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References and Notes

(1) M. Sellini, J. C. Maurizot, J. L. Dimicoli, and C. Helene, *FEBS Lett.*, **30**, 219 (1973).
(2) (a) G. Lancelot, *Biophys. J.*, **17**, 243–254 (1976); (b) G. Lancelot, submitted for publication.
(3) J. J. Fox, D. V. Van Pragg, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).
(4) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *J. Am. Chem. Soc.*, **86**, 4182 (1964).
(5) P. O. P. Ts'o, N. S. Kondo, M. P. Schweizer, and D. P. Hollin, *Biochemistry*, **8**, 997 (1969).
(6) M. D. Joesten and L. J. Schaad, "Hydrogen Bonding", Marcel Dekker, New York, N.Y., 1974.
(7) J. D. Engel and P. H. Von Hippel, *Biochemistry*, **13**, 4143 (1974).
(8) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **91**, 2703 (1969).
(9) R. L. Scott, *Recl. Trav. Chim. Pays-Bas*, **75**, 787 (1956).
(10) W. B. Person, *J. Am. Chem. Soc.*, **87**, 167 (1965).
(11) J. Pitha and R. N. Jones, *Can. J. Chem.*, **44**, 1045 (1966).
(12) R. A. Newmark and C. R. Cantor, *J. Am. Chem. Soc.*, **90**, 5010 (1968).
(13) J. G. C. M. Van Duijneveldt-Van de Rijdt and F. B. Van Duijneveldt, *Theor. Chim. Acta*, **19**, 83 (1972).
(14) H. A. Gielbic, W. J. Burroughs, J. Chamberlain, J. E. Harries, and R. G. Jones, *Nature (London)*, **221**, 143 (1969).
(15) J. E. Del Bene and W. L. Kochenour, *J. Am. Chem. Soc.*, **98**, 2041 (1976).
(16) J. A. Pople, in "Aspect de la Chimie Quantique Contemporaine", edited by CNRS, Paris, 1971, p 17.
(17) D. M. Blow and T. A. Steiz, *Annu. Rev. Biochem.*, **39**, 63 (1970).
(18) R. F. Stewart and L. H. Jensen, *J. Chem. Phys.*, **40**, 2071 (1964).
(19) R. G. Lener, J. P. Friend, and B. P. Dailey, *J. Chem. Phys.*, **23**, 201 (1955).
(20) A. Kvich, T. F. Koetzle, and R. Thomas, *J. Chem. Phys.*, **61**, 2711 (1974).
(21) V. Thewalt, C. E. Bugg, and R. E. Marsh, *Acta Crystallogr., Sect. B*, **26**, 1089 (1970).
(22) D. L. Barker and R. E. Marsh, *Acta Crystallogr.*, **17**, 1581 (1975).
(23) E. Clementi, J. M. Andre, M-Cl. Andre, D. Klint, and D. Hahn, *Acta Phys. Acad. Sci. Hung.*, **27**, 493 (1969).

Theoretical Studies of Environmental Effects on Protein Conformation. 1. Flexibility of the Peptide Bond

Steve Scheiner[1] and C. W. Kern*

Contribution from the Department of Chemistry, Ohio State University, Columbus, Ohio 43210. *Received April 20, 1977*

Abstract: The effects of hydrogen bonding on peptide bonds are studied by quantum mechanical methods. The peptide unit is modeled by *trans*-*N*-methylacetamide (NMA) which is allowed to interact with various hydrogen bonding species that are similar to those typically found in the environment of a peptide within a protein molecule. These species include waters of hydration, other peptide units, and the side chains of amino acid residues. The effects of these species on the flexibility and electronic charge distribution of NMA can be interpreted in terms of resonance structures and atomic orbital overlap. All species are found to have a restricting influence on the conformation of a peptide bond. Nonplanar deformations of the peptide unit require more energy in the presence of these species. The effects of partial as well as full hydration of the peptide are considered. It is found that many of the effects of a full hydration shell can be simulated by the interaction with two water molecules. A correlation is found between the increased rigidity of the peptide and several parameters of charge redistribution. Interpeptide hydrogen bonding is found to have much the same effects as hydration. Interaction of a peptide unit with the electrically charged side chains of several residues is also predicted to result in a significantly more rigid peptide.

Hydrogen bonding is one of the most important determinants of three-dimensional protein structure. The peptide units of the various residues normally engage in extensive hydrogen bonding with one another. They also interact with waters of hydration which are found both within the protein and along its periphery. These effects on the peptide linkage must be understood before the principles underlying protein conformation can be ensured.

Quantum mechanical methods are useful for studying these interactions on a molecular level. The interaction between amide units, which serve as models for the peptide units within proteins, has been studied by both ab initio[2-7] and semiempirical[8,9] theory. The hydration of an amide has also been examined by ab initio techniques.[5,6,10-13] Although such studies provide useful information about the nature of the interactions, they have dealt mostly with amide units in their fully planar conformations. It has become increasingly more evident from x-ray crystal-structure determinations of proteins,[14] as well as open and cyclic polypeptides and other amides,[15-20] that the peptide units within proteins deviate significantly from planarity. Distortions of "isolated" peptide units from planar form have been examined by both semiempirical[9,15,16,21-24] and ab initio[21,25-28] techniques which predict[15,16,21] that the preferred conformation is the fully planar one but that relatively little energy is required for significant deviations from planarity to occur.

A major limitation of previous studies is that the amide unit was modeled in vacuo, much unlike the true environment of a protein. In the present paper, we examine the effects that various hydrogen bonding species (HBS) produce on a model peptide unit, particularly the role they play in altering its flexibility, and the possible implications on the secondary protein structure. The molecule chosen to model the peptide unit is *trans*-*N*-methylacetamide (NMA). The trans arrangement is chosen because the overwhelming majority of peptide units in proteins is found in this configuration. Of more than 20 globular proteins whose crystal structures have been determined, the cis configuration is observed only rarely and is usually associated with the presence of a proline residue.[29]

Planar Systems

The primary computational method used in our study is partial retention of diatomic differential overlap (PRDDO) which has been proven[30] to simulate accurately and rapidly ab initio minimum Slater basis-set results. Optimization of the geometry of NMA (Figure 1) with PRDDO yielded a planar molecule with the bond lengths and bond angles shown in Table

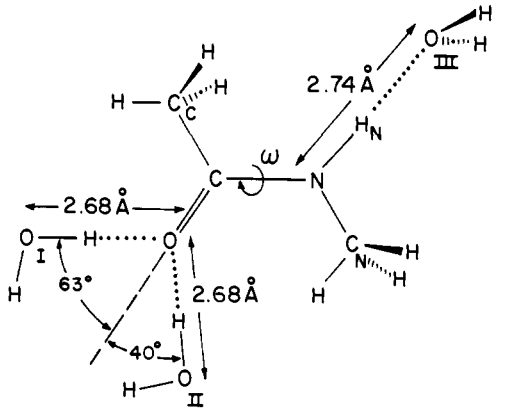


Figure 1. Optimal configuration of NMA·3H_2O. All atoms of waters I and II lie in the amide plane. The two hydrogens of H_2O(III) are symmetrically disposed about this plane. The N-H_N axis makes an angle of 40° with the plane of H_2O(III).

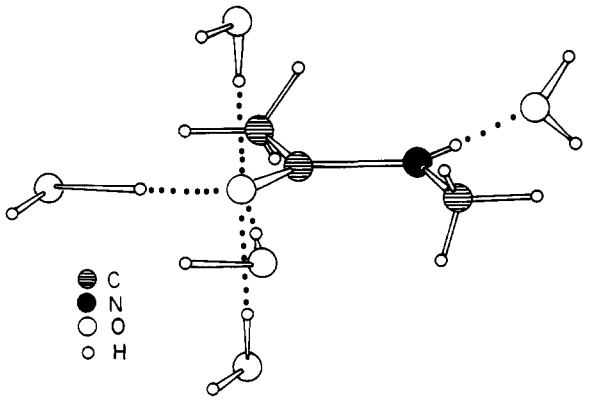


Figure 2. Full hydration sphere of NMA. The system shown has C_S symmetry. For waters IV and V, the projection into the amide plane of the bond axis between the oxygen and hydrogen not involved in the hydrogen bond is parallel to the C=O axis of NMA.

Table I. Optimized Geometry of NMA

Bond lengths, Å	Bond angles, deg
C-N, 1.42	O-C-N, 119
C-O, 1.24	C_C-C-N, 116
C-C_C, 1.55	C-N-H_N, 120
N-C_N, 1.46	C-N-C_N, 122
N-H_N, 1.02	

Table II. Optimized Geometry of Formamide

Bond lengths, Å	Bond angles, deg
C-N, 1.41	O-C-N, 124
C-O, 1.24	H-C-N, 113
C-H, 1.13	C-N-H_t,[a] 123
N-H, 1.02	C-N-H_c,[a] 120

[a] H_t and H_c refer to the hydrogens trans and cis to the O atom, respectively.

I. The two methyl groups were assumed to be tetrahedral with C-H bond lengths of 1.10 Å. The most stable conformation of NMA was found to be that in which one C—H bond of each methyl group eclipses the C=O bond. The most stable orientation of the *C*-methyl group is better established than is that of the *N*-methyl group. The eclipsed conformation of the *C*-methyl group is more stable than the staggered conformation by 1.3 kcal/mol. The corresponding value for the *N*-methyl group is only 0.2 kcal/mol. An ab initio study[27] of NMA has also found the eclipsed conformation of the *N*-methyl group to be most stable while another study based on the PCILO method[22] has found that the *N*-methyl group prefers a staggered conformation. In addition, an ab initio procedure that employed a minimum basis set has found[25] a preference for the staggered conformation in *N*-methylformamide. These studies were all performed using idealized geometries which included a C-N bond length of 1.3 Å. Upon optimization, this bond length is found to be 1.4 Å in NMA (Table I). When the C-N bond of NMA is shortened to 1.3 Å, the PRDDO method finds the staggered conformation of the *N*-methyl group to be most stable. Other studies indicate that the methyl group orientations have very little effect on nonplanar distortions.[15] We therefore use the conformation shown in Figure 1 in which both methyl groups eclipse the carbonyl oxygen.

Several previous ab initio studies[5,10] have investigated possible hydration sites of amides. It was found that the oxygen atom can accommodate two water molecules and the NH group a third, as shown in Figure 1.[31] Accordingly, in our study on NMA, the water molecules were added to these sites such that the geometries of individual molecules were preserved in their isolated configurations (for H_2O, r(OH) = 1.00 Å; ∠HOH = 100°). The water molecules were added to NMA one at a time, and their orientations and hydrogen bond lengths were optimized. The presence of a second water molecule was found to have very little effect on the hydrogen bond length of another. The nonlinearity of each hydrogen bond was also found to be quite small and had a negligible effect on the total energy. Linear hydrogen bonds are assumed throughout this study. Upon adding H_2O(II), the *N*-methyl group of NMA must be rotated into a staggered conformation to avoid steric interactions with this water. In our calculations discussed below, it is to be understood that the *N*-methyl group is in this orientation whenever H_2O(II) is present. The group is otherwise in the eclipsed conformation.

Several studies[5,10a] have found a possibility of water hydrogen bonding to the π system of an amide. The calculations of Kollman et al.[5] indicate that the strongest interaction would occur when the water is directly above the O atom of the amide. Our calculations confirm these results. A single water molecule (H_2O(IV)) was allowed to approach the O atom of NMA such that the O-O(IV) axis was perpendicular to the amide plane (see Figure 2). The interaction energy between NMA and H_2O(IV) was found to be −0.6 kcal/mol at the optimum O-O(IV) distance of 3.2 Å. In addition, an energy barrier of 1.3 kcal/mol to dissociation of the NMA·H_2O(IV) complex was found. By contrast, the interaction potential between NMA and a water analogously positioned above the N atom was found to be repulsive for all distances.

Inclusion of waters I, II, and III results in a weakening of the interaction between NMA and H_2O(IV). The interaction energy of H_2O(IV) with [NMA·3H_2O] was calculated to be zero. However, there still exists an energy barrier (of 0.6 kcal/mol) to dissociation of H_2O(IV) in the resulting complex. The combined interaction energy of H_2O(IV) and its reflection through the amide plane, H_2O(V), with NMA is −1.2 kcal/mol, twice that of NMA with H_2O(IV) alone. The full hydration sphere of five water molecules about NMA is shown in Figure 2.

The predominant form of hydrogen bonding within a folded globular protein is that between different peptide units of the backbone. These C=O···H—N bonds were modeled by the interaction between NMA and formamide. (Formamide may serve also as a suitable model for the side chains of the glutamine and asparagine residues.) The geometry of isolated formamide was fully optimized under the constraint that all atoms lie in a common plane, with the results in Table II. One formamide molecule, f_O, was allowed to interact with the CO group of NMA and another, f_N, with the NH group as shown in Figure 3. Both formamide molecules were added to the

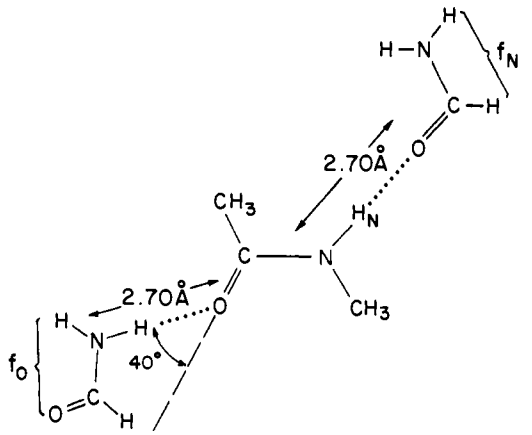


Figure 3. Geometry of NMA·2HCONH$_2$. All three amides are coplanar. The angle between the C=O axis of NMA and the NH axis of f_O was optimized to 40°.

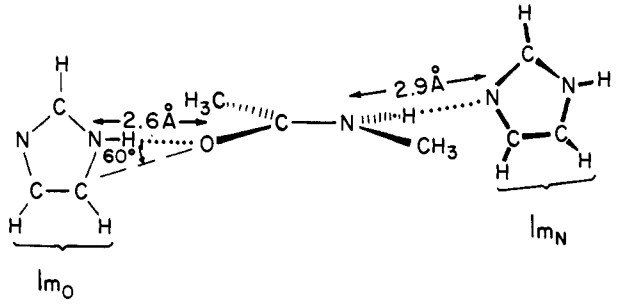


Figure 4. NMA and two imidazoles. The planes of both imidazoles are perpendicular to the amide plane of NMA. The optimum orientation of the plane of Im$_N$ was found to be parallel to the NH axis of NMA.

system such that they lay completely in the amide plane. This relative orientation of amide units has been found by an initio study[4] to be the most stable one. Each formamide molecule was added individually to NMA and the hydrogen bond distance optimized. For either f_N or f_O, the bond length was virtually unaffected by the addition of the second formamide molecule.

A second type of hydrogen bonding found within proteins is that between peptide units and the side chains of various residues. Histidine is capable of bonding to both CO and NH groups. We model the side chain of this residue by imidazole. The geometry of imidazole was taken from a neutron diffraction study[32] of histidine which found the imidazole unit to be planar. Imidazole units were places so as to interact with both the CO and NH groups of NMA (Figure 4). The hydrogen bond distances were optimized for each imidazole individually.[33]

The cationic side chains of lysine or arginine were modeled by $CH_3NH_3^+$. The internal geometry of this ion was assumed completely tetrahedral with the CH_3 group staggered with respect to the NH_3 group. The CH and NH bond lengths were taken as 1.1 and 1.02 Å, respectively, and the CN bond length was optimized to 1.50 Å. This cation was added to NMA as shown in Figure 5 and the hydrogen bond length and angle optimized.

We have also modeled the negatively charged side chains of aspartic and glutamic acids by $HCOO^-$. The geometry of this planar anion was optimized and added to NMA as shown in Figure 5. In an alternate mode of bonding, $HCOO^-$ was added to the system so as to form a bifurcated hydrogen bond. In this configuration, the CH bond of the anion was coincident with the N–H axis of NMA. The two oxygen atoms were symmetrically disposed above and below the amide plane. In

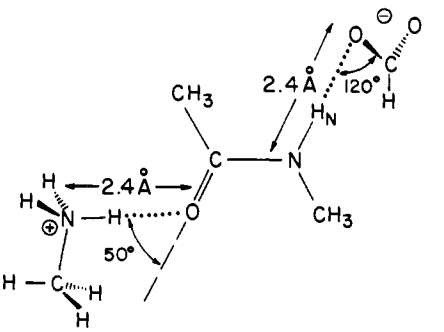


Figure 5. NMA + $CH_3NH_3^+$ + $HCOO^-$. The plane of $HCOO^-$ is perpendicular to the amide plane and parallel to the N–H$_N$ axis. The internal geometry of $HCOO^-$ is: r(CO) = 1.29 Å, r(CH) = 1.09 Å, ∠(OCO) = 127°. The angles whose numerical values are shown in this and preceding figures have been optimized.

the bifurcated configuration the distance from either O atom of $HCOO^-$ to the N of NMA was optimized to 2.81 Å.

The atoms of each ligand that form σ-type hydrogen bonds with NMA have been placed in the amide plane. This position was found to be the most stable for all cases considered.

Charge Redistributions. The electronic charge redistributions within NMA, resulting from the presence of the various HBS, are shown in Table III. All of the species increase the negative charge of the oxygen atom. Both the carbonyl carbon and nitrogen atoms become more electropositive when a single hydrogen bond [e.g., H_2O(I or II)] is formed with the CO group of NMA. The opposite is true when the NH group is involved in a hydrogen bond [H_2O(III)]. The charge of the C atom (as well as that of the *C*-methyl group) is a measure of the total charge transferred to, or withdrawn from, NMA when hydrogen bonds are formed. The amount of negative charge transferred to the N atom as a result of formation of a hydrogen bond to the NH group is considerably larger than the negative charge lost by the same atom as a result of a hydrogen bond to the CO group. The nitrogen atom therefore acquires some negative charge as a result of multiple hydrogen bonding provided there exists at least one H bond to the NH group. The 2p–π atomic orbital of C follows the same trend as the carbon atom as a whole (though the sign changes if the HBS is $HCOO^-$). The 2p–π orbital of nitrogen, on the other hand, loses electron density regardless of the location and number of hydrogen bonds to NMA. Similarly, the π atomic orbital of oxygen acquires additional electron density in all cases.

The hydrogen atom H_N becomes more electropositive for all HBS. For hydrogen bonds to the CO group, this may be understood in several ways, one of which is in terms of the electronic charge that tends to build up at the CO end of NMA. It has been found previously[2,5] that the hydrogen atom involved in a hydrogen bond will tend to lose electron density. This effect is responsible for the increase of positive charge of H_N when a hydrogen bond to the NH group is formed.

The effects, as measured by Mulliken's overlap populations, of the various HBS on the internal bond strengths of NMA are shown in Table IV. In general, a strengthening of the CN bond is found along with a concomitant weakening of the CO bond. These changes are associated almost exclusively with the π system. Very small changes are observed in the σ overlap populations.

Perhaps one of the more interesting aspects of the information contained in Tables III and IV is the additivity of the effects of the various HBS. The charge redistributions and bond strength changes produced by several species hydrogen bonded to NMA simultaneously are nearly equal to the sum of the effects produced by the same species when hydrogen bonded individually. Kollman et al.[5] have observed a similar additivity of charge redistribution effects in an ab initio study

Table III. Electron Redistribution in NMA

HBS	Charge changes[a]									
	C	C_π[b]	O	O_π[b]	N	N_π[b]	H_N	$(CH_3)_N$[c]	$(CH_3)_C$[c]	CT[d]
H_2O(III)	−10	−1	−19	−10	−28	+12	+45	−34	−11	−56
H_2O(I)	+30	+43	−8	−64	+4	+17	+7	+7	+23	+64
H_2O(I + III)	+21	+43	−27	−76	−24	+31	+49	−27	+15	+7
H_2O(II)	+27	+33	−5	−52	+3	+17	+4	+21	+9	+60
H_2O(I + II + III)	+46	+74	−32	−130	−20	+52	+53	−5	+23	+65
H_2O(IV + V)	+19	+34	−22	−28	+8	+18	+7	+5	+11	+28
H_2O(I-V)	+61	+97	−52	−151	−11	+72	+59	0	+33	+90
$(NH_2CHO)_N$ (f_N)	−12	−3	−21	−8	−25	+12	+53	−36	−12	−52
$(NH_2CHO)_O$ (f_O)	+32	+40	−5	−64	+5	+19	+6	+11	+14	+63
$f_N + f_O$	+21	+39	−26	−76	−19	+35	+59	−24	+2	+14
$Imidazole_N$	−14	−3	−27	−9	−20	+13	+63	−43	−10	−50
$Imidazole_O$	+46	+68	−13	−110	+8	+35	+12	+16	+35	+104
$CH_3NH_3^+$	+76	+113	−56	−207	+25	+80	+39	+48	+49	+182
$HCOO^-$	−28	+7	−78	−63	−65	+61	+98	−95	−31	−198
$HCOO^-$(bif)[e]	−12	+12	−66	−50	−13	+41	+101	−68	−18	−76

[a] Mulliken population changes in units of millielectrons. Negative sign denotes increase in electron density. [b] Atomic orbital 2p-π only. [c] Group charge is computed as the sum of atomic charges. [d] Total charge transferred to NMA from HBS. [e] Bifurcated hydrogen bond between $HCOO^-$ and NMA.

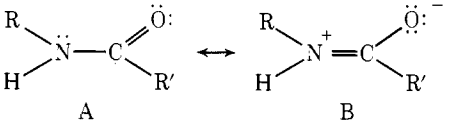
Table IV. Effects of HBS on Peptide Bond Strengths

	Changes in Mulliken overlap populations, millielectrons			
	C-N[a]	$(C-N)\pi$[b]	C-O[a]	$(C-O)\pi$[b]
H_2O(III)	7	6	−4	−2
H_2O(I)	16	15	−9	−8
H_2O(I + III)	23	21	−13	−11
H_2O(II)	17	15	−3	−7
H_2O(I + II + III)	40	38	−20	−20
H_2O(IV + V)	12	14	−18	−12
H_2O(I-V)	50	51	−38	−34
$(NH_2CHO)_N(f_N)$	8	6	−3	−2
$(NH_2CHO)_O(f_O)$	17	15	+2	−8
$f_N + f_O$	26	23	−2	−12
$Imidazole_N$	10	6	−4	−2
$Imidazole_O$	30	28	−14	−15
$CH_3NH_3^+$	62	57	−8	−36
$HCOO^-$	20	30	−15	−14
$HCOO^-$(bif)	21	22	−9	−10

[a] Over atoms. [b] Over atomic orbitals orthogonal to amide plane.

of hydrogen bonding to formamide.

These effects of HBS on NMA are consistent with the resonance structures:



A ⟷ B (1)

One would expect a hydrogen bond to either the CO or NH groups of the peptide linkage to preferentially stabilize structure B. The N and O atoms will thus tend to become more electropositive and electronegative, respectively. This trend will be more pronounced in the π system. A consequence will also be greater double bond character in the CN bond as well as a weaker CO bond. These expectations are in agreement with the data shown in Tables III and IV.

Nonplanar Deformations

To examine the flexibility of the peptide bond, we define several modes of deformation. The conventional parameter used for measuring the distortion of the peptide unit from planarity in polypeptides is the dihedral angle ω. For NMA, ω is the dihedral angle $\varphi(C_CCNC_N)$ in Figure 1 and takes the value of 180° in a planar trans-peptide unit. The deviation of ω from 180° is defined as $\Delta\omega = [\varphi(C_CCNC_N) - \pi]$ which takes a positive value when the *C*-methyl group moves up out of the amide plane.

Winkler and Dunitz[18] have examined distortions of the peptide bond in terms of the separate deviations from planarity of the C and N atoms. Following Ramachandran et al.[16] these deviations, denoted θ_C and θ_N, are defined by:

$$\theta_C = \Delta\omega - \varphi(OCNC_N)$$

$$\theta_N = \varphi(C_CCNH_N) - \Delta\omega$$

and measure out of plane torsion of the two atoms. For example, both θ_C and θ_N are equal to zero for the fully planar conformation. Puckering of N such that both $(CH_3)_N$ and H_N are rotated by 10° above the plane of the paper in Figure 1 corresponds to $\theta_N = -20°$.

Previous semiempirical[15,16] and ab initio quantum mechanical[21] calculations indicate that the fully planar conformation of NMA is the most stable. Small deviations from planarity were calculated to require little additional energy. The most facile mode of deformation was found to be that for which $\theta_N = -2\Delta\omega$, while θ_C is small and uncorrelated with $\Delta\omega$. This prediction was shown[15] to be in good agreement with x-ray and neutron diffraction studies of crystals of various polypeptides. The principal mode of deformation defines a pathway whereby H_N and $(CH_3)_N$ rotate in *opposite* directions about the C-N axis and in *equal* amounts. Since this motion tends to preserve maximum overlap between the π atomic orbitals of the C and N atoms, we define a parameter:

$$\omega' = \Delta\omega + (1/2)\theta_N$$

which measures the dihedral angle made by these orbitals about the C-N axis. It is clear that ω' is equal to zero for the planar configuration as well as for any in which $\theta_N = -2\Delta\omega$. To illustrate the use of these parameters further, let us assume that H_N and $(CH_3)_N$ both rotate up out of the amide plane by 15 and by 5°, respectively. This conformation is characterized by $\Delta\omega = 5°$, $\theta_N = -20°$, and $\omega' = -5°$.

Hydration. The increase in energy that occurs upon a rigid rotation of NMA about its C-N bond axis is shown in Figure 6. These rotations were performed such that $\Delta\omega = \omega'$ varied, but θ_C and θ_N were held fixed at 0°. Analogous curves for NMA in various degrees of hydration are also shown. For all changes from the optimized planar geometries reported here, the substituents, e.g., H_N and $(CH_3)_N$, were rotated about the

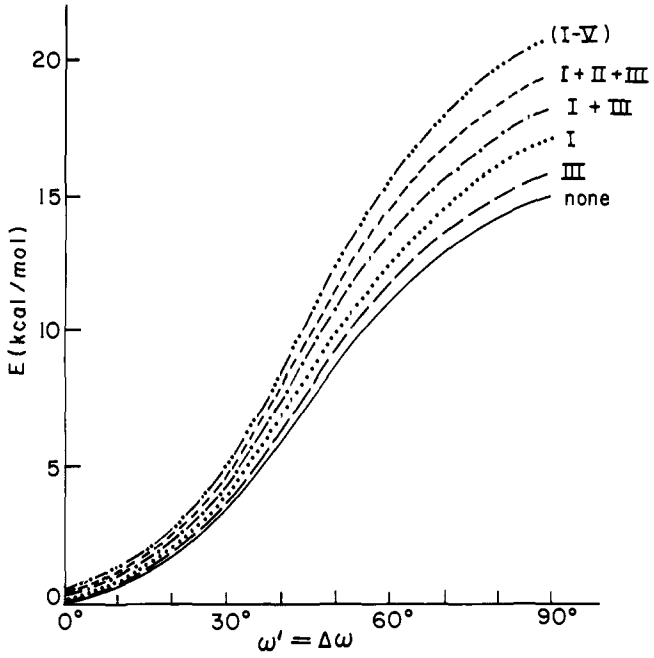


Figure 6. Energy as a function of $\omega' = \Delta\omega$ for NMA·nH$_2$O when $\theta_C = \theta_N = 0°$. Roman numerals correspond to designation of various water molecules.

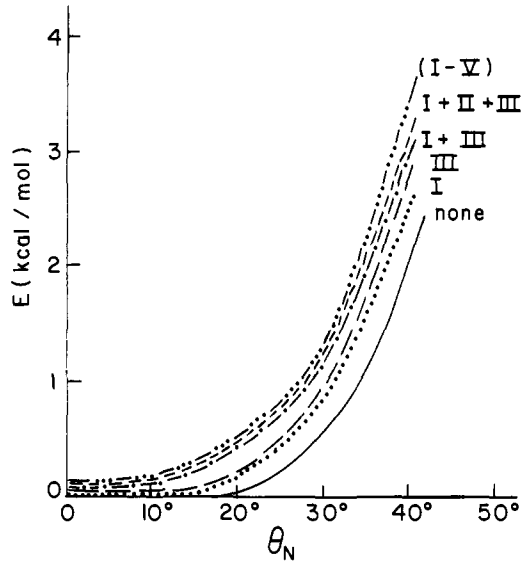


Figure 7. Energy as a function of the puckering of the nitrogen atom of NMA·nH$_2$O when $\omega' = \theta_C = 0°$.

C–N axis. A ligand (e.g., H_2O) considered hydrogen bonded to H_N or O performed all rotations in an identical manner as H_N or O. All bond lengths and noninvolved bond angles were held constant during the rotation. The results show that hydrogen-bonded water molecules increase the rigidity of the peptide bond. This effect is rather small for small rotations but increases for larger values of ω'. A full complement of five water molecules increases the barrier to rotation about the C–N bond by 40%. It is also noted that the effects of the water molecules appear to be cumulative; that is, each water added to the hydration shell incrementally increases the energy required for a given rotation.

Figure 7 shows the variation of energy as the nitrogen atom of NMA is puckered under the constraint that $\theta_C = \omega' = 0$. The energy changes very slowly at first with no appreciable increase until θ_N reaches values greater than 20°. Adding water molecules to the hydration shell of NMA has the effect of making the curve progressively steeper. These effects are significant even for small values of θ_N. By contrast, puckering the C atom of an isolated NMA molecule results in a more drastic energy increase. The energy minimum occurs at $\theta_C = 0°$ and follows approximately the quadratic relationship $\Delta E = 0.01\theta_C^2$ kcal/mol when θ_C is expressed in degrees. Addition of water molecules I or III results in a small diminution of the steepness of this curve.

The effects of hydration on these modes of distortion involving ω', θ_N, and θ_C can be explained by referring to resonance structures A and B. As shown above, hydrogen bonds from NMA to species such as water result in a stronger C–N bond and increased overlap between their π atomic orbitals. Deformations ω' and θ_N both produce disalignments of these two orbitals. The presence of HBS thus makes it more energetically costly for the peptide unit to deviate from planarity in these two modes. Distortion θ_C, on the other hand, is more determined by the pair C–O than by C–N. By removing double bond character in the CO bond, the waters can facilitate the θ_C deformation. But in the free state, structure A is dominant and controls the relative energy required for nonplanar distortions of all modes.

These energy changes are in good agreement with other theoretical studies as well as experiments. The energy required to distort θ_C by 20° was found here to be twice that for an identical distortion of ω' and an order of magnitude greater than that for $\theta_N = 20°$. Another study has found a similar large energy increase associated with the parameter θ_C.[16] The crystal structures of various polypeptides reveal a clustering of values of θ_C about 0° with a deviation of approximately ±5°. By contrast, the deviations of ω' and θ_N from zero are found to be approximately ±15 and ±30°, respectively.[15]

Charge Redistributions. We now examine the electronic redistributions that take place as a result of the distortions from planarity. One would expect the oxygen atom to become more electropositive since resonance structure B should contribute less in a distorted rather than a planar amide, thereby opposing the effects of HBS addition to NMA. This is indeed found to be the case. The N atom becomes increasingly negatively charged as the ω' distortion progresses. The presence of waters I, II, and III increases this charge transfer by ~50%. By contrast, the charge on N remains nearly constant as θ_C and θ_N deviate from zero. A similar result is found in the presence of the waters. The presence of the water molecules has little effect on the amount of electron density lost by the O atom during all of these distortions. These results are consistent with the fact that the ω' distortion produces the most drastic disalignment of the π orbitals of N and C, which in turn makes less important any contribution from resonance structure B. Although the carbonyl carbon atom of NMA acquires additional electron density during the course of all three of the distortions, particularly θ_C, the waters of hydration tend to ameliorate this charge transfer. In the case of ω', the waters cause a reversal in the transfer and the C atom becomes more electropositive rather than electronegative as the distortion increases. The total charge transferred to NMA as well as the charges of the individual water molecules remain quite constant during the course of any of the three modes of distortion.

The changes in bond strength that arise from the deformations can be interpreted in terms of atomic orbitals. The θ_N and ω' distortions, which cause a disalignment of the N_π orbital and the $(CO)_\pi$ system, produce a weakening of the C–N bond and a concomitant strengthening of the C–O bond. The θ_C distortion which produces a disalignment of all three π atomic orbitals leads to a weakening of both the C–O and C–N bonds. The addition of waters I, II, and III enhances these trends, the most drastic of which is observed in the weakening of the C–N bond during the ω' distortion. The C–N overlap population decreased by 6 millielectrons when ω' varied from 0 to 90° for

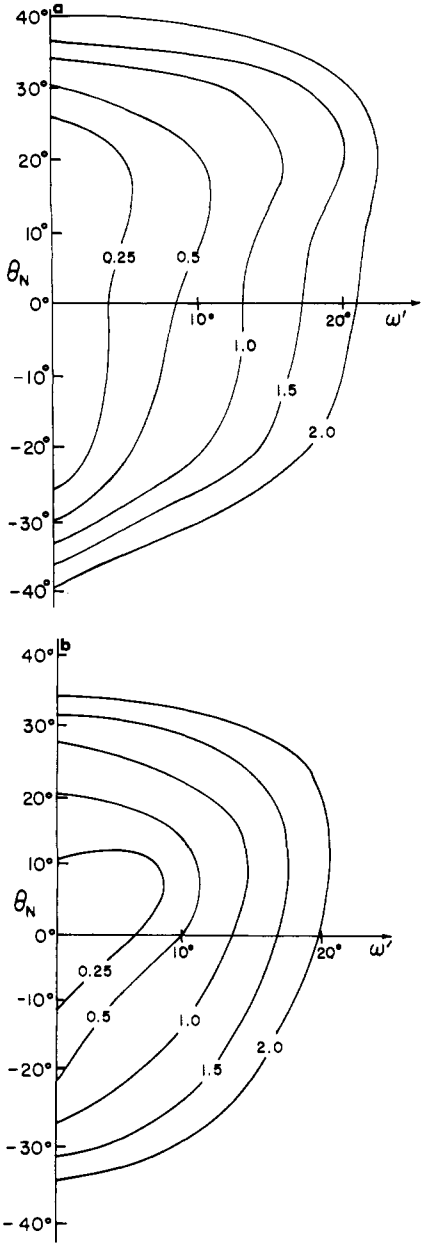


Figure 8. Isoenergy contour plot of (a) NMA and (b) NMA·3H_2O(I + II + III). The zeroes of energy are at the planar conformations $\omega' = 0, \theta_N = 0$. Energy units are kcal/mol.

NMA. This decrease became 38 millielectrons when the three water molecules were added. The overlap populations between the waters and NMA were observed to remain nearly constant throughout all three modes of distortion.

Figure 8a is an isoenergy contour plot for NMA. The two axes represent the modes of distortion ω' and θ_N. Negative values of ω' are not shown here but the energies of these points can be obtained by: $E(\omega',\theta_N) = E(-\omega',-\theta_N)$. (This relation is due to the symmetry of NMA but would not be expected to be generally true for polypeptides.) Figure 8b is a similar plot for the hydrated species (NMA·3H_2O). Comparison of these two figures demonstrates the restricting influence of hydration on the conformation of NMA. The area enclosed by a given isoenergy curve is considerably reduced when the water of hydration is present. This trend has the greatest effect on values of θ_N and is most marked for contours of lower energy. It is the shapes of these curves in the lower energy regions that should be most important in determining the populations of the peptide bonds in each configuration. Let us assume a Boltzmann distribution:

$$P(\omega',\theta_N) = \frac{\exp(-E(\omega',\theta_N)/RT)}{\sum_{\omega'}\sum_{\theta_N}\exp(-E(\omega',\theta_N)/RT)}$$

where $E(\omega',\theta_N)$ is the energy of the configuration (ω',θ_N) and $P(\omega',\theta_N)$ is the fraction of molecules in that configuration. For both Figures 8a and 8b the total populations of the configurations contained within the 0.25-kcal contours are greater than 50% at 25 °C. Similarly, more than 80% of the total populations are located within the 0.5-kcal contours. Whereas values of θ_N of as much as 25–30° are quite likely for NMA in vacuo, solvation would have the effect of making values of θ_N greater than 10–20° rather unlikely. Completing the hydration shell by adding waters IV and V has little effect on the contours shown in Figure 8b.

As has already been pointed out, the minimum energy configuration of NMA, both isolated and solvated, is the fully planar one. For any given value of θ_N, the optimum value of ω' is zero which yields the most favorable overlap between the π atomic orbitals of the C and N atoms. However, for given nonzero values of ω', the optimum values of θ_N are greater than zero. Thus, given an already existing disalignment of the π orbitals of C and N via ω', the N atom will choose to pucker. Of the two possible directions of puckering, the more favorable one is that in which the stabilizing interaction between the carbonyl oxygen and the *N*-methyl group is maximized. For larger values of ω', θ_N appears to approach asymptotically an optimum value between 20 and 30°. Greater puckering of the nitrogen atom results in steric interaction between H_N and the *N*-methyl group.

Table V. Conformational Energies of HBS (kcal/mol)

HBS(X)	$-E_{int}$[a]	$\Delta E(\theta_N = 30°)$[b]	$\Delta E(\omega' = 90°)$[c]
1. (NMA)	0	0.5	14.9
2. H_2O(III)	11.7	0.9	15.7
3. H_2O(I)	6.9	0.8	17.0
4. H_2O(I + III)	20.7	1.1	18.4
5. H_2O(II)	6.4	0.8	16.7
6. H_2O(I + II + III)	25.9	1.1	19.5
7. H_2O(IV + V)	1.2	0.9	16.4
8. H_2O(I-V)	24.4	1.2	20.8
9. $(NH_2CHO)_N$ (f_N)	7.8	0.9	15.6
10. $(NH_2CHO)_O$(f_O)	9.2	0.9	17.0
11. $f_N + f_O$	18.7	1.2	17.5
12. $Imidazole_N$	11.5	0.9	15.3
13. $Imidazole_O$	18.3	1.1	19.0
14. $CH_3NH_3^+$	42.6	1.6	24.5
15. $HCOO^-$	41.2	1.2	19.1
16. $HCOO^-$ (bif)	24.8	1.0	19.7

[a] $E_{int}(X) = E(NMA{\cdot}X) - [E(NMA) + E(X)]$. [b] $\Delta E(\theta_N = 30°) = E(\theta_N = 30°, \omega' = 0°, \theta_C = 0°) - E(\theta_N = 0°, \omega' = 0°, \theta_C = 0°)$. [c] $\Delta E(\omega' = 90°) = E(\theta_N = 0°, \omega' = 90°, \theta_C = 0°) - E(\theta_N = 0°, \omega' = 0°, \theta_C = 0°)$.

Comparative Effects and Reliability of the Model

The interaction energies of the various HBS and NMA are shown in Table V. There appears to be some cooperativity in the hydrogen bonding of the first two waters (I and III) to NMA. That is, the dihydration energy of NMA is greater than the sum of the two monohydration energies. A similar trend is found when the two waters are replaced by the formamide molecules, f_N and f_O. Kollman et al.[5] have noticed a similar cooperativity in the dihydration of formamide. This trend reverses, however, when the first hydration shell of NMA is filled beyond the first two waters. The extra stabilization energy produced as a result of the subsequent addition of the third, fourth, and fifth water molecules is less than the interaction energy of isolated NMA with each of these waters.

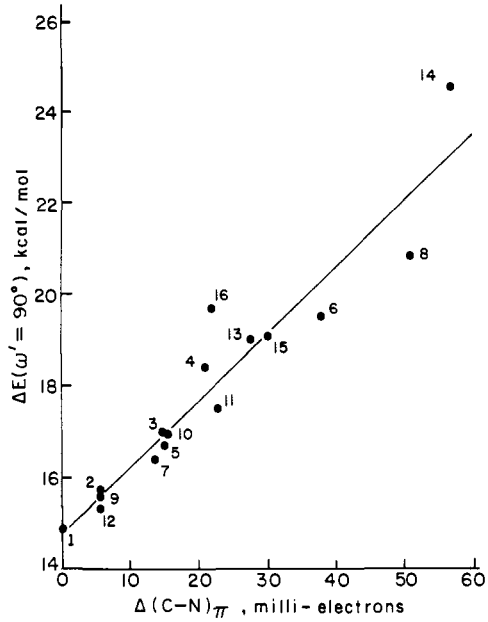


Figure 9. Internal barrier to rotation of NMA in kcal/mol vs. increased C–N π-bond overlap population in millielectrons. The numbered points correspond to the HBS index number in Table V. The equation of the straight line fit is: $\Delta E(\omega' = 90°) = 14.8 + 0.15[\Delta(CN)_\pi]$.

A similar trend is observed in the effect of hydration on the flexibility associated with the puckering of the N atom. As shown in Table V, the energy required to distort the N atom of dihydrated NMA by 30° is 0.6 kcal greater than the analogous quantity for isolated NMA. Addition of three more waters to complete the first hydration shell results in a further increase of only 0.1 kcal. In fact, it was found that for all calculated values of θ_N, the presence of the first two water molecules accounted for nearly all of the increased rigidity of NMA with a complete first hydration shell. The presence of these two water molecules would thus appear to be sufficient to simulate the energetic effects of hydration on the θ_N distortion. As shown in Figure 8, it is upon this mode of distortion in which hydration appears to exert the greatest influence on the conformation of a peptide bond.

One might expect that there would exist some correlation between the flexibility of the peptide unit and the charge redistribution produced by the HBS. The increases in the π-overlap population of the C–N bond, $\Delta(C-N)_\pi$, and the population of the N_π atomic orbital, ΔN_π (Tables III and IV), were found to be good measures of the decreased flexibility. The internal barrier to rigid rotation in NMA, $\Delta E(\omega' = 90°)$, is plotted against $\Delta(C-N)_\pi$ for the various HBS in Figure 9. The best fit of these data to a straight line, as calculated by a least-squares linear regression analysis, is given in the figure caption. The correlation coefficient of the fit is 0.95.

One may note some grouping of points in Figure 9. One cluster consists of points 2, 9, and 12 which correspond to single hydrogen bonds between the NH group of NMA and H_2O, $HCONH_2$, and imidazole, respectively. Another group, 3, 5, 7, and 10, is associated with hydrogen bonds to the CO group of NMA. Note that the combined effect of waters IV and V (point 7) is similar to that of either I or II alone (points 3 and 5). The remaining points, which correspond for the most part to multiple hydrogen bonds and electrically charged HBS, are scattered over the remaining length of the line. An analysis of the barrier to rotation vs. ΔN_π leads to a similar grouping of data points and a correlation coefficient of 0.94. There is also correlation between the charge redistribution and the effects on the puckering of nitrogen as measured by $\Delta E(\theta_N = 30°)$. These data also fit a linear relation but less well. The correlation coefficients for $\Delta E(\theta_N = 30°)$ vs. $\Delta(C-N)_\pi$ and ΔN_π are 0.86 and 0.89, respectively.

These correlations indicate that the binding of formamide to the peptide bond has much the same effects as does water. This similarity applies to charge redistribution as well as flexibility. In either case, a hydrogen bond to the CO group of NMA produces a greater increase in the rotational barrier than does a bond to the NH group. Little differentiation of this sort is observed for the θ_N distortion. The effects of imidazole when bonded to the NH group are nearly identical with those of H_2O or H_2NCHO when bonded to the same group. When bonded to the CO group, however, imidazole produces substantially greater effects in all categories including the interaction energy.

The electrically charged HBS, particularly $CH_3NH_3^+$, produce significantly larger effects than do the uncharged species. It is interesting to note that $HCOO^-$ produces more of an effect on both charge redistribution and the energetics of puckering the nitrogen when it is hydrogen bonded in a conventional manner to NMA than when there exists a bifurcated hydrogen bond. An anomolously high barrier to rotation is found, however, in the case of the bifurcated bond.

It is possible to compare some of our theoretical results with experiment. An NMR study[34] has been made of the barrier to internal rotation of formamide in the neat liquid and in the solvents water, acetone, and dioxane. For liquid formamide, hydrogen bonding to both the CO and NH groups of each amide is expected. The situation shown in Figure 3 may thus be a suitable model for the liquid phase. An amide in aqueous solution may be modeled by all or part of the first hydration shell (Figure 2). A ketone would be expected to form a hydrogen bond to only the NH group of an amide. We may therefore model the situation in ketone solvent as [NMA·H_2O(III)]. The NMR study found the barrier height in the neat liquid greater than that in ketone solvent by ~2 kcal/mol. We calculate the barrier of [NMA·2$HCONH_2$] (Figure 3) to be higher than that of [NMA·H_2O(III)] by a similar amount. Similarly, the barrier was found by the NMR study to be higher in aqueous solution than in the neat phase by 2 kcal/mol. Our calculated barrier of NMA in the presence of three waters (Figure 1) is 2 kcal/mol higher than that of [NMA·2$HCONH_2$]. Completing the hydration shell with a total of five waters increases this difference to 3 kcal/mol.

The barrier to internal rotation in the amide has been calculated here assuming a rigid rotation about the C–N bond. In order to obtain a more accurate value, one might expect it necessary to optimize the geometry during the course of the rotation. However, theoretical studies[24,26] have found the activation barrier heights of several amides to be rather insensitive to optimizations of this sort. We are of course including in our calculations incomplete models of the various liquid phases. Bulk solvent effects have been neglected. It is also true that the experimental work was performed on formamide and our calculations on NMA. However, the fact that our calculations predict the correct relative magnitudes of the barriers in the various solvents as well as approximately correct energy differences augurs well for the essential correctness of our model.

All hydrogen bonds dealt with thus far have been idealized ones. That is, the position and orientation of each HBS with respect to the amide have been optimized to some degree. Within a real folded protein one would expect some constraints upon the various groups which would prevent the formation of these ideal hydrogen bonds. It thus becomes necessary to consider how deviations from ideality might affect our results.

We first consider the dihydrated system NMA·2H_2O(I + III). When the two waters were pulled away from NMA such

that the two hydrogen bond lengths stretched to 3.0 Å, ΔN_π and $\Delta(C\text{-}N)_\pi$ were reduced to one-half their values in Tables III and IV. This bond stretching resulted in a decrease of $\Delta E(\theta_N = 30°)$ from 1.1 to 0.9 kcal/mol. The two waters were then rotated, distorting the previously linear X–H···O arrangement of each hydrogen bond, such that ∠OXH = 30°. This resulted in a further reduction of the charge redistribution parameters and a value of $\Delta E(\theta_N = 30°)$ equal to that of isolated NMA. Similar results were obtained when the two waters were replaced by either one or two formamide molecules. In all cases examined, stretching of hydrogen bond lengths to 3.0 Å coupled with 30° distortions of the bonds resulted in a complete loss of the effects of the HBS on the θ_N flexibility.

Out-of-plane hydrogen bonding also favors peptide planarity. When $H_2O(I)$ was rotated out of the peptide plane by 20°, $\Delta E(\theta_N = 30°)$ was found to be equal to 0.6 kcal/mol. This value is slightly higher than that in which there is no ligand present. When the out-of-plane rotation of $H_2O(I)$ was increased to 45°, the value of $\Delta E(\theta_N = 30°)$ was calculated to be the same as that for isolated NMA.

So far, we have considered the effects of only those groups directly bonded to the amide. We have thus neglected the possible effects of those species which interact with the amide through the intermediacy of a second moiety, e.g., a second hydration shell. Calculations were performed that included a water molecule in a position such that it could act as a proton donor to $H_2O(I)$ which, in turn, acts as a proton donor to the CO group of NMA. The additional water had very little effect on either the charge redistribution or distortion energetics of NMA. In the same manner, one might wish to consider the effects of a secondary hydrogen bond within the interior of the folded protein. Calculations involving three consecutive amide units have been performed (cf. Figure 3). It was found that the addition of f_O to the NMA–f_N system produced very little charge redistribution within f_N. Similarly, only very small changes in f_O were found when f_N was added to the NMA–f_O system. We therefore conclude that secondary hydrogen bonds play a minor role in influencing the flexibility of a peptide bond.

In order to assess the accuracy of the approximate PRDDO method, several calculations were performed utilizing the ab initio GAUSSIAN 70 program.[35] The energy required to pucker the nitrogen atom of NMA is shown in Table VI for both the PRDDO method and the STO-3G basis set. Also shown are the same quantities for the dihydrated molecule which approximate the puckering of the N atom of the fully hydrated amide (see above). The two methods predict a similar energy increase (of ~0.5 kcal/mol) as a result of dihydration.

Analogous comparisons for formamide are also shown in Table VI. The geometry of this molecule was obtained by replacing the two methyl groups of NMA by hydrogen atoms and by setting r(CH) and r(NH) equal to 1.10 and 1.02 Å, respectively. The geometry of the formamide molecule was otherwise identical with that of NMA. The charge densities and bond strengths within formamide were found to be quite similar to those of NMA. Both PRDDO and STO-3G utilize minimum basis sets and predict the equilibrium configuration of formamide to be that with a puckered nitrogen. The 4–31 G method, which employs a split valence shell, predicts the fully planar conformation to be more stable. Calculations performed using larger basis sets that included polarization functions find that the energy difference between the two configurations is quite small (<0.1 kcal/mol).[26] No definite conclusion can yet be drawn as to which configuration is the more stable.[36] However, all three methods shown in the table predict that hydration will preferentially stabilize the fully planar conformation. In fact, all three methods predict that hydration will result in an increase of the energy of puckering of either amide by approximately equal amounts (0.5–0.7 kcal/mol). It might also be noted that the effects of the waters on the charge distributions and bond strengths of the two amides are quite similar in all basis sets studied.

Table VI. Energy Required to Pucker N Atom by 30° for Different Wave Functions[a]

Species	PRDDO	STO-3G	4-31 G
NMA	0.5	0.2	
NMA·2H₂O(I + III)	1.1	0.7	
NH_2CHO	−0.7	−0.5	1.5
$NH_2CHO{\cdot}2H_2O$(I + III)	0.0	0.0	2.0

[a] $[E(\theta_N = 30°) - E(\theta_N = 0°)]$ in kcal/mol.

Discussion

We envision the peptide units of an unfolded protein in aqueous solution to be fully hydrated. As the protein folds into its globular form, many of these contacts with water are replaced by interactions between various parts of the polypeptide chain. In the case of a peptide bond located within a hydrophobic region of the protein interior, the hydration shell is replaced primarily by hydrogen bonds to other peptide units. This situation may be modeled in our study by replacing the full hydration shell of waters I–V by formamides f_N and f_O. The results in Table V indicate that this substitution will have little effect upon the θ_N mode of distortion. However, the flexibility of the ω' mode, as reflected by the barrier to internal rotation, will show some change. Those peptide units that become buried in a hydrophobic pocket as a result of protein folding are predicted to become more flexible. Should some structural constraints exist within the folded protein that prevent strong interactions between the various peptide units, this increased flexibility will be magnified. There are many peptide units within a folded protein that remain partially hydrated. These include those residues within hydrophilic regions and those on the surface of the folded protein. These peptide units are predicted to be less flexible than those buried in hydrophobic pockets.

The relative orientation of the amide units studied in Figure 3 corresponds to that expected for peptide units in β-antiparallel pleated sheet structures. In order to simulate the relative orientations found in helical structures, it would be necessary to rotate the plane of one amide relative to another. Berthod and Pullman[4] have investigated this rotation using an ab initio method. They found that the interaction energy and charge transfer as well as the charges on each atom are insensitive to the rotation. These results indicate that the flexibility of a peptide unit within a helical structure will be similar to that of one within a β-pleated sheet.

Upon folding of the protein, the peptide unit may also come into contact with the side chains of the various amino acids. Interactions with glutamine and asparagine side chains would be expected to be much like those with the polypeptide backbone. Similarly, hydrogen bonds to the hydroxyl groups of serine or threonine should be much like interactions with water molecules. As shown in Table V, all of these interactions should have much the same effect on a peptide bond. Of the other hydrogen bonding amino acids studied, the imidazole side chain of histidine is predicted to produce effects most like water and amides. This is particularly so when the imidazole is hydrogen bonded to the NH group of the peptide. A more rigid peptide unit is a result of hydrogen bonding to the CO group.

The electrically charged amino acids are predicted to effect the most rigid peptide units. The species $CH_3NH_3^+$, as a model for the cationic lysine or arginine side chains, produces a more rigid peptide unit than any other group considered, including a full hydration sphere. We thus expect any peptide unit hydrogen bonded to the cationic side chain of either of these two

residues to show preference for the planar conformation. The effects of anionic $HCOO^-$, which models aspartate or glutamate, are comparable to those of a full hydration shell and greater than those of a peptide–peptide hydrogen bond. In comparison, when the COO^- group forms a bifurcated hydrogen bond to the NH group of the peptide, the puckering of the nitrogen is predicted to be more facile but the rotation about the C–N bond less so.

The folding of a protein should thus have differing effects on the flexibility of each peptide unit. As a general rule, those residues located within the protein interior will tend to be more flexible than those on the surface. Exceptions to this rule are those peptides which are hydrogen bonded to electrically charged side chains of several amino acids. These peptides will be quite prone to adopt the planar configuration.

Various studies, including the present one, indicate that the planar conformation of a peptide is the most intrinsically stable. The question must therefore be considered as to what forces are responsible for causing a given peptide to deviate from planarity. One possibility is the combination of long-range, three-dimensional constraints on the protein. For example, one can conceive of a stabilizing lysine–glutamate salt bridge whose formation is dependent upon a certain motion of the peptide backbone. This motion may most easily occur as a result of a nonplanar distortion of one or several peptide units.

The possibility that a given peptide unit may form a hydrogen bond is another factor contributing to nonplanar deformations. For example, an electron donor may be located near the NH group but below the plane of a peptide unit. In order to form a hydrogen bond, the proton may move toward the electron donor and out of the peptide plane. As shown in Figure 8, the position of the H_N atom has some effect on the C^α atom bonded to the nitrogen. The motion of the hydrogen may therefore result in a corresponding motion of the C^α atom and thus the entire polypeptide backbone. Our calculations suggest that when the H_N atom rotates by 10° or less in a given direction about the C–N bond, C^α will tend to rotate a similar angle but in the opposite direction resulting in a puckering of the nitrogen. Greater angles of rotation of the hydrogen will result in a rotation of C^α in the same direction as H_N. The puckering of the nitrogen will be such that θ_N remains less than 20°.

There are many additional factors that we have not considered explicitly in this work. Bulk solvent effects may play a large role in determining peptide conformation in solution. Modeling an extensive polypeptide chain by *N*-methylacetamide may result in neglect of certain important long-range effects on protein conformation. Entropy effects, which have been largely neglected here, may also play a significant role. However, our results indeed demonstrate that the flexibility of a peptide bond is affected by its environment. Theoretical calculations performed on a peptide unit in vacuo will tend to exaggerate this flexibility. The introduction of a hydrogen bonding environment is necessary in order to obtain a more realistic picture of the peptide unit and its deformations.

References and Notes

(1) Weizmann Postdoctoral Fellow.
(2) (a) M. Dreyfus, B. Maigret, and A. Pullman, *Theor. Chim. Acta*, **17**, 109 (1970); (b) M. Dreyfus and A. Pullman, *ibid.*, **19**, 20 (1970).
(3) M. Dreyfus and A. Pullman, *C. R. Hebd. Seances Acad. Sci.*, **271**, 457 (1970).
(4) H. Berthod and A. Pullman, *Chem. Phys. Lett.*, **14**, 217 (1972).
(5) A. Johansson, P. Kollman, S. Rothenberg, and J. McKelvey, *J. Am Chem. Soc.*, **96**, 3794 (1974).
(6) J. F. Hinton and R. D. Harpool, *J. Am. Chem. Soc.*, **99**, 349 (1977).
(7) T. Ottersen, *J. Mol. Struct.*, **26**, 365 (1975).
(8) A. Pullman and H. Berthod, *Theor. Chim. Acta*, **10**, 461 (1968).
(9) F. A. Momany, R. F. McGuire, J. F. Yan, and H. A. Scheraga, *J. Phys. Chem.*, **74**, 2424 (1970).
(10) (a) G. Alagona, A. Pullman, E. Scrocco, and J. Tomasi, *Int. J. Pept. Protein Res.*, **5**, 251 (1973); (b) A. Pullman, G. Alagona, and J. Tomasi, *Theor. Chim. Acta*, **33**, 87 (1974).
(11) G. N. J. Port and A. Pullman, *Int. J. Quantum Chem., Quantum Biol. Symp.*, **No. 1**, 21 (1974).
(12) T. Ottersen and H. H. Jensen, *J. Mol. Struct.*, **26**, 375 (1975).
(13) J. E. DelBene, *J. Chem. Phys.*, **62**, 1961 (1970).
(14) (a) C. Ramakrishnan and R. Balasubramanian, *Int. J. Pept. Protein Res.*, **4**, 79 (1972); (b) R. Huber, W. Bode, D. Kukla, U. Kohl, and C. A. Ryan, *Biophys. Struct. Mech.*, **1**, 189 (1975); (c) J. Deisenhofer and W. Steigemann, *Acta Crystallogr. Sect. B*, **31**, 238 (1975).
(15) A. S. Kolaskar, A. V. Lakshminarayanan, K. P. Sarathy, and V. Sasisekharan, *Biopolymers*, **14**, 1081 (1975).
(16) G. N. Ramachandran, A. V. Lakshminarayanan, and A. S. Kolaskar, *Biochim. Biophys. Acta*, **303**, 8 (1973).
(17) G. N. Ramachandran and A. S. Kolaskar, *Biochim. Biophys. Acta*, **303**, 385 (1973).
(18) F. K. Winkler and J. D. Dunitz, *J. Mol. Biol.*, **59**, 169 (1971).
(19) M. R. Narasimhamurthy, K. Venkatesan, and F. Winkler, *J. Chem. Soc., Perkin Trans. 2, 768 (1976).*
(20) L. L. Reed and P. L. Johnson, *J. Am. Chem. Soc.*, **95**, 7523 (1973).
(21) V. Renugopalakrishnan and R. Rein, *Biochim. Biophys. Acta*, **434**, 164 (1976).
(22) B. Maigret, B. Pullman, and M. Dreyfus, *J. Theor. Biol.*, **26**, 321 (1970).
(23) J. F. Yan, F. A. Momany, R. Hoffmann, and H. A. Scheraga, *J. Phys. Chem.*, **74**, 420 (1970).
(24) P. R. Andrews, *Biopolymers*, **10**, 2253 (1971).
(25) M. Perricaudet and A. Pullman, *Int. J. Pept. Protein Res.*, **5**, 99 (1973).
(26) D. H. Christensen, R. N. Kortzeborn, B. Bak, and J. J. Led, *J. Chem. Phys.*, **53**, 3912 (1970).
(27) L. L. Shipman and R. E. Christoffersen, *J. Am. Chem. Soc.*, **95**, 1408 (1973).
(28) L. Radom, W. A. Lathan, W. J. Hehre, and J. A. Pople, *Austr. J. Chem.*, **25**, 1601 (1972).
(29) G. N. Ramachandran and A. K. Mitra, *J. Mol. Biol.*, **107**, 85 (1976).
(30) T. A. Halgren and W. N. Lipscomb, *J. Chem. Phys.*, **58**, 1569 (1973). We use a minimum set of Slater orbitals with standard orbital exponents including 1.2 for hydrogen.
(31) The numbering of the waters is in conformity with Pullman et al. in ref. 10b except that water IV has been renumbered III for the sake of continuity.
(32) M. S. Lehmann, T. F. Koetzle, and W. C. Hamilton, *Int. J. Pept. Protein Res.*, **4**, 229 (1972).
(33) The plane of both imidazole units was taken to be perpendicular to the amide plane of NMA. As in the case of the other ligands, the total energy was found to have insignificant dependence upon the relative orientations of the planes of NMA and the systems attached to it.
(34) H. Kamei, *Bull. Chem. Soc. Jpn.*, **41**, 2269 (1968).
(35) R. Ditchfield, W. J. Hehre, and J. A. Pople, *J. Chem. Phys.*, **54**, 724 (1971); W. J. Hehre, W. A. Lathan, R. Ditchfield, M. D. Newton, and J. A. Pople, GAUSSIAN 70, Program No. 236, Quantum Chemistry Program Exchange, Indiana University, Bloomington, Ind., 1974.
(36) A microwave geometry obtained for formamide includes a slightly puckered nitrogen; C. C. Costain and J. M. Dowling, *J. Chem. Phys.*, **32**, 158 (1960).