Combined Mass Spectrometric and ab Initio Study of the Point Contacts between 9-Methyladenine and the Amide Group

I. Galetich,[‡] S. G. Stepanian,[†,‡] V. Shelkovsky,[‡] M. Kosevich,[‡] Yu. P. Blagoi,[‡] and L. Adamowicz*[,†]

Department of Chemistry, University of Arizona, Tucson, Arizona 85721, and Institute for Low-Temperature Physics and Engineering, National Academy of Sciences of Ukraine, 47 Lenin Avenue, Kharkov 310164, Ukraine

Received: February 29, 2000; In Final Form: July 26, 2000

We applied the temperature-dependent field ionization mass spectrometry method to determine the interaction energy between 9-methyladenine and acrylamide. Acrylamide mimics the side chain amide group of the natural amino acids asparagine and glutamine. The experimental enthalpy of the dimer formation derived from van't Hoff's plot is -52.0 ± 5.0 kJ mol^{-1}. This value is close to the interaction energy between acrylamide and 1-methylcytosine (-57.0 kJ mol^{-1}) and much higher than the interaction energy between acrylamide and 1-methyluracil (-40.6 kJ mol^{-1}). The DFT/B3LYP/6-31++G** and MP2/6-31++G** quantum-chemical methods were used to determine the structures and the interaction energies of the 9-methyladenine−acrylamide dimers. In the calculations, two energetically almost equivalent equilibrium structures were found. They are stabilized by intermolecular H-bonds formed between the acrylamide amide group and the Watson−Crick and Hoogsteen sites of 9-methyladenine, respectively. The calculated interaction energies of the dimers are -41.4 and -39.2 kJ mol^{-1}, respectively, at the DFT level of theory and -40.9 and -38.8 kJ mol^{-1}, respectively, at the MP2 level. We also performed calculations for 9-methyladenine−glutamine dimers at the DFT/B3LYP/6-31++G** level of theory. The interaction energy differences between the corresponding 9-methyladenine−acrylamide and 9-methyladenine−glutamine dimers of less than 1 kJ mol^{-1} were obtained in the calculations. This result demonstrates that acrylamide is a good model for the amino acid amide group. The experimantal and the calculated data indicate that the amino acid amide group can interact with the adenine residue in the single-stranded DNA, as well as in the major groove of double-stranded DNA. The influence of the interaction with the amide group on the stability of the adenine−uracil base pair is discussed.

1. Introduction

In this work we continue the investigation of the point contacts between nucleic acid bases and amino acid side chain models. The main aim of the work is to establish a detailed molecular mechanism of the specific recognition of the nucleic acid bases by the amino acid side chains. Interactions between nucleic acids and proteins are among the most important biological processes, but despite their importance detailed information about the molecular mechanisms of the specific recognition patterns is still very limited. This lack of information is mainly due to the large size of the systems and the complexity of the interaction effects. To determine how specific the interactions between the protein polar groups and nucleic acid sites are, we have investigated simpler model systems, which contain some characteristic structural features of the protein−DNA systems. The models are intermolecular complexes formed by the nucleic acid bases and systems with amino acid polar side chains. The approach allows determination of the local thermodynamic and structural parameters of the interacting sites. This information will be used in considerations of more complex models and eventually in considerations of real biological systems. In the studies, we employ the temperature-dependent field ionization (TD FI) mass spectrometry to measure the enthalpy of formation of the H−bonded complexes of the nucleic acid bases and the systems modeling the amino acid side chains. The experimental data are correlated and assigned to specific structures of the complexes based on the results of quantum mechanical calculations.

Recently we studied the complexes between acrylamide (Acr) and the pyrimidine nucleic acid bases − 1-methyluracil (m^1 Ura)[1] and 1-methylcytosine (m^1 Cyt).[2] We demonstrated that acrylamide is a suitable model for the amide group of the amino acids asparagine and glutamine. We also determined the interaction energies between acrylamide and the pyrimidine bases, as well as the structures of the complexes. As we showed, both cytosine and uracil can form two H−bonds with acrylamide and the enthalpy of formation of the Acr−m^1 Cyt dimer (58.7 kJ mol^{-1}) is much higher than for the Acr−m^1 Ura dimer (40.6 kJ mol^{-1}). We concluded that this difference should allow the peptide amide group to distinguish between the two pyrimidine bases.

In the present work we undertook an investigation of the complexes between the purine base adenine (Ade) and acrylamide, which models the side chains of asparagine and glutamine. Unfortunately it is not possible to perform experimental gas-phase studies of the amino acid−base dimers since both asparagine and glutamine are thermally unstable. The low thermal stability precludes TD FI mass-spectrometry study

* Corresponding author. E-mail: ludwik@u.arizona.edu. Fax: (520) 621-8407.
[†] University of Arizona.
[‡] National Academy of Sciences of Ukraine.

10.1021/jp000755s CCC: $19.00
Published on Web 09/13/2000

because it is not possible to obtaine a concentration of the amino acids in the gas phase which is sufficient to register their mass-spectra. However it is possible to calculate interaction energies for the 9-methyladenine−amino acid (glutamine or asparagine) system. The comparison of the structures and interaction energies of the 9-methyladenine−amino acid and 9-methyladenine−acrylamide dimers may provide a direct evidence whether acrylamide is a good model of the glutamine and asparagine side chains. The natural amino acids asparagine and glutamine are among the six amino acids which are present in the peptide recognition sites.[3] Their neutral amide groups can form at least two H−bonds with all monomeric nucleic acid bases, as well as with the base pairs. The formation of two H−bonds is an important property since formation of a single H−bond between the amino acid side chain and the base would be insufficiently specific for recognition, but formation of two H-bonds should suffice to recognize all the bases.[4] The direct interaction between the adenine residue and the asparagine and glutamine amide groups was observed experimentally. For example, the specific interaction between these amino acids and adenine was observed in the process of DNA recognition by yeast transcription activators GCN4.[5] Another example concerns the crystal structure of ribonuclease S (bovine pancreatic ribonuclease) and dinucleoside monophosphate $C_{2'p5'}A$ studied by Wodak et al.[6] They found that the specific H−bonds are formed between the NH_2 group of adenine and the C═O moiety of the amide group of asparagine and glutamine. Yet another example of the specific interaction of asparagine−adenine is the complex between DNA and the zinc-finger recognition domain of the Tramtrack protein, which is the transcriptional regulator of the *fushi-tarazu* gene. In this complex, a specific bond is formed between asparagine and adenine in one of the zinc fingers which is involved in the formation of the recognition structure.[7] To understand these types of processes, it is important to obtain direct information on the thermodynamic parameters of the point contacts between the H−bonding groups in these systems. The comparison of the interaction energies between the amino acid amide group and different nucleic acid bases, as well as analysis of the structure of the complexes formed by these systems, can provide information at the molecular level about the mechanisms governing the specific recognition patterns. The aim of this work is to contribute to the understanding of these mechanisms.

2. Experimental Method

The experimental measurements of the mass spectra were performed using a magnetic sector mass spectrometer MI-1201, ('Electron Works', Sumy, Ukraine) equipped with a laboratory built FI ion source.[8] The methodology of temperature dependent field ionization (TD FI) mass spectrometry, and the procedure used for measurements of enthalpies of interaction, has been described in detail elsewhere.[8−10] Here we only briefly outline the main points of the experimental procedure. Monomolecular fluxes of the compounds under study (obtained by heating of the individual crystalline samples in separate glass ampules) were mixed in the gas-phase reaction zone in the cavity of a cylindrical counter-electrode. The FI emitter (an electrolytically etched tungsten needle, with the radius of the tip of ≈4000 Å) was located in the center of the zone and kept at a potential of +5 kV. The density of the gas-phase mixture near the FI emitter was adequate to facilitate a sufficient rate for the association reaction between the monomers. Experimental conditions were adjusted to ensure that the thermodynamic equilibrium is reached by the system at the fixed temperature of the experiment.[9,11] Ionization of the components of the reaction mixture occurred near the FI emitter surface by a tunneling mechanism. The ionization process did not cause the weakly bound noncovalent complexes to dissociate.

The temperature change in the reactive zone was achieved by resistive heating of the FI emitter. The temperature of the emitter was measured using a copper−constantan thermocouple firmly attached at the point of welding of the tungsten needle to the emitter metal holder. The gradient of the temperature between the welding point and the tip of the needle with the lengths of 2−3 mm was assumed to be negligibly small.

It was shown earlier,[9] that the abundance of the ion current is proportional to the concentrations of the components (the monomers and the complexes) in the reactive system. This dependency allows one to directly determine the relative association constants, K_{assoc}, on the basis of the mass spectral data. Variation of the temperature of the reactive zone (by resistive heating of the FI emitter) permits measurement of the temperature dependencies of K_{assoc}, construction of the van't-Hoff plots and calculation of the enthalpies of association, ΔH, according to the following equation:

$$\Delta H = -R[\delta(\ln K_{assoc})/\delta(1/T)] \quad (1)$$

The data of four independent experiments were averaged, leading to the estimated precision in the enthalpy determination of ±5.0 kJ mol^{-1}. The samples of 9-methyladenine were synthesized at the Kharkov State University (Ukraine). Acrylamide was purchased from Reanal, Hungary.

3. Computational Details

The interaction energies in the complexes of acrylamide and 9-methyladenine have been calculated using the DFT (Density Functional Theory) and MP2 (Møller−Plesset second-order perturbation theory) methods.[12,13] The DFT method was also employed in the calculations of the IR frequencies using the harmonic approximation. The frequencies were used to determine the ZPVE (zero-point vibrational energy) corrections. The DFT calculations were carried out with the three-parameter density functional, usually abbreviated as B3LYP, which includes Becke's gradient exchange correction,[14] the Lee, Yang, Parr correlation functional[15] and the Vosko, Wilk, and Nusair correlation functional.[16]

As we demonstrated earlier,[1,2] the DFT method is capable of producing the interaction energies between acrylamide and pyrimidine bases which are very similar to the ones obtained at the MP2 level of theory. The geometries of the Acr−m^9 Ade dimers were determined at the DFT/B3LYP/6-31++G** level of theory and used to calculate the DFT harmonic frequencies. The DFT/B3LYP/6-31++G** geometries were also used to calculate the MP2/6-31++G** energies of the dimers. The similar calculations were performed for the glutamine **(**Glu**)**−m^9 Ade system. Two structures were found for each of the Acr−m^9 Ade and Glu−m^9 Ade complexes at the DFT/B3LYP/6-31++G** level. They are denoted as dimer A and dimer B.

The interaction energies of the dimers at both the DFT and MP2 levels were calculated with the account of the basis set superposition error (BSSE) by the counterpoise method of Boys and Bernardi.[17] The method involves a calculation of the total energy of the dimer and two calculations of the monomer energies performed with the basis set of the dimer. The calculations of monomers are done using their respective equilibrium geometries. The BSSE- -corrected interaction energies were calculated for each dimer as the difference of the dimer energy and the monomer energies calculated with the

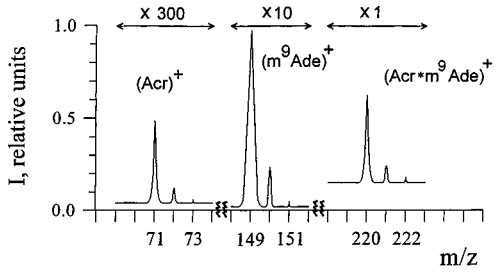

Figure 1. Field ionization mass spectra of the reaction mixture of acrylamide with m^9 Ade at the emitter potential $U = +5$ kV and the emitter temperature $T = 298$ K.

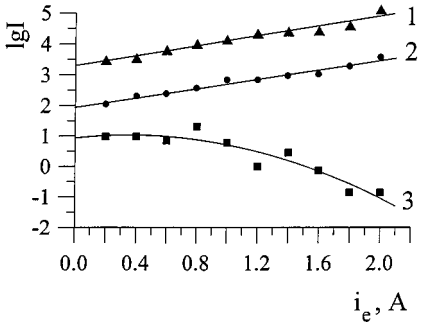

Figure 2. Dependencies of the ion currents (I) vs currentie of the emitter resistive heating (I_e) for acrylamide (1), m^9 Ade (2) and their dimer (3) at the emitter potential $U = +5$ kV.

dimer basis set. The BSSE corrections for the monomers were calculated as the difference of the monomer energies calculated with the basis sets of the monomer and the dimer. All calculations were done on IBM RS6000 and SGI ORIGIN 2000 computers using the Gaussian94 quantum-chemical program package.[18]

4. Results and Discussion

Enthalpies of Formation. The field-ionization mass spectrum of the mixture of Acr and m^9 Ade is shown in Figure 1. It contains peaks corresponding to the molecular ions of the monomers, peaks corresponding to the Acr and m^9 Ade homodimers and peaks due to the Acr−m^9 Ade heterodimers. For each of the ions the spectrum also contains a peak located at one mass unit higher than the peak of the ion which is a superposition of the ^{13}C- isotope-containing ion and the protonated ion. The mass spectrum of the Acr−m^9 Ade mixture was measured at different temperatures and the temperature dependencies of the ion currents of the monomers and of the heterodimer are presented in Figure 2. The intensities of the peaks in the FI mass spectra are related to the concentrations of the molecules in the reactive zone through the following relation:

$$I_x(F) = \sigma_x(F) n_x(F) \tag{2}$$

where $I_x(F)$ is intensity of the peak due to ion x at the field strength F; $\sigma_x(F)$ is the ionization coefficient; and $n_x(F)$ is the concentration of the neutral molecules. The concentration of the neutral molecules (expressed in terms of the number of molecules per cm^3) can be evaluated using the barometric equation:

$$n(F) = n_0(T) \exp[(\mu F + (\alpha/2)F^2)/kT] \tag{3}$$

where the symbols used in the equation indicate the following quantities: μ = the dipole moment, α = the polarizability, and F = the emitter potential. In the case of the nucleic acid bases and their methylated derivatives, the density, $n(F)$, calculated

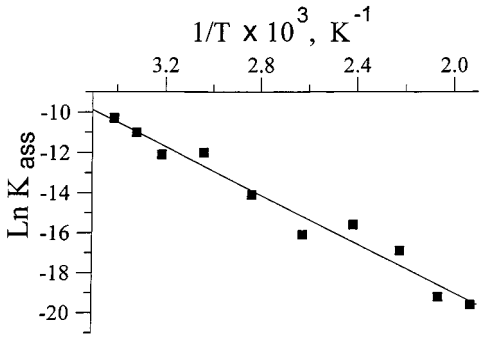

Figure 3. Temperature dependencies of K_{assoc} for the Acr−m^9 Ade dimer at the emitter potential $U = +5$ kV.

for the temperature in the range of 300−400 K is $\approx 10^{19} - 10^{20}$ molecules/cm^3. (At $F = 0$ the density $n_0(T)$ was determined using the vapor pressure and the heat of sublimation.)

The relative constant of association, K_{assoc}, for the Acr−m^9 Ade dimer is

$$K_{assoc} = n_{Acr-m^9Ade}/(n_{Acr} \cdot n_{m^9Ade}) = I_{Acr-m^9Ade}/(I_{Acr} \cdot I_{m^9Ade}) \cdot \sigma^*(F) \tag{4}$$

where $\sigma^*(F)$ denotes the effective ionization coefficient. The real constant of the chemical equilibrium, K'_{assoc}, and the relative association constant, K_{assoc}, are connected via the ratio

$$K_{assoc} = K'_{assoc} \sigma^*(F) \tag{5}$$

It was shown,[11,19] that, in the case of close values of the ionization potentials of the studied molecules at constant experimental conditions, the temperature dependence of $\sigma^*(F)$ could be neglected. The association constant, K_{assoc}, was calculated at each point of the $[\log(I) - f(i_e)]$ dependency. Temperature dependencies of K_{assoc}, calculated as

$$\ln K_{assoc} - (\Delta H/(RT)) + \text{const} \tag{6}$$

allowed to construct the van't Hoff plot (Figure 3) and evaluate the enthalpy of formation of the Acr−m^9 Ade hydrogen-bonded complex. The enthalpy value, ΔH, derived from this analysis is -52.0 ± 5.0 kJ mol^{-1} at the emitter potential of +5 kV.

Since the enthalpy of the Acr−m^9 Ade dimer formation was obtained in the presence of a nongomogeneous electric field created in the reaction zone by the field ionization emitter, one needs to consider that the electric field may have influenced the enthalpy value. A dependency of the enthalpy on the field strength was observed in our previous studies of the cytosine homodimers[20,21] and the 1-methylcytosine−acrylamide heterodimers.[2] However, in the study of the 1-methyluracil−acrylamide dimers no field dependency was observed[1] and this effect was attributed to small differences between the dipole moments of the monomers and the dimers. To elucidate this point for the Acr−m^9 Ade complex, we performed measurements at additional two different values of the emitter potential (+4 kV, +3 kV) and at different field strengths. The experiments showed no field dependency of the enthalpy of the Acr−m^9 Ade dimer formation. Therefore, the enthalpy value mentioned above should provide an accurate estimation for the field-free enthalpy of the dimerization.

Structures of the Dimers. Two almost planar equilibrium structures of the Acr−m^9 Ade dimers were found in the DFT/B3LYP/6-31++G** calculations. These structures (dimer A and dimer B) are depicted in Figure 4. Two equilibrium structures

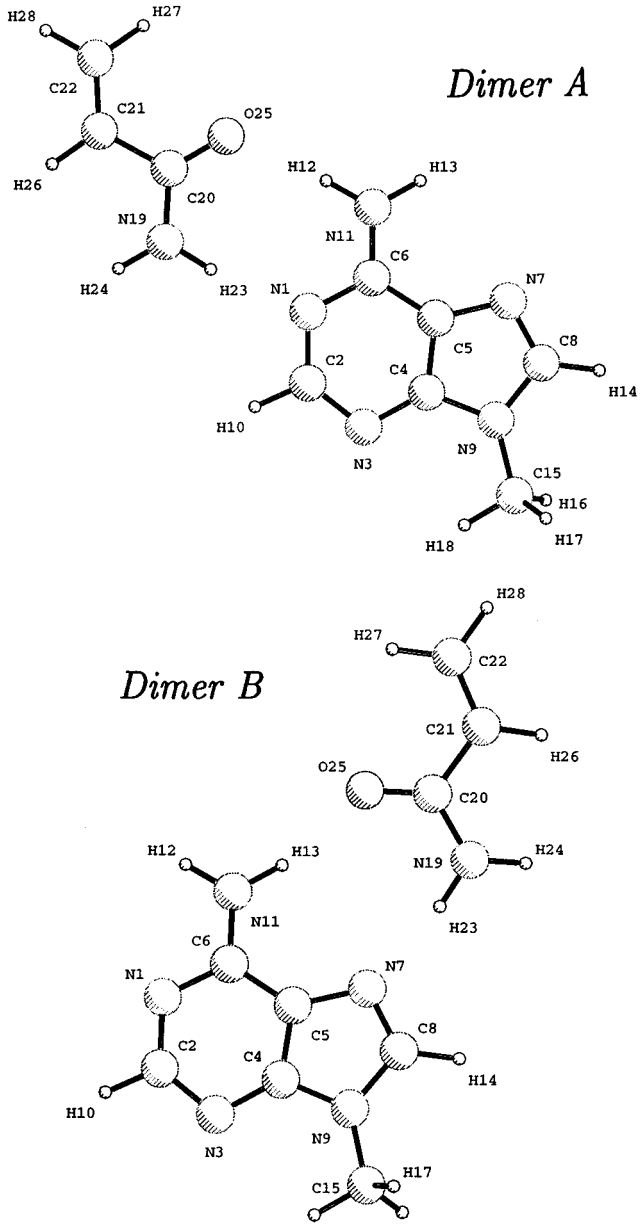

Figure 4. Equilibrium geometries of the Acr−m^9 Ade dimer A (top) and Acr−m^9 Ade dimer B (bottom) calculated at the DFT/6-31++G** level of theory. (The geometries of the dimers are available from the corresponding author.)

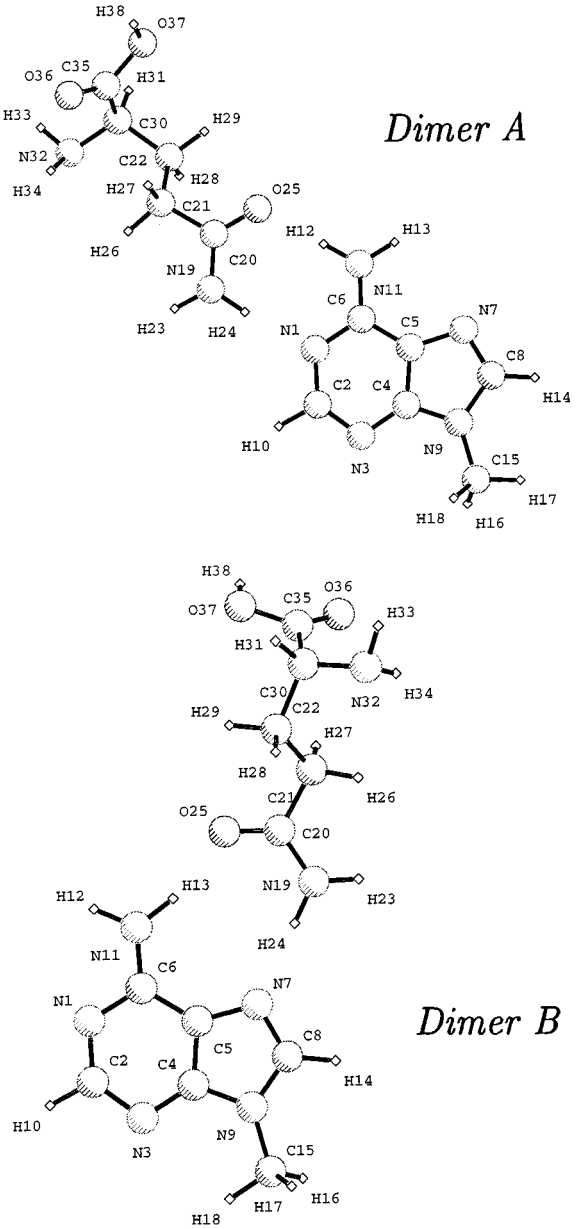

Figure 5. Equilibrium geometries of the Glu−m^9 Ade dimer A (top) and Glu−m^9 Ade dimer B (bottom) calculated at the DFT/6-31++G** level of theory. (The geometries of the dimers are available from the corresponding author.)

(dimer A and dimer B) were also found for the Glu−m^9 Ade system and they are depicted in Figure 5. In these dimers acrylamide and glutamine interact with the Watson−Crick (in dimers A) and Hoogsteen (in dimers B) sites of m^9 Ade via the N−H···O and N−H···N H−bonds. The calculations predicted rather short H−bonds in all the dimers. The calculated bond lengths for the 9-methyladenine complexes with acrylamide and glutamide are: 1.977 (Acr) and 1.982 Å (Glu) for the N−H···N H bonds (dimers A); 1.863 (Acr) and 1.867 Å (Glu) for the N−H···O H bonds (dimers A); 1.980 (Acr) and 1.985 Å (Glu) for the N−H···N H bonds (dimers B); 1.876 (Acr) and 1.882 Å (Glu) for the N−H···O H bonds (dimers B). The average difference between the H−bond lengths in the acrylamide and glutamine dimers with 9-methyladenine is only 0.004−0.006 Å.

The total energies, the ZPVE's, the BSSE corrections and the interaction energies of the Acr−m^9 Ade dimers calculated at the DFT/B3LYP/6-31++G** and MP2/6-31++G**//DFT/B3LYP/6-31++G** levels of theory are presented in Tables 1 and 2, respectively. Analysis of the data allows one to draw the following conclusions:

•Both methods predict very close interaction energies of the Acr−m^9 Ade dimer A and dimer B. Dimer A is slightly more stable by 1.6 kJ mol^{-1} at the DFT level and by 2.1 kJ mol^{-1} at the MP2 level than dimer B. It indicates that both dimers can be formed in gas phase and that the peak observed in the mass spectrum is probably a superposition of two dimer peaks. Since the dimers have identical masses they are indistinguishable in our experiments.

•The total energies, the BSSE corrections and the interaction energies of the Glu−m^9 Ade dimers calculated at the DFT/B3LYP/6-31++G** and MP2/6-31++G**//DFT/B3LYP/6-31++G** levels of theory are shown in Tables 3 and 4, respectively. Comparison of these data with the energies obtained for the Acr−m^9 Ade dimers demonstrates that the interaction energies of m^9 Ade with Glu and Acr are almost

TABLE 1: Total Energies (in hartrees), Interaction Energies (IE, in kJ mol^{-1}) and Zero-Point Vibration Energies (ZPVE, in hartrees) Calculated at the DFT/B3LYP/6-31++G Level of Theory for Dimer A and Dimer B of Acr−m^9 Ade and for the m^1 Ura−m^9 Ade Dimer[a]**

	Acr−m^{9}Ade		
	dimer A	dimer B	m^{1}Ura−m^{9}Ade
energy	−754.006573	−754.005717	−960.847441
IE	−51.0	−48.8	−54.3
ZPVE[b]	0.210154	0.210129	0.243302
ZPVE correction	0.002766	0.002741	0.001986
BSSE correction[c]			
Acr or m^{1}Ura	0.000499	0.000542	0.000666
m^{9}Ade	0.000388	0.000377	0.000510
total	0.000887	0.000919	0.001176
IE (BSSE corrected)	−48.7	−46.4	−51.2
IE (BSSE and ZPVE corrected)	−41.4	−39.2	−46.0

[a] All energies were obtained at the DFT/B3LYP/6-31++G** dimer equilibrium geometries. [b] Calculated based on the DFT/B3LYP/6-31++G** frequencies scaled by a factor of 0.95. [c] Energy difference calculated for the equilibrium monomer geometries in the monomer and dimer basis sets.

TABLE 2: Total Energies (in hartrees), Interaction Energies (IE, in kJ mol^{-1}) and Zero-Point Vibration Energies (ZPVE, in hartrees) Calculated at the MP2/6-31++G Level of Theory for Dimer A and Dimer B of Acr−m^{9}Ade and for the m^{1}Ura−m^{9}Ade Dimer[a]**

	Acr−m^{9}Ade		
	dimer A	dimer B	m^{1}Ura−m^{9}Ade
energy	−751.822071	−751.821312	−958.104829
IE	−59.7	−57.7	−67.9
ZPVE[b]	0.210154	0.210129	0.243302
ZPVE correction	0.002766	0.002741	0.001986
BSSE correction[c]			
Acr or m^{1}Ura	0.002320	0.002366	0.002951
m^{9}Ade	0.002062	0.002100	0.002745
total	0.004382	0.004466	0.005696
IE (BSSE corrected)	−48.2	−46.0	−53.0
IE (BSSE and ZPVE corrected)	−40.9	−38.8	−47.8

[a] The calculations performed at the dimer equilibrium geometries obtained at the DFT/B3LYP/6-31++G** level. [b] calculated based on the DFT/B3LYP/6-31++G** frequencies scaled by a factor of 0.95. [c] Energy difference calculated for the equilibrium monomer geometries in the monomer and dimer basis sets.

TABLE 3: Calculated at the DFT/B3LYP/6-31++G Level of Theory Energies (au), Interaction Energies (IE, kJ mol^{-1}) and Zero-Point Vibration Energies (ZPVE, au) for the Glu−m^{9}Ade Dimer A and Dimer B, Together with BSSE Corrected Interaction Energies[a]**

	Glu−m^{9}Ade	
	dimer A	dimer B
energy	−1038.492833	−1038.491895
IE	−50.4	−47.9
BSSE correction[a]		
Glu	0.000535	0.000536
m^{9}Ade	0.000406	0.000398
total	0.000941	0.000934
IE (BSSE corrected)	−47.9	−45.5

[a] All the energies were obtained for the dimer geometries fully optimized at the DFT/B3LYP/6-31++G** level. [a] Energy difference calculated for the equilibrium monomer geometries in the monomer and dimer basis sets.

identical. Since the differences are less than 1 kJ mol^{-1}, we can conclude that acrilamide is a suitable model of the amino-acid amide group.

TABLE 4: Calculated at the MP2/6-31++G Level of Theory Energies (au), Interaction Energies (IE, kJ mol^{-1}) and Zero-Point Vibration Energies (ZPVE, au) for the Glu−m^{9}Ade Dimer A and Dimer B, Together with BSSE Corrected Interaction Energies[a]**

	Glu−m^{9}Ade	
	dimer A	dimer B
energy	−1035.536756	−1035.535657
IE	−60.8	−57.9
BSSE correction[b]		
Glu	0.002580	0.002621
m^{9}Ade	0.002203	0.002252
total	0.004783	0.003873
IE (BSSE corrected)	−48.2	−45.1

[a] All the energies were obtained for the dimer geometries fully optimized at the DFT/B3LYP/6-31++G** level. [b] Energy difference calculated for the equilibrium monomer geometries in the monomer and dimer basis sets.

•Acrylamide interacts with the Watson−Crick or Hoogsteen site of adenine. This indicates that adenine can be recognized by the amide group of the amino acid both in the single and double stranded DNA. In the latter case the interaction occurs in the major groove of DNA. It should be noted that pyrimidine bases cannot be recognized in the double stranded DNA. This is an important result since the interaction with the amide group in the major groove lowers the stability of the adenine−uracil base pair.[22]

•The enthalpies of the dimer formation, (ΔH), between acrylamide and m^1 Ura, m^1 Cyt and m^9 Ade, which are −40.6, −57.0 and −52.0 kJ, respectively, form the following relation: $-\Delta H_{\mathbf{A}\text{cr}-\text{m}^1\text{Cyt}} - \Delta H_{\text{Acr}-\text{m}^9\text{Ade}} \gg -\Delta H_{\text{Acr}-\text{m}^1\text{Ura}}$. It indicates that, based on thermodynamics, the amide group can distinguish between the adenine and uracil residues in the single stranded DNA.

The experimental heat of formation of the Acr−m^9 Ade dimer (−52.0 ± 5.0 kJ mol^{-1}) are different from the calculated interaction energies obtained for the most stable dimer A (−41.4 kJ mol^{-1} at the DFT/B3LYP/6-31++G** level and −40.9 kJ mol^{-1} at the MP2/6-31++G**//DFT/B3LYP/6-31++G** level) by ca. 10 kJ mol^{-1}. Analyzing reasons which may cause the difference we should firstly note that the harmonic approximtion used for the frequency calculation leads to the respectively higher overestimation of the frequencies of the stretching vibrations of N−H bonds which form the H-bonds in the Acr−m^9 Ade dimers. For example, in the case of the strong H-bonds in the amino acids the difference between the experimental and calculated at the DFT level frequencies may reach few hundreds revers centimeters.[23,24] This results in the increasing of the calculated zero-point vibration energies of the dimers and consequently in the decreasing of the calculated interaction energies by 3−5 kJ mol^{-1}.

Other two reasons which can influence the accuracy of the obtained interection energies are the truncation of the basis set and underestimation of the correlation energy contributions. The size of the dimers studied precludes direct calculation with larger basis sets or at higher level of theory. In this situation to evaluate the influence we can only refer to the investigations of the simplier systems. The comparison of the interaction energies obtained for the water and ammonia dimers at the MP2/6-31++G** level of theory and at the higher levels (until MP4-(SDTQ)/aug-cc-VTZ and CCSD+T(CCSD)/aug-cc-pvdz)[25] demonstrates that due to the truncation of the basis set and neglecting of the higher correlation energy contributions the real interaction energy of the dimers should be about 10−20% larger than the calculated at the MP2/6-31++G** level.

TABLE 5: Rotational Constants (MHz) and Dipole Moments (Debye) of the Acr−m^{9}Ade Dimer A and Dimer B Determined at the DFT/B3LYP/6-31++G Level of Theory**

	A_c	B_c	C_c	μ
dimer A	1732.4	201.7	180.9	2.45
dimer B	1027.3	253.8	203.8	6.25

It is interesting to compare the calculated and the experimental interaction energies in the Acr−m^9 Ade and Acr−m^1 Ura dimers and those in the m^1 Ura - - m^9 Ade Watson−Crick base pair. To do this, we performed additional calculations of the base pair at the DFT/B3LYP/6-31++G** and MP2/6-31++G**//DFT/B3LYP/6-31++G** levels. The results of the calculations are included in Tables 1 and 2. The experimental interaction energy of the m^1 Ura−m^9 Ade base pair derived from the mass spectrometry experiment is −54.6 kJ mol^{-1}.[26,27] This interaction energy is close to the interaction energy in the Acr−m^9 Ade dimers. It means that the amide group can compete with uracil in pairing with adenine. This may explain the experimentally observed lowering of the transition temperature of poly(rA)·poly(rU) in the presence of acetamide.[28]

Spectral Characterization of the Acr−m^9 Ade Dimers. As was mentioned before, the Acr−m^9 Ade dimers are indistinguishable in the mass-spectromeric experiments since they have identical masses. In this section we provide spectral characteristics which can assist in identifying the dimers using other specroscopic methods.

The rotational constants of dimer A and dimer B are presented in Table 5. As it is seen, the dimers have significantly different rotational constants which may allow to distinguish between them using the microwave spectroscopy. One should also note that dimer B has much higher dipole moment (6.25 D) than dimer A (2.45 D). Therefore, the dimer B bands should be more intense than those of dimer A in the microvave spectrum.

The matrix-isolation IR spectroscopy is another method which may be used to study the Acr−m^9 Ade dimers. Although most of the dimer frequencies are very close to the frequencies of the monomers (the differences are within 10 cm^{-1}) there are some dimer vibrational frequencies which are more distinct from the monomer frequencies and also different for dimer A and dimer B. These frequencies are summarized in Table 6. The most distinct spectral manifestation of the dimers should be observe in the high-frequency region of the IR spectra for the N−H stretching vibrations (3600−3000 cm^{-1}). Both 9-methyladenine and acrylamide have two stretching vibrations of the amino group, i.e., a higher frequency antisymmetric vibration and a lower frequency symmetrical vibration (see Table 6). The H-bonding in the dimers changes the frequencies of these vibrations. The bands of the stretching vibrations of the free NH bonds in the dimers should appear at around 3500 cm^{-1}. These vibrations have close frequencies [3504, 3499 cm^{-1} (for dimer A) and 3497, 3500 cm^{-1} (for dimer B)] and cannot be used to distinguish the dimers. But the frequencies of the stretching vibrations of H-bonded NH bonds are significantly different. They are 3177 and 3099 cm^{-1} for dimer A and 3230 and 3142 cm^{-1} for dimer B. The H-bonding also leads to a strong increase of the IR intensities of these vibrations by 10−20 times. These features should enable identification of the dimers with the matrix-isolation IR spectroscopy.

The frequencies of the C═O stretching vibrations of the dimers are downshifted with respect to the corresponding acrylamide monomer band by ca. 17 cm^{-1}. However both dimers have close C═O stretching vibration frequencies (see Table 6). Other vibrations which are different for dimer A and dimer B are the NH bending vibration, the C2N3 stretching vibration and the NH_2 torsion vibration of 9-methyladenine. Their frequencies and intensities are given in Table 6. These modes may provide additional means to distinguish the dimers.

TABLE 6: Selected Harmonic Frequencies (ν, cm^{-1})[a] and Intensities (I, km mol^{-1}) of the Acr and m^{9}Ade Monomers, and of Acr−m^{9}Ade Dimer A and Dimer B Calculated at the DFT/B3LYP/6-31++G Level of Theory[b]**

ν/I assignment			
m^{9}Ade	dimer A	dimer B	Acr
3569/62.0	3504/111.3	3497/98.6	
$N11H_2$ str as	N11H13 str	N11H12 str	
	3499/75.4	3500/96.3	3556/44.0
	N19H24 str	N19H24 str	$N19H_2$ str
3435/101.3	*3177*/2000.4	*3230*/1437.8	
$N11H_2$ str s	N11H12 str	N11H13 str	
	3099/726.3	*3142*/845.8	3423/50.1
	N19H23 str	N19H23 str	$N19H_2$ str
	1709/270.0	1710/275.6	1727/288.8
	C20O25 str	C20O25 str	C20O25 str
1580/13.7	1665/220.6	1660/161.4	
NH_2 bend	N11H12 bend	N11H13 bend	
	1609/288.2	*1599*/292.9	
	N11H13 bend	N11H12 bend	
	1661/186.3	1661/237.9	1593/125.4
	N19H23 bend	N19H23 bend	NH_2 bend
	1625/203.3	1625/221.7	
	N19H24 bend	N19H24 bend	
1313/72.2	*1331*/84.0	*1313*/75.8	
C2N3 str	C2N3 str	C2N3 str	
526/0.7	*764*/24.8	*741*/18.9	
NH_2 tor	N11H12 tor	N11H13 tor	
	450/152.4	*480*/138.1	
	N11H13 tor	N11H12 tor	

[a] Frequencies are scaled with the following scaling factors: 0.95 for the N−H stretching vibrations and 0.98 for all other vibrations. [b] Frequencies which may be used for the IR spectral identification of the dimer A and dimer B are shown in italic.

In conclusion, the above analysis of the IR spectral characteristics of dimer A and dimer B of Acr−m^9 Ade shows that each dimer has at least six strong bands which may be used for their identification. Thus, the matrix-isolation IR spectroscopy seems to be a suitable tool for investigating these and other complexes between nucleic acid bases and amino acids. This is mainly due to the sensitivity of the IR spectra to the intermolecular H-bonding.

Acknowledgment. This work was supported in part by a NATO grant CRG973389 allowing the visit of Dr. S. G. Stepanian to the University of Arizona.

References and Notes

(1) Galetich, I.; Kosevich, M.; Shelkovsky, V.; Stepanian, S. G.; Blagoi, Yu. P.; Adamowicz, L. *J. Mol. Struct.* **1999**, *478*, 155.

(2) Galetich, I.; Stepanian, S. G.; Shelkovsky, V.; Kosevich, M.; Blagoi, Yu. P.; Adamowicz, L. *J. Phys. Chem. B* **1999**, *103*, 11211.

(3) Helene, C.; Lancelot, G. *Prog. Biophys. Mol. Biol.* **1982**, *39*, 1.

(4) Seeman, N. C.; Rosenberg, J. M.; Suddath, F. *J. Mol. Biol.* **1976**, *104*, 109.

(5) O'Neil, K. T.; Hoess, R. H.; DeGrado, W. F. *Science* **1990**, *249*, 774.

(6) Wodak, S. Y.; Lin, M. Y.; Wyckoff, H. W. *J. Mol. Biol.* **1977**, *116*, 855.

(7) Fairall, L.; Schwabe, J. W. R.; Chapman, L.; Finch, J. T.; Rhodes, D. *Nature* **1993**, *366*, 483.

(8) Sukhodub, L. F. *Chem. Rev.* **1987**, *87*, 589.

(9) Verkin, B. I.; Yanson, I. K.; Sukhodub, L. F.; Teplitsky, A. B. *Interactions of biomolecules. New experimental approaches and methods*; Naukova Dumka: Kiev, 1985 (in Russian).

(10) Sukhodub, L. F.; Shelkovsky, V. S.; Wierzchovsky, K. L. *Biophys. Chem.* **1984**, *19*, 191.

(11) Korol, T.; Lobanov, V. V.; Nazarenko, V. A.; Pokrovski, V. A. *Physical Principles of Field Mass Spectrometry*; Naukova Dumka: Kiev, 1978 (in Russian).
(12) Binkley, J. S.; Pople, J. A. *Int. J. Quantum Chem.* **1975**, *9*, 229.
(13) Pople, J. A.; Binkley, J. S.; Seeger, R. *Int. J. Quantum Chem., Quantum. Chem. Symp.* **1976**, *10*, 1.
(14) Becke, A. D. *Phys. Rev. B* **1988**, *38*, 3098.
(15) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(16) Vosko, S. H.; Wilk, L.; Nusair, M. *Can. J. Phys.* **1980**, *58*, 1200.
(17) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553.
(18) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V, G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94,* Revision E.2; Gaussian Inc.: Pittsburgh, PA, 1994.
(19) Verkin, B. I.; Yanson, I. K.; Sukhodub, L. F.; Teplitsky, A. B. *Interactions of biomolecules. New experimental approaches and methods*; Naukova Dumka: Kiev, 1985; 164 pp (in Russian).
(20) Teplitsky, A. B.; Galetich, I. K.; Sukhodub, L. F. *Mol. Biol.* **1990**, *25*, 709 (in Russian).
(21) Yanson, I. K.; Teplitsky, A. B.; Sukhodub, L. F. *Biopolymers* **1979**, *18*, 1149.
(22) Sarai, A.; Saito, M. *Int. J. Quantum Chem* **1984**, *25*, 527.
(23) Stepanian, S. G.; Reva, I. D.; Rosado, M. T. S.; Duarte, M. L. T. S.; Fausto, R.; Radchenko, E. D.; Adamowicz, L. *J. Phys. Chem. A* **1998**, *102*, 1041.
(24) Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Adamowicz, L. *J. Phys. Chem. A* **1998**, *102*, 4623.
(25) Del Bene, J. E.; Shavitt, I. *J. Mol. Sctruct. (THEOCHEM)* **1994**, *307*, 27.
(26) Sukhodub, L. F.; Yanson, I. K. *Nature* **1976**, *264*, 245.
(27) Yanson, I. K.; Teplitsky, A. B.; Sukhodub, L. F. *Biopolymers* **1979**, *18*, 1149.
(28) Molina, M.; Carmona, P. *J. Mol. Struct.* **1988**, *175*, 283.