Communication to the Editor

Conformational Energy Map of a Dipeptide Unit in Relation to Infrared and Nuclear Magnetic Resonance Data

The variation of energy of the conformation of a dipeptide unit with the dihedral angles ϕ and ψ is a fundamental aspect, which is of great importance to the study of protein structure. Good reviews are available[1-4] dealing with the energy changes associated with the variation of different parameters such as bond lengths, bond angles, dihedral angles etc. More recently, attempts have been made to obtain satifactory expressions for hydrogen bond energy as a function of the parameters relating to it.[5-8] Although various results of interest in relation to the conformation of polypeptides and proteins have been worked out from such theory, the theoretical results have not been directly tested in many cases in relation to available data from physicochemical studies on a dipeptide unit, or fragments of simple compounds which sufficiently approximate to it. The purpose of this preliminary note is to point out the possibility of making such tests and to indicate some results which show reasonable agreement with data obtained from infrared and NMR studies. Possible further experiments are also indicated. The full details will be published elsewhere.

The distribution of states in the (ϕ,ψ)-plane can be readily worked out by using the Boltzmann relation:

$$P(\phi,\psi) \propto e^{-V(\phi,\psi)/RT} \tag{1}$$

where $P(\phi,\psi)$ is the probability of occurrence of the state (ϕ,ψ) with total energy $V(\phi,\psi)$ per mole. By using this it is possible to obtain, for instance, two quantities which can be tested against infrared and NMR data.

Since a hydrogen bond between N_2H_2 and C_1O_1 can occur in the backbone sequence C_1^{α}–C_1O_1–N_1H_1–C_2^{α}(H,R)–C_2O_2–N_2H_2–C_3^{α} (I)* for certain regions of (ϕ,ψ), the fraction n_H of hydrogen-bonded molecules can be obtained from the formula

$$n_H = \iint_{\text{H-bonded}} P(\phi,\psi)\, d\phi\, d\psi \Big/ \iint_{\text{Total}} P(\phi,\psi)\, d\phi\, d\psi \tag{2}$$

in which the integration may be replaced by a summation over a grid of points, if necessary for computational purposes.

Similarly, by using the partition functions (as given by the formulae of Flory[9]) for the hydrogen-bonded and the non-hydrogen-bonded states, the values of ΔH and ΔS between the two can be calculated.

By integrating eq (1), the relative probability distribution $P(\phi)$ can be obtained. This is of interest in relation to the coupling constant J_{NH-CH} between NH and $C^{\alpha}H$ protons, since J is a function of θ, the dihedral angle between the NH and $C^{\alpha}H$ bonds. With the definition that $\theta = 0$ when the two are *cis* to each other, and by using the standard definition of ϕ according to Edsall et al.,[10] we have the relation $|\theta| = |240° - \phi|$. In fact, on writing θ for $|\theta|$, the expression for J has the well-known form

$$J = a + b \cos\theta + c \cos 2\theta \quad (0 \leq \theta \leq 180°) \tag{3}$$

which may also be written in the form

$$J = A \cos^2\theta + B \cos\theta + C \sin^2\theta \tag{4}$$

* We use the same subscript for atoms in a peptide unit.

Thus, the value of the coupling constant for a distribution of states as mentioned above will be given by

$$J = A \langle \cos^2 \theta \rangle + B \langle \cos \theta \rangle + C \langle \sin^2 \theta \rangle \tag{5}$$

where the averaging is illustrated by the formula

$$\langle \cos^2 \theta \rangle = \int_0^{360^\circ} \cos^2 (|240 - \phi|)\, P(\phi)\, d\phi \Big/ \int_0^{360^\circ} P(\phi)\, d\phi \tag{6}$$

Some results computed by use of potential functions based on parameters as given by Ramachandran and Sasisekharan[1] and hydrogen-bond potential functions[8] modified for the case of peptide NH···O=C bonds are given below, in relation to available infrared and NMR data.

Infrared Data on Hydrogen Bonding

Hydrogen bonds can occur near about the values (100°, 240°) and (260°, 120°) for (ϕ,ψ). Of these, the former is found to have a much higher probability. (Pullman and co-workers[11] have calculated from *a priori* quantum mechanical theory that the depths of the energy minima of the two types of hydrogen bonds are nearly the same—the latter in fact slightly lower—but we do not find this to be so, in agreement with a recent

TABLE I
Data in Relation to Internal Hydrogen Bonding in a Dipeptide Unit Compared with Results from IR Data

Nature of residue[a]	Hydrogen bonded n_H, %	ΔH, kcal/mole	ΔS, e.u.	
Gly	62	−2.2	6.2	Theory
Ala	47	−1.9	6.6	Theory
	70	−3.1	9.8	Portnova et al.[13]
	20	−0.7	5.2	Theory
N-Me-Ala	70	—	—	Portnova et al.[13]
	—	−1.7[b]	5.4[b]	Mizushima et al.[14]

[a] This refers to the group of atoms $-N_1H_1$ (or Me)$-C_2^\alpha$(H,R)$-C_2O_2-$.

[b] The data of Mizushima et al.[14] actually refer to *N*-methylnorleucyl residue, but the values are not expected to differ much from those for *N*-Me-Ala.

report by Crippen and Scheraga.[12]) By using the criterion that a hydrogen bond exists when the N···O distance is between 2.6 and 3.2 Å and the angle NH∧N···O is less than 35°, the values of n_H, ΔH, and ΔS calculated for different cases are given in Table I. It will be seen that, while the theoretical results have the same trend as experimental data, the agreement between the two is poor. In fact, for *N*-Me-Ala, attempts at varying the theoretical parameters over a wide range did not yield a value of n_H appreciably larger than 20%.

It appears that careful experiments must be performed with model systems even simpler than those studied by Portnova et al.[13] and Mizushima et al.,[14] e.g., compounds of the following general type are worthy of study: *N*-acetyl-(Gly, Ala, ···)-*N*-methylamide (II); *N*-(Ac,Me)-(Gly, Ala···,)-*N*-methylamide (III). Since these compounds will contain just the group of atoms listed in (I), the theoretical calculations can be made exact for comparison with experiment in these cases.

NMR Data on Coupling Constants

The calculations were made in the cases of both Gly and Ala C_2^α atoms, in each case assuming that there is a hydrogen bond and that the internal hydrogen bond is absent. The theoretical results are summarized in Table II. An observed value of 7–7.5 cps is reported by Bystrov et al.[15] for Ala, both in $CDCl_3$ and $(CH_3)_2SO$. Although these authors have interpreted their results in terms of a hydrogen-bonded state with $\phi \approx 240°$, it appears that the data are perfectly consistent with the results of conformational theory based on conventional formulae obtained by us. Our potential energy map in the (ϕ,ψ) plane is not appreciably different from that of Crippen and Scheraga.[12] In fact, J values for Ala, calculated with three different types of interatomic potential functions, analogous to the so-called F, S, and $K2$ functions of Venkatachalam and Ramachandran[19] gave J values differing by not more than 0.15–0.3 cps from those listed in Table II.

TABLE II
Coupling Constants for Different Residues, with and without Hydrogen Bonding, Calculated from Theory

Residue	Nature	J, cps	
		Set 1[a]	Set 2[b,c]
L-Ala	H-bonded	7.3	6.6
	Non-H-bonded	7.7	7.0
Gly	H-bonded	6.7	6.4
	Non-H-bonded	6.2	5.8

[a] Set 1 corresponds to values of $A = 9$, $B = -0.5$, $C = 1.0$, which are close to those given by Bystrov et al.[15,16] Although we have some doubts about the interpretations of Bystrov et al.[15] of their NMR data in relation to the formation and type of H-bonds, values of A, B, and C close to those proposed by them appear to be valid. Later NMR studies on cyclic systems, making use of measured coupling constants (e.g., on the K-complex of valinomycin[17]) bear out the essential correctness of the numerical formula for $J(\theta)$.

[b] Set 2 is calculated by using $A = 9.0$, $B = 0.0$, and $C = 0.0$.

[c] Kopple has used $A = 8.9$, $B = -0.2$, and $C = 0.5$ in his recent NMR study of evolidine.[18]

The function $P(\phi)$ for Ala with and without hydrogen bonds is shown in Table III for different values of ϕ. Although the distribution is somewhat different, the calculated coupling constants come out to be very nearly the same. We shall therefore not comment definitely on the question whether the DMSO solutions studied by Bystrov et al.[15] have an internal hydrogen bond.*

Bystrov et al.[16] report also that J(Phe) in DMSO is about 1 cps larger than J(Ala), and this is borne out by our preliminary calculations for this side chain. On the other hand, the theoretical results in Table II show that J(Gly) is epxected to be about 2 cps lower than J(Ala) in the non-hydrogen-bonded state. We learn from Dr. K. D. Kopple[18] that J(Gly) in DMSO and water solutions is of the order of 5–6 cps, while J(Leu) and J(Phe,Tyr) are of the order of 8–8.5 cps, in small peptides containing these residues. Both these are in agreement with the theoretical trend. A detailed comparison of theory with more data with a variety of residues is being made.

It should be mentioned that the values of A, B, and C used in Table II are highly tentative. More precise values are being obtained by making measurements on model compounds (of either known or theoretically calculable geometry) in collaboration with Dr.

* DMSO is known to break hydrogen bonds of DNA, and is in fact used to obtain single-stranded DNA.

TABLE III
Distribution Function $P(\phi)$ for Hydrogen-Bonded and Non-Hydrogen-Bonded Alanyl Dipeptide Unit

	$P(\phi)$, %	
ϕ	H-bonded	Non-H-bonded
0°	0.5	0.9
10°	1.1	2.3
20°	1.8	3.7
30°	2.6	5.4
40°	3.6	7.4
50°	4.6	9.4
60°	5.5	11.2
70°	6.3	12.9
80°	8.7	14.3
90°	37.7	13.0
100°	22.3	8.7
110°	2.6	5.4
120°	1.4	2.9
130°	0.6	1.2
140°	0.1	0.3
a		
a		
230°	0.1	0.2
240°	0.1	0.3
a		
a		
350°	0.0	0.1

[a] The values of $P(\phi)$ for intermediate values are less than 0.1%.

Kopple, and these will be reported elsewhere. They should be useful in obtaining accurately the value of ϕ in unknown systems from measurements of the coupling constants $J_{NH-C^{\alpha}H}$.

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