HYDROGEN BONDING OF ADENINE DERIVATIVES TO TYROSINE SIDE CHAIN

G. LANCELOT, *Centre de Biophysique Moléculaire, 45045 Orleans Cedex, France*

ABSTRACT High resolution proton magnetic resonance measurements provide evidence for the formation of hydrogen-bonded complexes between 9-ethyladenine and *p*-cresol used as a model of tyrosine side chain in $CDCl_3$. We have calculated the sum of the association constants corresponding to the three existing 1:1 complexes: $K = 6.3 \pm 0.15$. By methylation of the amino group of adenine, we were able to calculate the ratio of the two strongest equilibrium constants $K_7/K_1 = 1.6 \pm 0.3$. Theoretical computations by the complete neglect of differential overlap (CNDO/2) method indicate that several hydrogen-bonded planar complexes can form between 9-methyladenine and phenol. The computed energy of the complexes with 6-dimethylamino adenine removes some ambiguity concerning the computed ratio of the association constants. Comparison of the calculated energies with free energies experimentally determined in organic solvent shows that despite the competition with $CDCl_3$, which associates with both solute molecules, the preferential order of association is conserved. The small variations of charge density of adenine carbon atoms when complexed with phenol are in agreement with very small chemical shifts observed by ^{13}C-nuclear magnetic resonance.

INTRODUCTION

During the past few years much work has been devoted to the investigation of a fundamental problem in molecular biology, namely the specificity of recognition of nucleic acids by proteins and enzymes. Several direct interactions might take place: electrostatic interactions, formation of stacked complexes, and hydrogen bonding. Although several papers have been published on electrostatic interactions between polypeptides and nucleic acids (Shapiro et al., 1969; Olins et al., 1967) or on the formation of stacked complexes between aromatic amino acids and polynucleotide bases (Brun et al., 1975; Dimicoli and Hélène, 1974; Toulmé et al., 1974; Gabbay et al., 1976*a,b*), little work has been devoted to hydrogen bonding between amino acids side chains and nucleic acid bases. However, interactions involving OH groups have been demonstrated in crystal complexes of ribonuclease and cytidine 3'-monophosphate between threonine 45, serine 123, and the pyrimidine ring (Richards et al., 1969), and proposed for uridine (Blow and Steitz, 1970). Preliminary results from our laboratory have provided evidence for such interactions in organic solvents (Sellini et al., 1973): hydrogen bonding interactions of phenol and carboxylic groups with uracil and

adenine bases were demonstrated. Recently,[1] we have extended these investigations by measuring association constants between derivatives of adenine, uracil, and cytosine and amino acid side chains of serine or threonine, aspartic, or glutamic acid, lysine, cysteine, and methionine in cyclohexane by ultraviolet absorption measurements. Unfortunately the overlap of the absorption spectra of tyrosine and nucleic acid bases precludes using absorption spectroscopy to study interactions between these molecules. But by measuring the increase of solubility of 9-ethyladenine in the presence of *p*-cresol, we were able to calculate an association constant of 1,000 ± 200 M^{-1} in cyclohexane at 20°C. Since several 1:1 complexes can exist, the constants reported in such studies are the sum of the different association constants. On the other hand, hydrogen bonding sites could not be altogether available during the recognition of nucleic acids by proteins and enzymes. It is therefore very important to know the specificity of interaction of each amino acid side chain with different sites of each nucleic acid base. The present paper is aimed at answering these questions in the system 9-ethyladenine *p*-cresol, used as a model for the side chain of tyrosine.

MATERIALS

9-ethyladenine (9 EA), 6-methylamino-9-methyladenine (m^6A), and 6-dimethylamino-9-ethyladenine (m_2^6A) were purchased from Cyclo Chemical (New York). Para-cresol (Prolabo, Paris, France) was distilled and purified by sublimation. During this work, deuterated chloroform (Commissariat à l'Energie Atomique, Saclay, France) was kept on appropriate molecular sieves to eliminate water or polar impurities. Nuclear magnetic resonance (NMR) spectra were obtained with a Bruker WH 90 Fourier transform spectrometer (Bruker Instruments, Inc., Billerica, Mass.). The spectrometer magnetic field was locked on an internal deuterium reference (deuterated chloroform) for all experiments. Temperature was regulated at ±0.5°C and known at ±2°C. The positions are given in parts per million (ppm) downfield from tetramethylsilane (TMS = 0.0) at 303°K. The H_2 and H_8 proton resonances of the adenine analogues were assigned by deuterium replacement of the slightly acid H_8 proton, by the method of Chan et al. (1964). All chemical shifts of carbon-13 spectra were calculated with respect to the internal reference ($CDCl_3$). Complete neglect of differential overlap (CNDO) calculations were performed on an IBM 370-168 computer (International Business Machines Corp., Armonk, N.Y.) with a FORTRAN program written by J. M. Leclercq (1972).

RESULTS

9-EA and p-Cresol

Fig. 1 shows the paramagnetic resonance (PMR) spectra of 9 EA, *p*-cresol, and 9 EA + *p*-cresol in $CDCl_3$ at 303°K. The binding of *p*-cresol to 9 EA leads to a downfield shift of amino, H_2 and H_8 protons of 9 EA, as well as of the hydroxylic proton of *p*-cresol. The downfield shift of proton resonances is a general characteristic of protons participating in hydrogen bonds (Joesten and Schaad, 1974). At low *p*-cresol concentra-

[1]Lancelot, G. 1976. Hydrogen bonding of amino acid side chains to nucleic acid bases. Submitted for publication.

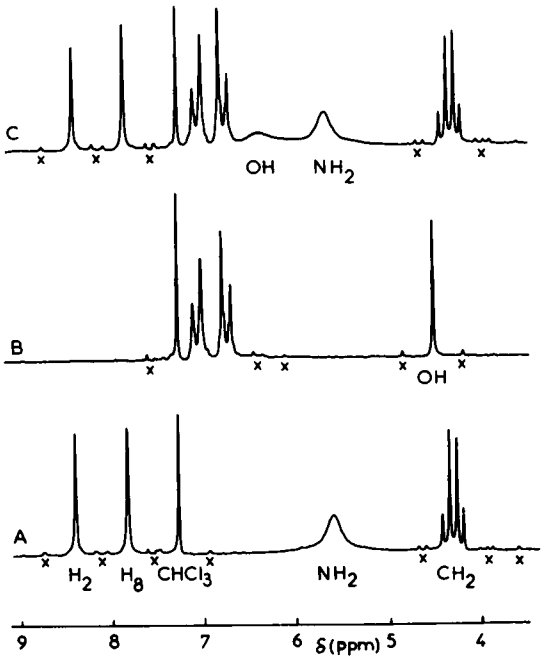

FIGURE 1 PMR spectra of 9 EA (A), *p*-cresol (B), and 9 EA + *p*-cresol (C) in $CDCl_3$ at 303°K. Spinning side bands are indicated by ×. 9 EA = 2×10^{-2} M and *p*-cresol = 2×10^{-2} M.

tion, 9 EA (2×10^{-2} M) leads to a large downfield shift of the OH proton resonance (shift of 2.11 ppm for 7.6×10^{-3} M in *p*-cresol). If one assumes that a 1:1 complex is formed:

$$A + B \rightleftharpoons AB \tag{1}$$

with

$$K = [AB]/[A] \times [B]. \tag{2}$$

If a_0 and b_0 refer to the initial concentrations of A and B, complex concentration (under the assumption that A and B are not self-associated) is given by:

$$[AB] = \tfrac{1}{2}[a_0 + b_0 + K^{-1} - \sqrt{(a_0 + b_0 + K^{-1})^2 - 4a_0b_0}]. \tag{3}$$

If K^{-1} is sufficiently large with respect to the sum of reactant concentrations ($K^{-1} \gg a_0 + b_0$) then $[AB]$ is given by the relation:

$$[AB] = (a_0 \times b_0)/(a_0 + b_0 + K^{-1}). \tag{4}$$

The chemical shift of a proton in molecule B is given by:

$$\delta = \delta_0 \frac{[B]}{b_0} + \frac{a_0}{a_0 + b_0 + K^{-1}} \delta_c, \tag{5}$$

where δ, δ_0, and δ_c are the observed chemical shift, the chemical shift of B proton in the free state ($a_0 = 0$), and in the AB complex, respectively.

So the reciprocal of the change in chemical shift of a proton is linearly related to B concentration by:

$$\frac{1}{\delta - \delta_0} = \frac{b_0}{a_0}(\Delta\delta_c)^{-1} + \left(1 + \frac{K^{-1}}{a_0}\right)(\Delta\delta_c)^{-1} \text{ with } \Delta\delta_c = \delta_c - \delta_0 \tag{6}$$

Plotting $1/(\delta - \delta_0)$ versus b_0 will allow us to calculate both $\Delta\delta_c$ and K from spectrometric measurement if $K^{-1} \gg a_0 + b_0$.

By this method, which neglects self-association of 9 EA and *p*-cresol, we have obtained (Fig. 2) $K = 6.8 \pm 0.4\ M^{-1}$ and $\delta_c^{OH} = 16.9 \pm 1.3$ ppm, with $\delta_0^{OH} = 4.44$ ppm. By using a least square program to fit δ as a function of K and $\Delta\delta_c$, including self-association of 9 EA ($K_{a2} = 2.28\ M^{-1}$) and self-association of *p*-cresol ($K_{c2} = 1\ M^{-1}$, $\delta_{c2} = 0.53$ ppm), we obtain the best fit with the parameters $K = 6.32 \pm 0.15\ M^{-1}$, $\Delta\delta_c^{OH} = 19.5 \pm 0.4$ ppm. For the amino protons of 9 EA (with the assumption $K^{-1} \gg a_0 + b_0$), $(\delta - \delta_0)_a$ depends linearly on c_0 (with a_0 constant) as shown by the relation:

$$(\delta - \delta_0)a = c_0/(a_0 + c_0 + K^{-1})\,\Delta\delta_c^a. \tag{7}$$

We have calculated the following parameters:

NH_2 of 9 EA:	$\delta_0 = 5.54$ ppm,	$\Delta\delta_c = 0.74 \pm 0.07$ ppm;
H_2 of 9 EA:	$\delta_0 = 8.37$ ppm,	$\Delta\delta_c = 0.12 \pm 0.01$ ppm;
H_8 of 9 EA:	$\delta_0 = 7.81$ ppm,	$\Delta\delta_c = 0.28 \pm 0.02$ ppm.

The change in chemical shift of the OH proton resonance of *p*-cresol is very large

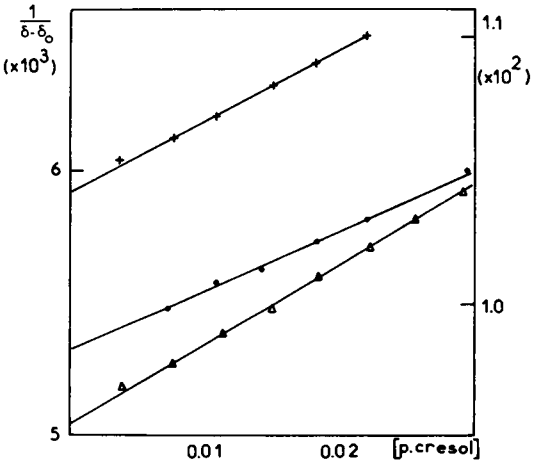

FIGURE 2 Plot of $1/(\delta_0 - \delta_0)$ (δ in hertz) for hydroxylic resonance line versus concentration in *p*-cresol mixing with 9 EA (Δ, left axis), m^6A (+right axis). The concentration of all the derivatives of adenine is 2×10^{-2} M.

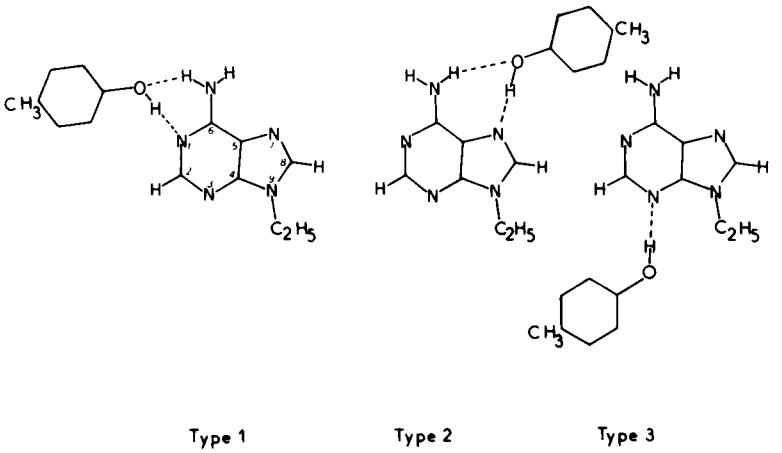

FIGURE 3 The three types of 1:1 complexes between 9 EA and *p*-cresol associated by H bonds.

(~ 19.5 ppm). Such a chemical shift due to H bonding interaction has already been reported for acetic acid and benzoic acid self-association (Davis and Pitzer, 1960).

Association of 9 EA and *p*-cresol leads to three 1:1 complexes (Fig. 3) with association constants K_1, K_3, K_7, named after the position of the H-bond-acceptor nitrogen atom. The constant calculated above is the sum $K = K_1 + K_3 + K_7$. To obtain the three constants separately, it is necessary to prevent some of the possible interactions, e.g., by methylation of the adenine amino group.

m^6A and p-Cresol

As shown by Engel and Von Hippel (1974), the PMR spectrum of m^6A reveals a restricted rotation of the amino group. A study of interactions of 9 EA and m^6A with 1-cyclohexyluracil (Engel and Von Hippel, 1974) or with butyric acid[2] indicated that the methyl group is maintained in *syn* conformation (CH_3 substitutes for H_{12} in Fig. 4). Engel and Von Hippel have computed an equilibrium population ratio of 96:4 (*syn:anti*) in CD_3OD, in agreement with our work, which leads to a ratio of 95:5 in $CHCl_3$. Since complex in position N_1 forms only one hydrogen bond with m^6A and since its formation is hindered by steric effect of CH_3, the most important complexation of m^6A will take place in position N_7. Using Eq. 6, we have computed an equilibrium constant $K = 5.5 \pm 0.4\ M^{-1}$ (Table I, Fig. 2).

The change in chemical shift of the OH proton of *p*-cresol due to H bonding to m^6A is nearly the same as that obtained with 9 EA. However, as can be seen in Table 1, the NH proton of m^6A is shifted downfield about twice as much as in the case of 9 EA. This results from the fact that the exchange between bound and free molecules is always fast and that the two NH_2 protons of 9 EA are experiencing the same magnetic

[2]Lancelot, G. 1976. Hydrogen bonding between nucleic acid bases and carboxylic acids. Submitted for publication.

TABLE I

PARAMETERS COMPUTED FOR *p*-CRESOL-ADENINE DERIVATIVES SYSTEM IN $CDCl_3$ AT 303°K.

	K	$\Delta\delta c$(OH)	$\Delta\delta c(NH_2)$ or (NH)	$\Delta\delta c(H_2)$	$\Delta\delta c(_8)$
9 EA	6.32 ± 0.15	19.5 ± 0.4	0.74 ± 0.07	0.12 ± 0.01	0.28 ± 0.02
m^6A	5.5 ± 0.4	20.5 ± 2.8	1.8 ± 0.3	0.06 ± 0.02	0.33 ± 0.02
m_2^6A	2.4 ± 0.2	23.0 ± 2.2		0.13 ± 0.02	0.17 ± 0.02

For 9 EA we used a least square program with K_{dimer} 9 EA = 2.28, K_{dimer} *p*-cresol = 1.0 and $\Delta\delta_{dimer}$ *p*-cresol = 4.44 ppm. For m^6A and m_2^6A we have used relation 6. All chemical shifts are given in ppm.

environment due to the rotation of the NH_2 group, which is fast on the NMR time scale even though it is restricted. The chemical shift measured for a 1:1 complex between 9 EA and *p*-cresol will be an average value for one free and one complexed NH proton, the latter proton chemical shift being itself an average over the two types of 1:1 complexes (N_1 or N_7). A change in chemical shift about half that obtained with m^6A is thus expected for the NH_2 protons of 9 EA as compared to the NH proton of m^6A.

m_2^6A and p-Cresol

The substitution of both amino protons of 9 EA by methyl groups precludes the formation of complexes with two hydrogen bonds and leads to the formation of the two new complexes with one hydrogen bond on N_1 and N_7 atoms. Although downfield shifts of *p*-cresol OH proton resonance line are less important in the presence of m_2^6A than with 9 EA or m^6A, the association constant is obtained with accuracy since the amino resonance line of adenine does not overlap the OH proton resonance line. By using relation 6, we obtained an equilibrium constant $K = 2.4 \pm 0.2\ M^{-1}$ (Table I, Fig. 2). The change in chemical shift of the OH *p*-cresol proton remains at the same order of magnitude as observed with 9 EA and m^6A.

Computation of the Three Association Constants

NMR studies of *p*-cresol association with 9 EA, m^6A, and m_2^6A led to a system of three equations with five unknowns (K_1, K_3, K_7, and the new association constants K_1' and K_7', which appear when the amino group is methylated). K_1, K_3, and K_7 can be computed with the help of some assumptions, as described in Table II, and the ratio K_7/K_1 is in the range 1.6–4.4. A critical survey of these hypothesis will be made in the discussion.

TABLE II

ASSOCIATION CONSTANTS BETWEEN 9 EA AND *p*-CRESOL COMPUTED ACCORDING TO DIFFERENT ASSUMPTIONS

Assumptions	$K_1(M^{-1})$	$K_3(M^{-1})$	$K_7(M^{-1})$	K_7/K_1
$K_3 = K_1' = K_7'$	1.49 ± 0.16	0.8 ± 0.07	4.0 ± 0.7	2.7 ± 0.75
$K_3 \ll K_1'$ or K_7'	1.7 ± 0.16	0.0	4.6 ± 0.10	2.7 ± 0.3
$K_3 \gg K_1'$ or K_7'	0.72 ± 0.08	2.4 ± 0.2	3.20 ± 0.64	4.4 ± 1.4
$K_7' \ll K_3$ or K_1'	2.0 ± 0.2	1.2 ± 0.1	3.2 ± 0.3	1.6 ± 0.3

Theoretical Computations

The relative stability of the different hydrogen-bonded complexes can be tested by another method. Theoretical computations should enable us to compute the energy of complexes with different geometries. Many such computations have been made by various methods, from Hückel approximation to more sophisticated *ab initio* calculations (Clementi et al., 1970). Several reviews of these methods exist (Bratoz, 1967; Kollman and Allen, 1972; Joesten and Schaad, 1974). We have used the CNDO/2 method (complete neglect of differential overlap) described by Pople and Segal (1966), a good compromise between too crude methods (that lead to fast computations) and too sophisticated methods that require very long computation time. Since CNDO/2 method include all-valence electrons, we can find evidence for bonding between lone pairs of nitrogen or oxygen atoms and hydrogen atoms. The geometry used for 9-methyladenine was determined by X-ray diffraction by Stewart and Jensen (1964). For phenol, we have used the structure obtained by neutron diffraction techniques for tyrosine (Frey et al., 1973) and adapted this geometry to phenol by symmetrization of the ring. Fig. 4 shows the isopotential map of the complex 9-methyladenine-phenol of type 1. For each studied geometry, we have computed the total energy of the supersystem and subtracted the energy of isolated compounds. Each point of the map corresponds to the position of an oxygen atom of phenol with the O-H directed to the N_1 atom of adenine. For this supersystem, defects of convergence of self-consistent field procedure occur currently but vanish if we use for the i^{th} iteration the new bond order:

$$[P]_i = \tfrac{1}{2}[[P]_{(i-1)} + [P]_{(i-2)}] \qquad (8)$$

By using this procedure, after five normal iterations using as starting wavefunction the function already computed for another geometry, convergence with precision of 10^{-3} is always obtained after 3–11 iterations. Two energy minima of -8 kcal M^{-1} have been found with complex of type 1 (Fig. 4). For comparable H bond length we determined that the strongest binding $E = -9.69$ kcal M^{-1} (Table III) occurs for complex formation of type 7. This difference in the formation energy of the two types of complex is explained in terms of repulsion between two hydrogen atoms of the supersystem (steric effect) as shown Fig. 4. The total charge density of different atoms for isolated or associated molecules is given in Table III. It should be noted that the binding of adenine and phenol leads to a small transfer of charge (about 0.05–0.06 electron) from adenine to phenol. These computations show that the nonlinear NH....O hydrogen bond distance [R(H....O) = 2.4 Å] is greater than generally found for a linear hydrogen bond (2.0 Å, Joesten and Schaad, 1974) but shorter than Van der Waals contact distance (2.6 Å, Hamilton and Ibers, 1968). The linear N....H-O[R(N....H) = 1.52–1.57 Å] is found shorter than usually reported (1.9 Å, Joesten and Schaad, 1974) but this results from a general failure of CNDO/2 method (Pople and Segal, 1966; Abdulnur and Flury, 1975). The energy minimum found for complex of type 3 is $E = -6.46$ kcal $\cdot M^{-1}$. Fig. 5 shows steric effect obtained by rotation of $N_9 - CH_3$ methyl group of 9-methyladenine. All results are given in Table III. The energy of for-

TABLE III

LENGTHS OF HYDROGEN BONDS, ENERGY OF FORMATION, ELECTRON TRANSFER, AND ELECTRONIC CHARGES ON ATOMS COMPUTED BY CNDO/2 METHOD FOR THE THREE TYPES OF COMPLEX BETWEEN 9-METHYLADENINE (9 MA) AND *p*-CRESOL

Complex	Type 1	Type 7	Type 3	Isolated molecules
R(O....H)	2.395	2.2180		
R(N....H)	1.519	1.5413	1.600	
ΔE, *kcal/mol*	−7.84	−9.69	−6.46	
Total electronic charge				
computed, 9 MA	55.9388	55.9468	55.9539	55.9980
p-Cresol	36.0564	36.0481	36.0399	35.9993
Electron transfer, 9 MA				
p-Cresol	0.059	0.051	0.043	
Electronic charges				
9 MA				
N_1	5.2855	5.2763	5.2777	5.2796
C_2	3.7754	3.7792	3.7749	3.7811
N_3	5.2464	5.2496	5.2577	5.2511
N_7	5.1991	5.2084	5.2009	5.2024
C_8	3.8381	3.8264	3.8393	3.8409
H_2	1.0272	1.0327	1.0317	1.0358
H_8	1.0063	1.0032	1.0063	1.0094
N_{10}	5.2357	5.2373	5.2305	5.2321
H_{11}	0.8805	0.8724	0.8835	0.8866
H_{12}	0.8712	0.8820	0.8814	0.8842
p-Cresol 0	6.3389	6.3315	6.3194	6.2655
H(OH)	0.7922	0.7985	0.8047	0.8523

mation of complex between cresol and m_2^6A were obtained by the following procedure: We used the isopotential map obtained for the supersystem 9-methyladenine-phenol and took into account the repulsive effect of the methyl group on oxygen and hydrogen atoms of phenol. For a complex of type N_3, we have determined an effect of repulsion of the $N_9 - CH_3$ group of adenine on an oxygen atom of phenol for a CO distance less than 3.0 Å, in agreement with the sum of Van der Waals radii reported by Bondi (1964). For the hydrogen atom we used a Van der Waals radius of 1.2 Å (Bondi, 1964). We have computed two energy minima for the complex between m_2^6A and phenol; $E = -5$ kcal $\cdot M^{-1}$ for type 1′ and $E = -2$ kcal $\cdot M^{-1}$ for type 7′.

DISCUSSION

In chloroform solutions adenine derivatives and *p*-cresol are un-ionized, as shown by comparison of their absorption spectra in chloroform and water as well as their PMR spectra. The very similar PMR spectra of the aromatic protons and absorption spectra of the three adenine derivatives indicate small perturbation of the electronic structure of 9 EA by substitution of amino protons by methyl groups. In agreement with these results, the equilibrium constants found for the three adenine derivatives can be used to

compute the equilibrium constant for association of phenol with each binding site of adenine.

Theoretical computations can help to raise the important uncertainty in the ratio of equilibrium constants for complex formation with two H-bond in 9 EA-*p*-cresol system (Table II). Using the energy of complex formation computed by CNDO/2 method ($E_1 = -8$ kcal · M^{-1}, $E_3 = -6.46$ kcal · M^{-1}, $E_7 = -9.69$ kcal · M^{-1}, $E_1' = -5$ kcal · M^{-1}, and $E_7' = -2$ kcal · M^{-1}), we can assume that $K_7' \ll K_1$, K_7, K_3, K_1' and $K_1' \cong K_3$. With these assumptions we compute $K_1 = 2.0 \pm 0.2$ M^{-1}, $K_3 = 1.2 \pm 0.1$ M^{-1}, $K_7 = 3.2 \pm 0.3$ M^{-1} and the ratio $K_7/K_1 = 1.6 \pm 0.3$. This conclusion agrees with computed energies ($E_7 > E_1 > E_3$). Of course the binding energies computed here

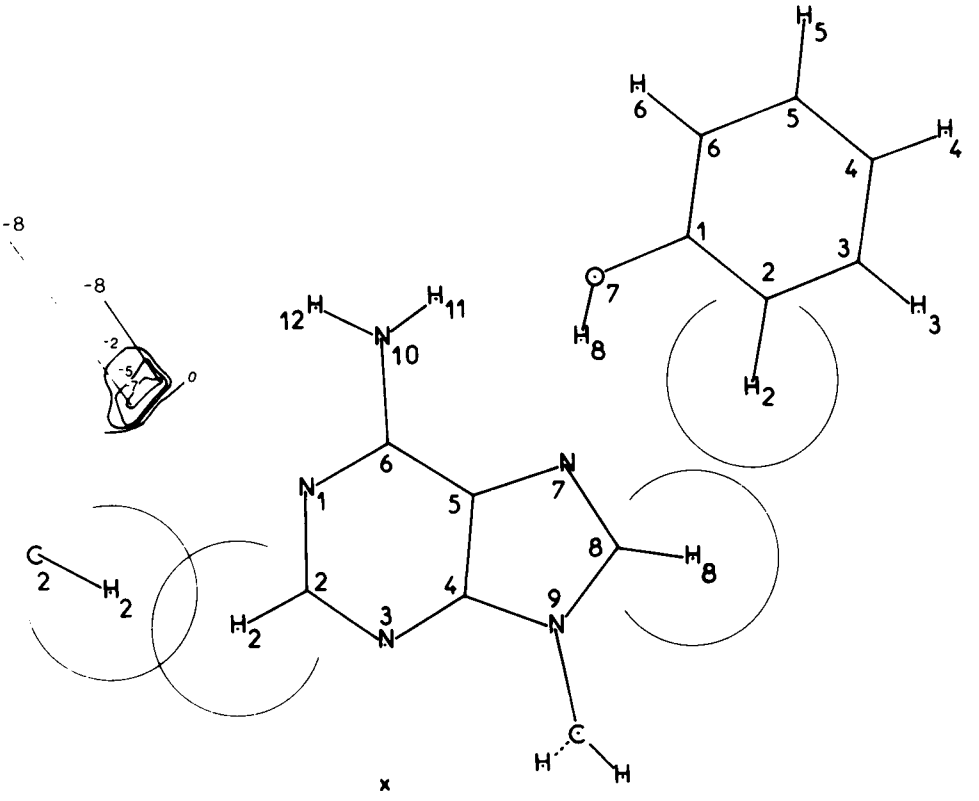

FIGURE 4 Isopotential map for phenol-9-methyladenine complex of type 1. All energies are computed with the O—H bond directed to the N_1 atom, as shown for complex of type 7 where O,H is directed to N_7. The lines of the map refer to the position of oxygen atom of phenol. The circles show Van der Waals' radii for proton H_2 and H_8 of 9-methyladenine and for proton H_2 of phenol. The figure shows how repulsion between H atoms destabilizes the energy of formation of complex type 1 (intersection of circles for the geometry corresponding to the most stable complex, $\Delta E = -5$ kcal · M^{-1}) and does not interact for complex of type 7 ($\Delta E = -9.7$ kcal · M^{-1}). × indicates the position of hydroxylic proton of phenol for the most stable complex of type 3 ($\Delta E = -6.46$ kcal · M^{-1}).

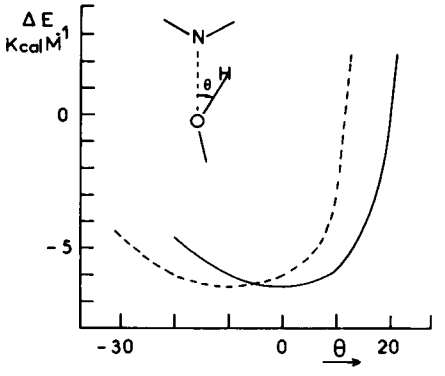

FIGURE 5

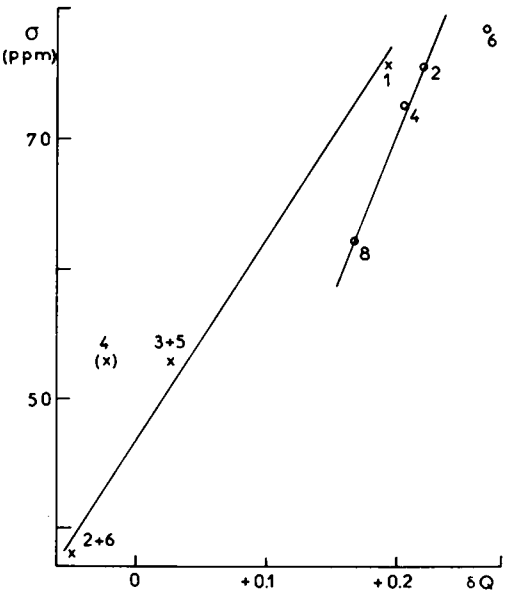

FIGURE 6

FIGURE 5 Variation of energy of complex (type 3) for an in-plane rotation of phenol computed by CNDO/2 method. Solid line is obtained with position of H atoms (for N_9 methyl group of adenine) as shown on Fig. 4. The dissymmetry of the curve shows the repulsion effect of methyl group. A rotation of hydrogen atoms of N_9 $-CH_3$ increases this repulsion and the minimum is obtained for $\theta = -10°$ (broken line).

FIGURE 6 Chemical shifts of ^{13}C atoms of 9 EA (right curve) and *p*-cresol (left curve) versus the net charge of carbon atoms of 9-methyladenine and phenol computed by CNDO/2. Parentheses for C_4 indicate that substitution is different between *p*-cresol (CH_3) and phenol (H).

can only provide information on the preferred order of association for the different binding sites on adenine. They cannot be used to compute equilibrium constants (we have taken into account the enthalpy of association but not the entropy).

It is now well known that a linear empirical relationship occurs between the ^{13}C chemical shift and the total electronic charge density of carbon atoms (for bases of nucleic acids see Jones et al., 1970). Fig. 6 shows such a relation for 9-methyladenine where the electronic densities are given by CNDO/2 computations. By using this relation and the variation of electronic density on C_2 and C_8 atoms (Table III) when 9-methyladenine is complexed with phenol, we are able to predict change in ^{13}C chemical shifts. From the relative values of K_1, K_3, and K_7 we computed $\Delta\delta C_2 = +1.10$ ppm and $\Delta\delta C_8 = +2.39$ ppm for 100% complex formation. These results show that chemical shifts of ^{13}C atoms are very small and cannot be used to determine quantitative parameters in agreement with our negative results when we attempted to measure changes in ^{13}C of chemical shifts when we mixed 9 EA and *p*-cresol in $CDCl_3$ (unpublished results). It should be noted that the order of complexation obtained by PMR

method in $CDCl_3$ is the same as that obtained by theoretical computations. This means that competition between solute and solvent molecules for H bond formation does not lead to a sufficiently strong perturbation to reverse the preferential order of association, as was already pointed out[1] by comparison of equilibrium constants determined in a nonpolar solvent (cyclohexane) and $CHCl_3$ for 9 EA-butyric acid and 1-cyclohexyluracil-butyric acid systems.

The comparison of the sum of the association constants in chloroform (6.3 M^{-1}) and in cyclohexane (1,000 M^{-1}) provides evidence for the strong competition of a slightly polar solvent such as chloroform as compared to a nonpolar solvent. As such, a strong competition was also shown in other systems such as nucleic acid bases complexes with side chains of glutamic or aspartic acid.[1] Relative rather than absolute values of association constants, however, have to be considered when one wants to compare the binding of different amino acid side chains to nucleic acid bases.

Carboxylic acids in their un-ionized forms bind more strongly to adenine than *p*-cresol.[2] On the other hand, *p*-cresol binds more strongly to adenine than amino side chains containing alcohol, thiol, sulphydryl, or amino groups.[1]

The results reported above show that tyrosine side chains of proteins might participate in the binding of proteins to nucleic acids by forming hydrogen bonds with bases. In double-stranded nucleic acids one should expect that such hydrogen bonding will be stronger in the large groove (N_7) than in the small groove (N_3). In the case of single stranded-nucleic acids, hydrogen bonding to the N(7) site of adenine should also be favored as compared to N(1) and N(3) sites.

We wish to thank Dr. C. Hélène for helpful discussions and Dr. J. M. Leclercq for a CNDO/2 program.

Received for publication 7 July 1976 and in revised form 30 November 1976.

REFERENCES

ABDULNUR, S. F., and R. L. FLURY. 1975. CNDO/2 calculations of the interactions of formamide and imidazole: another failure of CNDO/2. *Chem. Phys. Lett.* **36**:586.

BLOW, D. M., and T. A. STEITZ. 1970. X-ray diffraction studies of enzymes. *Annu. Rev. Biochem.* **39**:63.

BONDI, A. 1964. Van der Waals volumes and radii. *J. Phys. Chem.* **68**:441.

BRATOZ, S. 1967. Electronic theories of hydrogen bonding. *Adv. Quantum Chem.* **3**:209.

BRUN, F., J. J. TOULMÉ, and C. HELÈNE. 1975. Interactions of aromatic residues of proteins with nucleic acids. Fluorescence studies of the binding of oligopeptides containing tryptophan and tyrosine residues to polynucleotides. *Biochemistry.* **14**:558.

CHAN, S. I., M. P. SCHWEIZER, P. O. P. TS'O, and G. K. Helmkamp. 1964. Interaction and association of bases of nucleosides in aqueous solutions. III. A nuclear magnetic resonance study of the self-association of purine and 6-methylpurine. *J. Am. Chem. Soc.* **86**:4182.

CLEMENTI, E., J. MEHL, and W. VON NIESSEN. 1970. Study of the electronic structure of molecules. XIV. Hydrogen bridges in the guanine-cytosine pair and the dimeric form of formic acid. *J. Chem. Phys.* **54**: 508.

DAVIS, J. E., and K. S. PITZER. 1960. Nuclear magnetic resonance studies of hydrogen bonding. I. Carboxylic acids. *J. Phys. Chem.* **64**:886.

DIMICOLI, J. L., and C. HÉLÈNE. 1974. Interactions of aromatics residues of proteins with nucleic acid I and II. *Biochemistry.* **13**:714 and 724.

ENGLAND, W., and M. S. GORDON. 1971. Localized charge distribution. I. General theory, energy (partitioning and the internal rotation barrier in ethane. *J. Am. Chem. Soc.* **93**:4649.

ENGEL, J. D., and P. H. VON HIPPEL. 1974. Effect of methylation on the stability of nucleic acid conformations: studies at the monomer level. *Biochemistry.* **13**:4143.

FREY, M. N., T. F. KOETZLE, M. S. LEHMANN, and W. C. HAMILTON. 1973. Precision neutron diffraction structure determination of protein and nucleic acid components. X. A comparison between the crystal and molecular structures of L-tyrosine and L-tyrosine hydrochloride. *J.Chem. Phys.* **58**:2547.

GABBAY, E. J., P. D. ADAWADKAR, and W. D. WILSON. 1976*a*. Stereospecific binding of diastereometric peptides to salmon sperm DNA. *Biochemistry.* **15**:146.

GABBAY, E. J., P. D. ADAWADKAR, L. KAPICAK, S. PEARCE, and W. D. WILSON. 1976*a*. The interaction specificity of peptides with DNA. Evidence for peptide β-sheet-DNA binding. *Biochemistry.* **15**:152.

HAMILTON, W. C., and J. A. IBERS. 1968. Hydrogen bonding in solids. W. A. Benjamin, Menlo Park, Calif.

JOESTEN, M. D., and L. J. SCHAAD. 1974. Hydrogen Bonding. Marcel Dekker Inc., New York. p. 28 and references.

JONES, A. J., D. M. GRANT, M. W. WINKLEY, and K. R. ROBINS. 1970. Carbon-13 magnetic resonance. XVII. Pyrimidine and purine nucleosides. *J. Am. Chem. Soc.* **92**:4079.

KOLLMAN, P. A., and L. C. ALLEN. 1972. The theory of the hydrogen bond. *Chem. Rev.* **72**:283.

LECLERCQ, J. M. 1972. Contribution à l'étude théorique des transitions vibroniques des molécules polyatomiques. Ph.D. Thesis, University of Paris. Available CNRS Paris.

OLINS, O. E., A. OLINS, and P. H. VON HIPPEL. 1967. Model nucleoprotein complexes: studies on the interaction of cationic homopeptides with DNA. *J. Mol. Biol.* **24**:157.

POPLE, J. A., and G. A. SEGAL. 1966. Approximate self-consistent molecular orbital theory. III. *J. Chem. Phys.* **44**:3289.

RICHARDS, F. M., H. W. WYCKOFF, and N. ALLEWELL. 1970. The origin of specificity in binding: a detailed example in a protein-nucleic acid interaction. *In* The Neurosciences. Second Study Program. F. O. Schmitt, editor. The Rockefeller University Press, New York. 901.

SELLINI, H., J. C. MAURIZOT, J. L. DIMICOLI, and C.HÉLÈNE. 1973. Hydrogen bonding of amino acid side chains to nucleic acid bases. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **30**:219.

SHAPIRO, J. T., M. LENG, G. FELSENFELD. 1969. Deoxyribonucleic acid-polylysine complexes. Structure and nucleotide specificity. *Biochemistry.* **8**:3219.

STEWART, R. F., and L. H. JENSEN. 1964. Crystal structure of 9-methyladenine. *J. Chem. Phys.* **40**:2071.

TOULMÉ, J. J., M. CHARLIER, and C. HELENE. 1974. Specific recognition of single-stranded regions in ultraviolet. Irradiated and heat-denatured DNA by tryptophan-containing peptides. *Proc. Natl. Acad. Sci. U.S.A.* **71**:3185.