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Detection of Proton Acceptor Sites of Hydrogen Bonding between Nucleic Acid Bases by the Use of ^{13}C Magnetic Resonance

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Abstract: ^{13}C magnetic resonance spectra of 1-cyclohexyl derivatives of uracil, thymine, 5,6-dihydrouracil, 5-bromouracil, and 4-thiouracil and their 1:1 mixture with 9-ethyladenine were observed in chloroform solutions. The signals of the 2- and 4-carbonyl carbons show remarkable downfield shifts at higher concentrations. By computer analysis of the chemical shift–concentration curves, association constants and limiting shifts were obtained. From the values of the limiting shifts it can be inferred that the self-association using the 4-carbonyl group is more common in uracil and the ratio of the 4-carbonyl dimer to the 2-carbonyl dimer gradually falls in the order thymine, 5,6-dihydrouracil, 5-bromouracil, 4-thiouracil while that of the 2-carbonyl dimer rises in the order. However, with adenine association the ratio is highest in thymine and this is followed by uracil, 5-bromouracil, and 4-thiouracil in descending order of magnitude.

In the double-stranded structures of deoxyribonucleic acid and ribonucleic acid, adenine forms specific hydrogen bonds with thymine (or uracil) and guanine with cytosine. The specific bonds are believed to be the molecular basis of information transfer in nucleic acids. Much work has been done to find the basis of this specificity by the use of synthetic poly- and oligonucleotides and single base derivatives. Some infrared[1-8] and proton magnetic resonance studies[9-11] clearly showed that even single base residues interact by means of specific hydrogen bonds in solution. The data obtained in these experiments revealed the strength of interaction on the amino and imino protons, but little is known about the acceptor sites of the hydrogen bonds. In the base pair model proposed by Watson and Crick, thymine (or uracil) pairs with adenine by using the C-4 carbonyl group. However, x-ray analyses showed that 5-bromouracil[12] and 4-thiouracil[13] derivatives formed complexes with themselves and with adenine derivatives through the C-2 carbonyl group.

In the present experiment we observed ^{13}C magnetic resonances of single base derivatives in solution and tried to find interaction sites from the concentration dependency of chemical shifts. When hydrogen bonds are formed, ^{13}C nuclei at the nearer hydrogen bonded sites suffer perturbation in the electron distribution of the sites; this perturbation disturbs the diamagnetic circulation of electrons. The resultant downfield shift of the ^{13}C magnetic resonance is smaller than that of the ^1H resonance of the bonded proton, because the carbon atom does not participate directly in the hydrogen bonding and is located at least one bond from the site. Also the resonance frequency of ^{13}C is one-fourth that of ^1H. In spite of the smaller shifts, ^{13}C resonances promise to provide powerful information on the effect of the bonding on the molecule skeleton. The present experiment indicates the population of the carbonyl carbons bonded to hydrogen atoms and the population's dependency on substitution at the 5 position of the uracil residue.

Experimental Section

Materials. 9-Ethyladenine (A), 1-cyclohexyluracil (U), 1-cyclohexylthymine (TH), 1-cyclohexyl-5-bromouracil (BU), 1-cyclohexyl-5,6-dihydrouracil (DU), and 1-cyclohexyl-4-thiouracil (TU) were purchased from Cyclo Chemical Co., Los Angeles, Calif. 9-Ethyladenine was recrystallized from a mixture solvent of carbon tetrachloride and chloroform, and the other compounds were used without further purification. ^{13}C magnetic resonance spectra were measured for their chloroform-d_1 solutions. Chloroform-d_1 obtained from CEA, France, was dried by passing through an alumina gel column in a drybox. Chloroform is a favorable solvent for investigating intermolecular interaction because it has relatively weak polarity and scarcely interacts with adenine and uracil derivatives.

Methods. ^{13}C magnetic resonance spectra were obtained at 25 MHz with a JEOL PFT-100 pulse Fourier transform NMR system locked on deuterium. The protons were completely decoupled at 100 MHz for ^{13}C NMR spectra of all the uracil derivatives. Tetramethylsilane was used as an internal standard and chemical shifts were measured relative to the ^{13}C resonance of Me_4Si by data reduction. The spectrum of each uracil derivative was obtained in a concentration range from 0.2 to 0.025 M, and the spectra of mixtures with A were measured for solutions from 0.2 to 0.025 M. The temperature of the sample tubes was kept at 27 °C throughout the experiments.

Procedures for the Calculation of Association Constants and Population Differences. The equilibrium equation for the formation of a 1:1 complex of adenine and uracil derivatives is expressed as

$$A + U \rightleftharpoons AU \tag{1}$$

but in the present case, various conformations of dimers as shown in Figure 1 should be taken into account, because A and U have two proton acceptor sites each. Taking all the possibilities into account, the above relation can be rewritten as follows:

$$A + U \rightleftharpoons kA_1U_2 + lA_7U_2 + mA_1U_4 + nA_7U_4 \tag{2}$$

Here the relation of $k + l + m + n = 1$ holds for k, l, m, and n. Then the association constant K can be defined as follows:

$$K = C^k{}_{A_1U_2}C^l{}_{A_7U_2}C^m{}_{A_1U_4}C^n{}_{A_7U_4}/C_AC_U \tag{3}$$

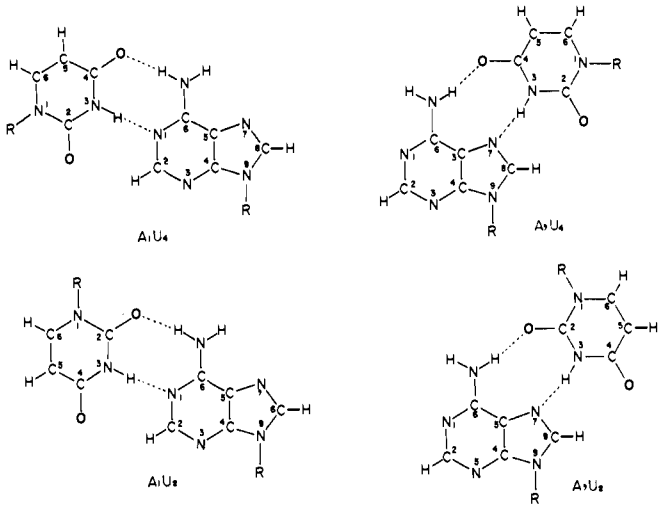

Figure 1. Structures of dimers between A and U.

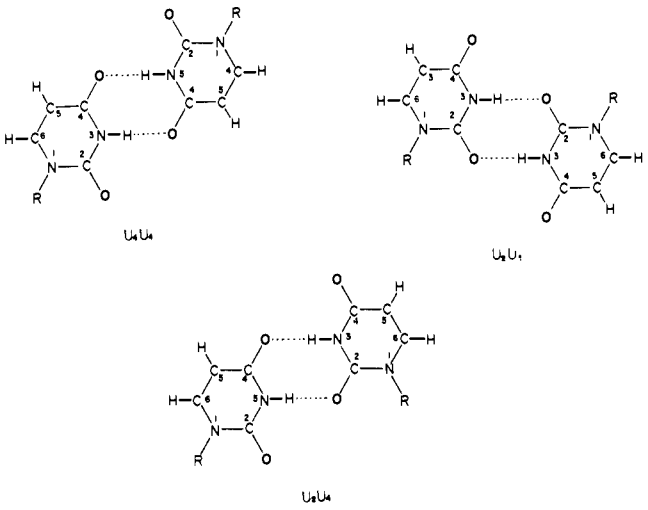

Figure 2. Structures of the self-associated dimers of U.

where C_A and C_U are the concentrations of free monomers and $C_{A_iU_j}$ is the concentration of each A_iU_j complex. If we take $C°_A$ and $C°_U$ as the total concentrations of A and U, then

$$C°_A = C_A + C_{A1U2} + C_{A7U2} + C_{A1U4} + C_{A7U4}$$
$$C°_U = C_U + C_{A1U2} + C_{A7U2} + C_{A1U4} + C_{A7U4} \quad (4)$$

If δ_U, δ_{A1U2}, δ_{A7U2}, δ_{A1U4}, and δ_{A7U4} are the limiting chemical shifts of U, A_1U_2, A_7U_2, A_1U_4, and A_7U_4, respectively, the observed chemical shift δ_m can be expressed by the next equation, assuming rapid exchange among complexes.

$$\delta_m = (\delta_U C_U + \delta_{A1U2}C_{A1U2} + \delta_{A7U2}C_{A7U2} + \delta_{A1U4}C_{A1U4} + \delta_{A7U4}C_{A7U4})/(C_U + C_{A1U2} + C_{A7U2} + C_{A1U4} + C_{A7U4}) \quad (5)$$

The limiting chemical shift δ_k is the shift which would be realized if all the molecules in the equilibrium system were in the state k. Taking eq 3 and 4 and the relation $C_{A1U2}:C_{A7U2}:C_{A1U4}:C_{A7U4} = k:l:m:n$ into account, we can obtain an equation which relates the binding constant K with the observed chemical shifts when $C°_U = C°_A$:

$$K = \frac{k^k l^l m^m n^n (\delta_m - \delta_U)(\delta_{AU} - \delta_U)}{C°_U(\delta_{AU} - \delta_m)^2} \quad (6)$$

where δ_{AU} is a newly defined variable which equals

$$k\delta_{A1U2} + l\delta_{A7U2} + m\delta_{A1U4} + n\delta_{A7U4} \quad (7)$$

The expression for K in eq 3 can be rewritten as follows:

$$\frac{K}{k^k l^l m^m n^n} = \frac{C_{A1U2}}{C_A C_U} + \frac{C_{A7U2}}{C_A C_U} + \frac{C_{A1U4}}{C_A C_U} + \frac{C_{A7U4}}{C_A C_U} = K_1 + K_2 + K_3 + K_4 \quad (8)$$

This equation shows that $K/(k^k l^l m^m n^n)$ is the sum of the association constants of the equilibrium between each type of complex and free monomer.

Uracil derivatives associate with each other, though the interactions are weak compared with that of the A-U association. Self-association is here treated in the same way as heteroassociation. The equilibrium relation for self-association is expressed as follows:

$$U + U \rightleftharpoons kU_2U_2 + lU_2U_4 + mU_4U_4 \quad (9)$$

Each complex has the structure shown in Figure 2. The equation relating the association constant with the observed chemical shifts is the following:

$$K = \frac{k^k l^l m^m n^n (\delta_m - \delta_U)(\delta_{UU} - \delta_U)}{2C°_U(\delta_{UU} - \delta_m)^2} \quad (10)$$

where $\delta_{UU} = k\delta_{U22} + l\delta_{U24} + m\delta_{U44}$. $K/k^k l^l m^m$ is the sum of the association constants between complexes and free monomers.

A computer program for a nonlinear least-square regression analysis[14] was written to calculate the limiting shifts (δ_U, δ_{UU}, and δ_{AU}) and apparent binding constants ($K/k^k l^l m^m$ or $K/k^k l^l m^m n^n$). This was done by feeding in the initial concentration ($C°_U$) and the observed chemical shifts (δ_m), and assuming that only 1:1 dimers are present and the other polymers are negligible.

Now we can define new values for Δ^2_{UU}, $\Delta^2_{U_2U_2}$, $\Delta^2_{U_2U_4}$, $\Delta^2_{U_4U_4}$, Δ^4_{UU}, $\Delta^4_{U_2U_2}$, $\Delta^4_{U_2U_4}$, and $\Delta^4_{U_4U_4}$. Δ^2_{UU} means the difference between the limiting shift of the C-2 carbonyl carbon of free monomer δ_U and that of dimer δ_{UU}. $\Delta^2_{U_2U_2}$ is the contribution from the U_2U_2 dimer to the difference. $\Delta^2_{U_2U_4}$, $\Delta^2_{U_4U_4}$, $\Delta^4_{U_2U_2}$, $\Delta^4_{U_2U_4}$, and $\Delta^4_{U_4U_4}$ are also defined in a similar manner to $\Delta^2_{U_2U_2}$. Thus we can obtain the following relation:

$$\Delta^2_{UU} = k\Delta^2_{U_2U_2} + l\Delta^2_{U_2U_4} + m\Delta^2_{U_4U_4} \quad (11)$$

For the C-4 signal a similar relation can be written:

$$\Delta^4_{UU} = k\Delta^4_{U_2U_2} + l\Delta^4_{U_2U_4} + m\Delta^4_{U_4U_4} \quad (12)$$

Subtracting eq 11 from eq 12 gives

$$\Delta^4_{UU} - \Delta^2_{UU} = -k(\Delta^2_{U_2U_2} - \Delta^4_{U_2U_2}) + l(\Delta^4_{U_2U_4} - \Delta^2_{U_2U_4}) + m(\Delta^4_{U_4U_4} - \Delta^2_{U_4U_4}) \quad (13)$$

It is safe to assume that the magnitude of perturbation at the C-4 carbon on the formation of a hydrogen bond using the C-2 carbonyl group is nearly identical with that at the C-2 carbon on the formation of a hydrogen bond using the C-4 carbonyl group. It is also reasonable to assume that the C-2 carbon participating in a hydrogen bond suffers the same perturbation as the C-4 carbon in a corresponding case. The above assumptions enable us to rewrite eq 13 as

$$\Delta^4_{UU} - \Delta^2_{UU} = (m - k)\Delta' \quad (14)$$

where

$$\Delta' = (\Delta^4_{U_4U_4} - \Delta^2_{U_4U_4}) = (\Delta^2_{U_2U_2} - \Delta^4_{U_2U_2})$$

If Δ' is the same for all the U derivatives, the value of $(\Delta^4_{UU} - \Delta^2_{UU})$ is determined by $(m - k)$. Therefore the larger value of $(\Delta^4_{UU} - \Delta^2_{UU})$ means that the C-4 carbonyl group is more often used as a proton acceptor in hydrogen bonding. We can also treat the case of association with adenine in a similar manner, and can say that the value of $(\Delta^4_{AU} - \Delta^2_{AU})$ represents semiquantitatively the population difference between the complexes which use the C-2 and C-4 carbonyl groups as proton acceptor sites.

MO Calculation. The electron charge densities of free uracil were calculated by the CNDO/2 method using the parameters employed in ref 15. The same procedure was applied to the system where a uracil molecule forms a cyclic hydrogen bonded dimer with amino aldehyde, a model of the cis amide part of uracil. The N-H bond length of free uracil was taken as 0.948 Å and the bonded one as 1.043 Å. The H···O distance in the dimer was 1.657 Å. The other bond lengths and bond angles were taken from the crystal structure of 1-cyclohexyluracil.[16]

Results and Discussion

Concentration Dependence of ^{13}C NMR Spectra. Concentration dependences of ^{13}C chemical shifts of the 1:1 mixtures

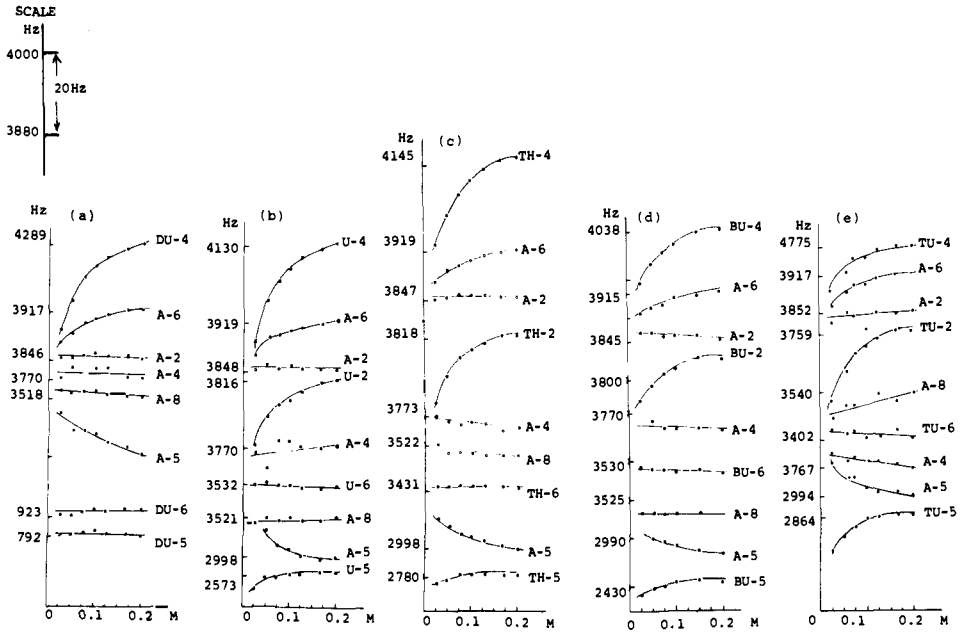

Figure 3. Concentration dependence of ^{13}C chemical shifts of the 1:1 mixtures of 9-ethyladenine (A) and 1-cyclohexyluracil derivatives: (a) 5,6-Dihydrouracil (DU) + A; (b) uracil (U) + A; (c) thymine (TH) + A; (d) 5-bromouracil (BU) + A; (e) 4-thiouracil (TU) + A. Each point was obtained by accumulating, 1000–15 000 times every 3 s.

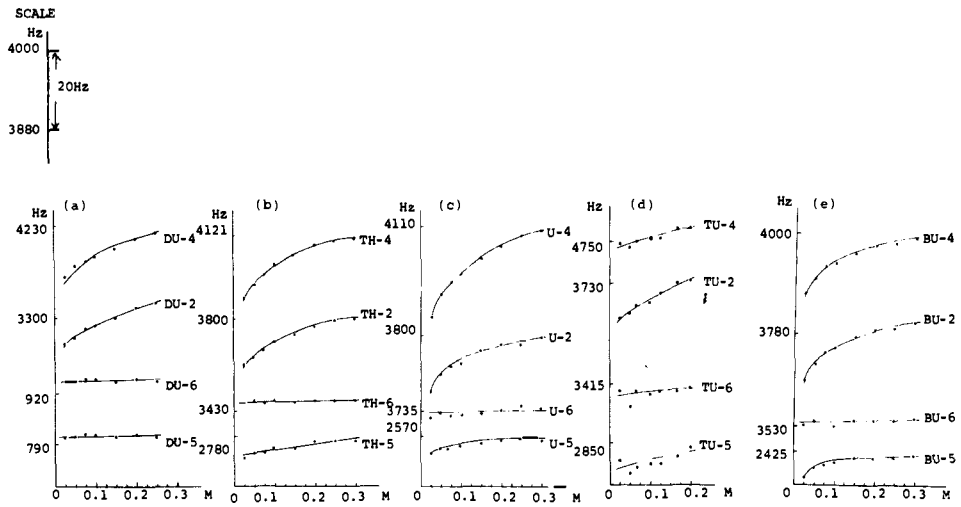

Figure 4. Concentration dependence of ^{13}C chemical shifts of 1-cyclohexyluracil derivatives: (a) 5,6-dihydrouracil; (b) thymine; (c) uracil; (d) 4-thiouracil; (e) 5-bromouracil.

of uracil derivatives with A are shown in Figure 3 and those of uracil derivatives are given in Figure 4. Assignments of the ^{13}C resonance peaks were made on the basis of analogies with previously published data.[17] The C-2 and C-4 signals of uracil derivatives shift downfield by a much larger amount than other signals. The downfield shifts are attributable to an increase in hydrogen bond formation with increase in concentration. The larger shifts of the C-2 and C-4 carbons of uracil derivatives indicate that the C-2 and C-4 carbonyl groups directly participate in hydrogen bond formation and suffer much more perturbation when bonding with hydrogen than do other carbons in the molecule. The result is consistent with the infrared absorption spectra in the carbonyl stretching region. The absorptions at 1680 and 1650 cm^{-1} in the spectrum of N-deuterated 1-cyclohexyluracil were assigned to the C-2 and C-4 carbonyl stretching vibrations, respectively, and their frequencies fall by 10–15 cm^{-1} with increased concentration or with added adenine derivative.[18]

The C-5 carbon of U and the C-6 carbon of A show relatively larger downfield shifts except in the case of the 1-cyclohexyl-5,6-dihydrouracil–A mixture. The C-5 carbon of A also shows a relatively large shift, but in this case the direction of the shift is opposite to that of carbonyl carbons of U. We cannot explain the result clearly at the present time, but the perturbation arising when hydrogen bonds are formed may induce changes in both electron density and bond anisotropy around these atoms.

Perturbation in Electron Density on Formation of Cyclic Hydrogen Bonds. The total electron density was calculated for the free uracil derivative and the hydrogen bonded uracil derivative with a cis amide group using the C_2 or C_4 carbonyl and the N_3 imino group. The differences in electron densities are given in Table I.

The result indicates that the formation of cyclic hydrogen bonds at C_2 and N_3 withdraws electrons from the C_2 carbon and results in the downfield shift of the ^{13}C resonance of C_2. Our calculations also indicated that the formation of hydrogen bonds at the C_2 carbonyl group does not affect the electron

Table I. Difference in Electron Charge Density between Free Uracil and Hydrogen-Bonded Uracil (Calculated by CNDO/2)

Atom no.	Free form	3,4 complex (Δ)	3,2 complex (Δ)
N-1	5.192	0.000	−0.001
C-2	3.552	0.000	−0.010
N-3	5.247	0.028	0.028
C-4	3.628	−0.014	−0.001
C-5	4.169	0.006	−0.003
C-6	3.823	−0.006	0.004
O-2	6.373	−0.001	0.026
O-4	6.351	0.029	−0.001
H-1	0.868	0.002	0.004
H-3	0.855	−0.048	−0.047
H-5	0.946	0.002	0.002
H-6	0.996	0.001	0.002

Table II. Association Constants for Self-Association and Association with 9-Ethyladenine of Uracil Derivatives Determined by ^{13}C Magnetic Resonance of Carbonyl Carbons[a]

Compd	Carbonyl position	Association constants, M^{-1} Self	(IR)[6]	With A	(IR)[6]
5,6-Dihydro-uracil	C-2	0.97 ± 0.08	(2.9)		(30)
	C-4	1.00 ± 0.08		28 ± 2	
Uracil	C-2	4.2 ± 0.6	(6.2)	50 ± 3	(100)
	C-4	3.2 ± 0.6		60 ± 3	
Thymine	C-2	2.2 ± 0.1	(3.2)	60 ± 5	(130)
	C-4	4.2 ± 0.2		73 ± 4	
5-Bromoura-cil	C-2	6.9 ± 0.4	(4.1)	300 ± 30	(240)
	C-4	9.0 ± 0.3		260 ± 10	
4-Thiouracil	C-2	0.69 ± 0.09	(2.7)	109 ± 11	(90)
	C-4	0.4 ± 0.2		140 ± 20	

[a] Measured at 27 °C for chloroform solutions.

Table III. Difference (Δ) between the Limiting Shifts of ^{13}C Resonances of Carbonyl Carbons in Free and Bound States

Compd	Carbonyl position	$\Delta_{UU} = \delta_{UU} - \delta_U$, Hz (self-association)	$\Delta_{AU} = \delta_{AU} - \delta_U$, Hz (association with adenine)
5,6-Dihydro-uracil	C-2	45.7 ± 0.5	
	C-4	45.6 ± 0.7	51.2 ± 0.7
Uracil	C-2	30.3 ± 1.2	46.1 ± 0.4
	C-4	55.2 ± 0.7	65.5 ± 0.5
Thymine	C-2	34.5 ± 0.6	45.9 ± 0.6
	C-4	41.7 ± 0.6	74.9 ± 0.5
5-Bromoura-cil	C-2	34.8 ± 0.4	50.6 ± 0.3
	C-4	32.4 ± 0.7	65.2 ± 0.2
4-Thiouracil	C-2	66.3 ± 1.0	51.1 ± 0.8
	C-4	53.4 ± 1.2	34.0 ± 0.7

Table IV. Semiquantitative Expression of Differences in Populations of Dimers Which Use the C-2 and C-4 Carbonyl Carbons

Compd	$(\Delta^4_{UU} - \Delta^2_{UU})/(\Delta^4_{UU} + \Delta^2_{UU})$ (self-association)	$(\Delta^4_{AU} - \delta^2_{AU})/(\Delta^4_{AU} + \Delta^2_{AU})$ (association with adenine)
5,6-Dihydro-uracil	0.00	
Uracil	0.29	0.17
Thymine	0.09	0.24
5-Bromo-uracil	−0.04	0.13
4-Thiouracil	−0.12	−0.20

density at the C_4 carbonyl group and vice versa. According to the empirical relationship (160 ppm/electron) proposed by Spiesecke and Schneider,[19] the present calculation predicts that $\Delta^4_{U_4U_4} = 56$ Hz, $\Delta^2_{U_2U_2} = 40$, $\Delta^4_{U_2U_2} = \Delta^2_{U_4U_4} = 0$, $\Delta^4_{U_2U_4} = 28$, and $\Delta^2_{U_2U_4} = 20$ Hz. The calculated shifts only explain half or one-third of the observed chemical shift differences when we assume $k = l = m = 1/3$. It may be due to the fact that the present calculation was not done for the complete dimer system or the shift is caused in part by other factors.

Binding Constants and Limiting Chemical Shifts. Computer analysis enables us to calculate the binding constants and limiting shifts for free monomers and associated complexes by using the plots of the relationship between concentration and chemical shift. The values obtained for binding constants and the limiting shift differences $\Delta = \delta_{UU} - \delta_U$ or $\delta_{AU} - \delta_U$ are given in Tables II and III. In Table II, binding constants calculated previously by infrared measurements are also given for comparison. The present data reveal similar results and so the validity of both procedures is proved.

Population Difference in the Dimers Using the C-2 and C-4 Carbonyl Groups. Table IV shows the changes in the limiting shift difference Δ for self- and complementary associations. To normalize the results $(\Delta^4_{AU} - \Delta^2_{AU})$ and $(\Delta^4_{UU} - \Delta^2_{UU})$ are here divided by $(\Delta^4_{AU} + \Delta^2_{AU})$ and $(\Delta^4_{UU} + \Delta^2_{UU})$, respectively. The table indicates that the self-association using the 4-carbonyl group is more common in uracil and the ratio of the 4-carbonyl dimer to the 2-carbonyl dimer gradually falls in the order thymine, 5,6-dihydrouracil, 5-bromouracil, 4-thiouracil while that of the 2-carbonyl dimer rises in the reverse order. However, with adenine association the ratio is highest in thymine and this is followed by uracil, 5-bromouracil, and 4-thiouracil in descending order of magnitude. In the association of U derivatives with A, the difference in availability of the C-4 carbonyl group in hydrogen bonds can be explained by the difference in electronegativity of the substituent at the 5 position. Since the methyl group is considered an electron-releasing group, the methyl group of thymine pushes electrons into the pyrimidine ring to increase the electron density at the C-4 carbonyl group. On the other hand, electron density of the pyrimidine ring of 5-bromouracil decreases, particularly at the C-4 site which is close to the strong electron-attractive group, bromine. Thus electron density at the C-4 carbonyl group increases in ascending order from thymine > uracil > 5-bromouracil; this is identical with the order of the relative induced shift of the C-4 carbon. Therefore we can say that the electron migration induced by substitution on the pyrimidine ring is an important factor in determining the population of the hydrogen-bonded complex. The 2-carbonyl group of 4-thiouracil is more often hydrogen bonded than the C-4 thiocarbonyl group because S is inferior to O as a proton acceptor.[20]

The data in Table IV can be used to express population differences only when the assumptions $\Delta^2_{U_2U_4} = \Delta^4_{U_2U_4}$ and $\Delta^4_{U_4U_4} - \Delta^2_{U_4U_4} = \Delta^2_{U_2U_2} - \Delta^4_{U_2U_2}$ are valid. There is no way to test them experimentally, but tentative calculation of electron charge density shows that the assumption is not far from the mark. Even when $\Delta^2_{U_2U_4} \neq \Delta^4_{U_2U_4}$ and $\Delta^4_{U_4U_4} \neq \Delta^2_{U_2U_2}$, the order of population differences is unaffected.

In spite of ambiguity over the quantitative population difference the C-2 carbonyl group is used with fairly high probability as a proton acceptor in dimer formation. Among the uracil derivatives uracil and thymine more often use the C-4 carbonyl group than the C-2 carbonyl group. It is reasonable to assume that the two most common bases are able to form hydrogen-bonded complexes by using the C-4 carbonyl group, just like the base pair models proposed by Watson and Crick[21] and by Hoogsteen.[22] However, even if the base pairs in nucleic acids are able to exist for most of the time using the C-4 carbonyl group, the possibility still exists of complexes being formed which use the C-2 carbonyl group, i.e., the so-called reversed Watson-Crick and Hoogsteen type base pairs. It is

quite surprising that misreading of the base sequences during the processes of transcription and translation is quite rare in spite of the apparent arbitrariness of the hydrogen bonded site. Steric hindrance at the decoding site should be severe. On the other hand, recent x-ray analyses on tRNA show the existence of extra base pairs which differ from usual A-U and A-T pairs.[23,24] Ability of the C-2 carbonyl group to form hydrogen bonds may be important in building up a three-dimensional structure of tRNA. It is known that in place of thymine, 5-bromouracil can easily be incorporated into DNA at the duplication process and induces mutation.[25] The present experiments show that in base pairing 5-bromouracil uses the C-2 carbonyl group as a proton acceptor much more frequently than thymine. This presumably means that 5-bromouracil is more likely to give rise to mispairs like G-BrU, where the C-2 carbonyl group is used. Thus the ability to form a hydrogen-bonded complex using the C-2 carbonyl group may be related to biological processes in several cases.[26]

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(26) The authors wish to express their thanks to Professor K. Nishimoto, Osaka Municipal University, and Dr. T. Katsura for their advice in calculation of electron charge densities.

Calculation of ^{13}C Relaxation Times and Nuclear Overhauser Enhancements in a Hydrocarbon Chain Undergoing Gauche–Trans Isomerism†

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Received December 29, 1976

Abstract: An analytically tractable model has been developed for calculating the relaxation times of a hydrocarbon chain undergoing gauche–trans isomerization about each carbon–carbon bond and overall isotropic motion. Each internal rotation is described by two parameters, τ_t, the lifetime of a trans state, and σ, the equilibrium ratio of gauche to trans states: $\sigma = ([g^+] + [g^-])/2[t]$. Spin lattice relaxation times (T_1), spin–spin relaxation times (T_2), and nuclear Overhauser enhancements (NOE) have been calculated for a six-bond chain. As a result of the restriction introduced into the internal motion, properties associated with the slowly tumbling end of the molecule such as a reduced NOE, $T_1 \gg T_2$, and a frequency dependence of T_1 propagate far into the chain in contrast to the free internal rotation model. Analysis of data obtained for *n*-hexadecyltrimethylammonium bromide micelles and for sonicated dimyristoyl lecithin lipid vesicles indicates that restriction of internal rotation is not sufficient to explain the observed T_1 and T_2 values. For this reason, the effects of correlated motions such as kink formation and diffusion have been treated semiquantitatively and a correlation factor f introduced to reflect the degree of motional correlation. The three possible contributions to the observed T_1 gradient (cumulative effects of successive internal gauche $\rightleftarrows$ trans isomerizations as modified by an appropriate correlation factor, a gradient in the isomerization rate as described by τ_t, and a gradient in σ) are evaluated in light of available experimental data. In general, T_1 values measured in systems undergoing multiple isomerizations are found to be sensitive to the order of the system as well as to the rate of internal motion. For typical parameters, T_2 values are found to be sensitive to the degree of motional correlation and to the value of σ, but not to the isomerization rate.

I. Introduction

Nuclear magnetic resonance studies of membranes and model systems have proliferated rapidly in recent years with ^1H, ^2H, ^{13}C, ^{19}F, and ^{31}P data reported. As discussed in the recent review articles by Lee and co-workers,[1,2] the theoretical interpretations of such studies have tended to vary with the particular nucleus studied. Primarily because of the greater resolution attainable by ^{13}C NMR and the dominance of the relaxation by the ^{13}C–^1H dipolar interaction between directly bonded nuclei, the ^{13}C NMR of membrane systems has been relatively simple to interpret—a situation which is in sharp contrast with the reported ^1H NMR studies.[1,3-6] The detailed description of the ^{13}C relaxation in such lipid systems has been

† Work performed under the auspices of the U.S. Energy Research and Development Administration.