	18
19	1454.0	44.0	1428.4	16.3	1433.3	9.2	1446.1	5.5	19
20	1461.0	41.0	1439.9	6.2	1450.5	14.5	1453.9	10.6	20
21	1469.8	6.4	1459.2	8.9	1467.4	10.5	1462.7	9.6	21
22	1518.5	128.9	1484.4	7.9	1482.0	9.8	1487.0	27.3	22
23	1719.3	239.9	1751.7	173.8	1771.2	208.0	1727.9	336.8	23
24	2922.4	60.3	2909.4	48.4	2866.7	72.7	2901.4	57.4	24
25	2940.5	10.4	2935.1	1.5	2930.6	5.3	2932.9	7.1	25
26	2970.6	38.9	2978.0	30.9	2971.4	34.0	2978.6	29.0	26
27	3012.1	21.7	2990.3	6.4	2987.3	9.2	2988.8	13.1	27
28	3016.5	7.0	3010.0	26.2	3010.7	20.9	3016.2	20.9	28
29	3047.7	1.0	3042.4	14.8	3041.4	10.8	3051.8	7.2	29
30	3492.7	16.3	3309.7	1.7	3328.8	1.6	3459.0	19.2	30

[a] Frequencies are scaled by 0.96

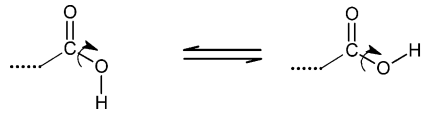
orientation of the C═O group against the lone electron pair of the amide nitrogen. The two methods are in fact equivalent but the current method is easier to define rigorously as the position of the lone pair is not uniquely defined. The tabulated dihedral angles (Table 1) for the TS structures are within the range of $90 \pm 30°$, which corresponds to a gauche orientation, and the lone pair is in either the syn or anti orientation of the C═O bond as can be inferred from Figure 2.

Thermodynamic functions (ΔH, ΔG, ΔS) were calculated at the G3MP2B3 level of theory and schematic reaction profiles are shown in Figure 3. The numerical values of the thermodynamic functions together with selected geometrical parameters are summarized in Table 1. The reaction profiles for ΔH and ΔG were both positive, with each having greater thermodynamic separation for the 1B TS than the 1A TS. The situation is the opposite for ΔS, where the 1A TS displays a greater thermodynamic separation than the 1B TS. Interestingly enough, the ΔS entropy changes computed for $3N - 6$ freedom of motion for the cis isomer and for $3N - 7$ freedom of motion for the transition states turned out to be negative with respect to the trans isomer.

According to Table 1, where the last two columns correspond to the TS structures of $1A_{left}$ and $1A_{right}$, the most favorable barrier height is computed to be 15.48 kcal/mol on the energy scale, which compares favorably to previously reported barrier heights of 16.34 kcal/mol.[4] The energetic trans→cis separation was found to be 2.21 kcal/mol, comparing favorably to a separation of 2.53 kcal/mol for a previously reported gas-phase study and 2.3 kcal/mol found experimentally.[4]

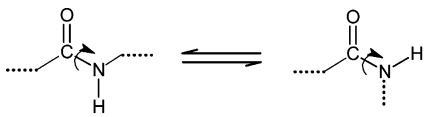
The corresponding spectra for the two isomers and the two different transition states 1A and 1B are depicted in Figure 4. Clearly the change of spectra along the torsional mode of motion involving the C−N bond is of great interest, which is shown in Figure 5. Most of the spectral shifts occur at the left-hand side, involving the frequency associated with the reaction coordinate, and on the right-hand side of the spectra, where the N−H stretching frequency is found. As far as the N−H bond is concerned, starting with the cis isomer ($\phi = 0$), there is a red shift from 3500 cm^{-1} to smaller values. However, after the TS, moving toward the trans isomer ($\phi = 180$), a blue shift occurs that gives a larger value than originally observed. These frequencies for the minima and transition structures are summarized in Table 2.

The trans→cis isomerization of peptide bonds is of great biological interest.[27−42] Since 1958 it has been studied experimentally[43−47] as well as theoretically.[48−52] More recent work[53,54] focused extensively on the energetics of the cis and trans isomers in small peptide models. Additionally, temperature-dependent infrared spectroscopy was used, successfully, to study the torsional mode of motion of a similar structure[55] [2],



[2]

which is analogous to the trans→cis isomerization mode of motion of peptides [3]. The results found herein no doubt add



[3]

additional information to the importance of trans→cis isomerization. Therefore, it is hoped that some IR spectroscopic technique may be developed to study the motion from stable

structures toward the transition state of a peptide bond isomerization, rather than only the two forms independently.

Acknowledgment. E.F.P. gratefully acknowledges support from the Canada Research Chairs Program, the Natural Sciences and Engineering Research Council of Canada, and the Canadian Institutes of Health Research.

Supporting Information Available: Important input geometry parameters and procedures for the ab initio quantum mechanical computations and the subsequent output parameters and molecular structures. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Poteau, R.; Trinquier, G. All-cis cyclic peptides. *J. Am. Chem. Soc.* **2005**, *127* (40), 13875−13889.

(2) Dugave, C.; Demange, L. Cis−trans isomerization of organic molecules and biomolecules: Implications and applications. *Chem. Rev.* **2003**, *103* (7), 2475−2532.

(3) Kang, Y. K. Ab initio MO and density functional studies on trans and cis conformers of *N*-methylacetamide. *THEOCHEM* **2001**, *546*, 183−193.

(4) Kang, Y. K.; Park, H. S. Internal rotation about the C−N bond of amides. *THEOCHEM* **2004**, *676* (1−3), 171−176.

(5) Scherer, G.; Kramer, M. L.; Schutkowski, M.; Reimer, U.; Fischer, G. Barriers to rotation of secondary amide peptide bonds. *J. Am. Chem. Soc.* **1998**, *120* (22), 5568−5574.

(6) Schiene-Fischer, C.; Fischer, G. Direct measurement indicates a slow cis/trans isomerization at the secondary amide peptide bond of glycylglycine. *J. Am. Chem. Soc.* **2001**, *123* (26), 6227−6231.

(7) Kang, Y. K. Conformational preferences of non-prolyl and prolyl residues. *J. Phys. Chem. B* **2006**, *110* (42), 21338−21348.

(8) Wiberg, K. B.; Rablen, P. R.; Rush, D. J.; Keith, T. A. Amides. 3. Experimental and Theoretical-Studies of the Effect of the Medium on the Rotational Barriers for *N*,*N*-Dimethylformamide and *N*,*N*-Dimethylacetamide. *J. Am. Chem. Soc.* **1995**, *117* (15), 4261−4270.

(9) Rablen, P. R. Computational analysis of the solvent effect on the barrier to rotation about the conjugated C−N bond in methyl *N*,*N*-dimethylcarbamate. *J. Org. Chem.* **2000**, *65* (23), 7930−7937.

(10) Polanyi, J. C. Some concepts in reaction dynamics. *Science* **1987**, *236* (4802), 680−690.

(11) Evans, M. G.; Polanyi, M. *Trans. Faraday Soc.* **1935**, *31*, 875.

(12) Eyring, H. *J. Chem. Phys.* **1935**, *3*, 107.

(13) Viskolcz, B.; Fejer, S. N.; Csizmadia, I. G. Thermodynamic functions of conformational changes. 2. Conformational entropy as a measure of information accumulation. *J. Phys. Chem. A* **2006**, *110* (10), 3808−3811.

(14) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, H.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukada, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Maliek, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, Revision B.01; Gaussian Inc.: Wallingford, CT, 2004.

(15) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Theory*; John Wiley & Sons: New York, 1986.

(16) Roothan, C. C. New developments in molecular orbital theory. *Rev. Mod. Phys.* **1951**, *23*, 69−89.

(17) Hehre, W. J.; Ditchfie, R.; Pople, J. A. Self-Consistent Molecular-Orbital Methods. 12. Further Extensions of Gaussian-Type Basis Sets for Use in Molecular-Orbital Studies of Organic-Molecules. *J. Chem. Phys.* **1972**, *56* (5), 2257ff.

(18) Ditchfie, R.; Hehre, W. J.; Pople, J. A. Self-Consistent Molecular-Orbital Methods. 9. Extended Gaussian-Type Basis for Molecular-Orbital Studies of Organic Molecules. *J. Chem. Phys.* **1971**, *54* (2), 724ff.

(19) Hariharan, P. C.; Pople, J. A. Influence of Polarization Functions on Molecular-Orbital Hydrogenation Energies. *Theor. Chim. Acta* **1973**, *28* (3), 213−222.

(20) Perczel, A.; Angyan, J. G.; Kajtar, M.; Viviani, W.; Rivail, J. L.; Marcoccia, J. F.; Csizmadia, I. G. Peptide Models. 1. Topology of Selected Peptide Conformational Potential-Energy Surfaces (Glycine and Alanine Derivatives). *J. Am. Chem. Soc.* **1991**, *113* (16), 6256−6265.

(21) Berg, M. A.; Chasse, G. A.; Deretey, E.; Fuzery, A. K.; Fung, B. M.; Fung, D. Y. K.; Henry-Riyad, H.; Lin, A. C.; Mak, M. L.; Mantas, A.; Patel, M.; Repyakh, I. V.; Staikova, M.; Salpietro, S. J.; Tang, T. H.; Vank, J. C.; Perczel, A.; Csonka, G. I.; Farkas, O.; Torday, L. L.; Szekely, Z.; Csizmadia, I. G. Prospects in computational molecular medicine: a millennial mega-project on peptide folding. *THEOCHEM* **2000**, *500*, 5−58.

(22) Becke, A. D. Density-functional thermochemistry. 4. A new dynamical correlation functional and implications for exact-exchange mixing. *J. Chem. Phys.* **1996**, *104* (3), 1040−1046.

(23) Lee, C. T.; Yang, W. T.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula Into A Functional of the Electron-Density. *Phys. Rev. B* **1988**, *37* (2), 785−789.

(24) Curtiss, L. A.; Redfern, P. C.; Raghavachari, K.; Rassolov, V.; Pople, J. A. Gaussian-3 theory using reduced Moller-Plesset order. *J. Chem. Phys.* **1999**, *110* (10), 4703−4709.

(25) Curtiss, L. A.; Raghavachari, K.; Redfern, P. C.; Rassolov, V.; Pople, J. A. Gaussian-3 (G3) theory for molecules containing first and second-row atoms. *J. Chem. Phys.* **1998**, *109* (18), 7764−7776.

(26) Baboul, A. G.; Curtiss, L. A.; Redfern, P. C.; Raghavachari, K. Gaussian-3 theory using density functional geometries and zero-point energies. *J. Chem. Phys.* **1999**, *110* (16), 7650−7657.

(27) Brandts, J. F.; Halvorson, H. R.; Brennan, M. Consideration of Possibility That Slow Step in Protein Denaturation Reactions Is Due to Cis−Trans Isomerism of Proline Residues. *Biochemistry* **1975**, *14* (22), 4953−4963.

(28) Borden, K. L. B.; Richards, F. M. Folding Kinetics of Phage-T4 Thioredoxin. *Biochemistry* **1990**, *29* (12), 3071−3077.

(29) Texter, F. L.; Spencer, D. B.; Rosenstein, R.; Matthews, C. R. Intramolecular Catalysis of A Proline Isomerization Reaction in the Folding of Dihydrofolate-Reductase. *Biochemistry* **1992**, *31* (25), 5687−5691.

(30) Shalongo, W.; Jagannadham, M. V.; Heid, P.; Stellwagen, E. A Kinetic-Study of the Folding of Staphylococcal Nuclease Using Size-Exclusion Chromatography. *Biochemistry* **1992**, *31* (46), 11390−11396.

(31) Lang, K.; Schmid, F. X. Role of 2 Proline-Containing Turns in the Folding of Porcine Ribonuclease. *J. Mol. Biol.* **1990**, *212* (1), 185−196.

(32) Kiefhaber, T.; Kohler, H. H.; Schmid, F. X. Kinetic Coupling Between Protein Folding and Prolyl Isomerization. 1. Theoretical-Models. *J. Mol. Biol.* **1992**, *224* (1), 217−229.

(33) Kiefhaber, T.; Schmid, F. X. Kinetic Coupling Between Protein Folding and Prolyl Isomerization. 2. Folding of Ribonuclease-A and Ribonuclease-T1. *J. Mol. Biol.* **1992**, *224* (1), 231−240.

(34) Brandl, C. J.; Deber, C. M. Hypothesis About the Function of Membrane-Buried Proline Residues in Transport Proteins. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83* (4), 917−921.

(35) Williams, K. A.; Deber, C. M. Proline Residues in Transmembrane Helices—Structural Or Dynamic Role. *Biochemistry* **1991**, *30* (37), 8919−8923.

(36) Vogel, H.; Nilsson, L.; Rigler, R.; Meder, S.; Boheim, G.; Beck, W.; Kurth, H. H.; Jung, G. Structural Fluctuations Between 2 Conformational States of A Transmembrane Helical Peptide Are Related to Its Channel-Forming Properties in Planar Lipid-Membranes. *Eur. J. Biochem.* **1993**, *212* (2), 305−313.

(37) Wess, J.; Nanavati, S.; Vogel, Z.; Maggio, R. Functional-Role of Proline and Tryptophan Residues Highly Conserved Among G-Protein-Coupled Receptors Studied by Mutational Analysis of the M3-Muscarinic-Receptor. *EMBO J.* **1993**, *12* (1), 331−338.

(38) Suchyna, T. M.; Xu, L. X.; Gao, F.; Fourtner, C. R.; Nicholson, B. J. Identification of A Proline Residue As A Transduction Element Involved in Voltage Gating of Gap-Junctions. *Nature* **1993**, *365* (6449), 847−849.

(39) Richards, N. G. J.; Hinds, M. G.; Brennand, D. M.; Glennie, M. J.; Welsh, J. M.; Robinson, J. A. Probing the Role of Proline As A Recognition Element in Peptide Antigens. *Biochem. Pharmacol.* **1990**, *40* (1), 119−123.

(40) Yaron, A.; Naider, F. Proline-Dependent Structural and Biological Properties of Peptides and Proteins. *Crit. Rev. Biochem. Mol. Biol.* **1993**, *28* (1), 31−81.

(41) London, R. E.; Stewart, J. M.; Cann, J. R. Probing the Role of Proline in Peptide-Hormones—Nmr-Studies of Bradykinin and Related Peptides. *Biochem. Pharmacol.* **1990**, *40* (1), 41−48.

(42) Yaron, A. The Role of Proline in the Proteolytic Regulation of Biologically-Active Peptides. *Biopolymers* **1987**, *26*, S215−S222.

(43) Steinberg, I. Z.; Berger, A.; Katchalski, E. Reverse Mutarotation of Poly-L-proline. *Biochim. Biophys. Acta* **1958**, *28* (3), 647−648.

(44) Steinberg, I. Z.; Harrington, W. F.; Berger, A.; Sela, M.; Katchalski, E. The Configurational Changes of Poly-L-proline in Solution. *J. Am. Chem. Soc.* **1960**, *82* (20), 5263−5279.

(45) Carver, J. P.; Blout, E. R. *Treatise on Collagen*; Ramachandran, E., Ed.; Academic Press: New York, 1967; pp 441−526.

(46) Deber, C. M.; Bovey, F. A.; Carver, J. P.; Blout, E. R. Nuclear Magnetic Resonance Evidence for Cis-Peptide Bonds in Proline Oligomers. *J. Am. Chem. Soc.* **1970**, *92* (21), 6191−&.

(47) Grathwohl, C.; Wuthrich, K. Nmr-Studies of the Rates of Proline Cis−Trans Isomerization in Oligopeptides. *Biopolymers* **1981**, *20* (12), 2623−2633.

(48) Maigret, B.; Perahia, D.; Pullman, B. Molecular Orbital Calculations on Conformation of Polypeptides and Proteins. 4. Conformation of Prolyl and Hydroxyprolyl Residues. *J. Theor. Biol.* **1970**, *29* (2), 275−&.

(49) Farmer, B. L.; Hopfinge, A. J. Nature of Molecular Motions of Poly(L-proline) for Cis Reversible Trans Conformational Transitions. *Macromolecules* **1974**, *7* (6), 793−796.

(50) Fischer, S.; Dunbrack, R. L.; Karplus, M. Cis−Trans Imide Isomerization of the Proline Dipeptide. *J. Am. Chem. Soc.* **1994**, *116* (26), 11931−11937.

(51) Robb, M. A.; Csizmadi, I. G. Non-Empirical Lcao-Mo-Scf-Ci Calculations on Organic Molecules with Gaussian Type Functions. 4. Preliminary Investigations on Formamide. *Theor. Chim. Acta* **1968**, *10* (3), 269−&.

(52) Andrews, P. R. Cis−Trans Isomerism of Peptide Bond. *Biopolymers* **1971**, *10* (11), 2253ff.

(53) Sahai, M. A.; Kehoe, T. A. K.; Koo, J. C. P.; Setiadi, D. H.; Chass, G. A.; Viskolcz, B.; Penke, B.; Pai, E. F.; Csizmadia, I. G. First principle computational study on the full conformational space of L-proline diamides. *J. Phys. Chem. A* **2005**, *109* (11), 2660−2679.

(54) Sahai, M. A.; Fejer, S. N.; Viskolcz, B.; Pai, E. F.; Csizmadia, I. G. First-principle computational study on the full conformational space of L-threonine diamide, the energetic stability of cis and trans isomers. *J. Phys. Chem. A* **2006**, *110* (40), 11527−11536.

(55) Havey, D. K.; Feierabend, K. J.; Black, J. C.; Vaida, V. Temperature-dependent infrared spectra of torsional vibrations in acetic acid. *J. Mol. Spectrosc.* **2005**, *229* (2), 151−157.