removed and combined with 1 mL of standard solution (1.32×10^{-4} M benzamide (internal standard) in methylene chloride), and 1 mL of NMN solution was removed and combined with 1 mL of second standard solution (1.32×10^{-3} M benzamide in methylene chloride); analysis was by HPLC (75% or 35% ether/Skelly F; 3.5 mL/min). Only the syn isomers **6** and **8** were examined. The relative quantum yields for NMP and NMN were determined to be 0.08 and 1.0, respectively.

Relative Quantum Yields of Photoaddition of Substituted Phenylcyclopropanes to NMN. Five milliliter solutions containing 0.028 M NMN and 0.59 M PC, *p*-chlorophenylcyclopropane, or *p*-methylphenylcyclopropane in acetonitrile were irradiated in parallel for 10 h on a merry-go-round. Aliquots were analyzed as outlined above.

Quantum Yield of Photoaddition of PC to NMN. Six milliliter solutions of 0.028 M NMN and 0.59 M PC were placed in a quartz cell, degassed, and irradiated at 360 nm for 8 h using a high-pressure mercury lamp in a black box apparatus. Aliquots were removed and analyzed as above. Einsteins of light were determined by standard ferrioxalate actinometry[21] before and after each run.

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Limiting Quantum Yields of PC Photoaddition to NMN. Ten milliliter solutions of 0.028 M NMN and varying concentrations of PC (0.30–1.48 M) were irradiated for 9 h on a merry-go-round apparatus. Aliquots were analyzed as above. The relative quantum yields were normalized to the absolute quantum yields and plotted $1/\Phi$ versus $1/[PC]$ to determine the limiting quantum yield.

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Registry No. **4**, 125928-12-3; **5**, 104506-74-3; **6**, 104506-73-2; **7**, 104506-76-5; **8**, 104506-75-4; **9**, 125928-13-4; **10**, 125928-14-5; **11**, 104506-78-7; **12**, 104506-77-6; **13**, 104506-79-8; PC, 873-49-4; NMP, 56788-11-5; NMN, 42896-23-1; *p*-methoxphenylcyclopropane, 4030-17-5; *p*-methylphenylcyclopropane, 6921-43-3; *p*-chlorophenylcyclopropane, 1798-84-1; *p*-cyanophenylcyclopropane, 1126-27-8.

Supplementary Material Available: Atom coordinates and temperature factors for 8 (1 page); structure factors for 8 (12 pages). Ordering information is given on any current masthead page.

Theoretical Study of the Hydroxyl Nucleophilic Attack on the 6-Aminopyrimidine Molecule: Functional Implications in the Reaction Mechanism of Nucleoside Deaminative Enzymes

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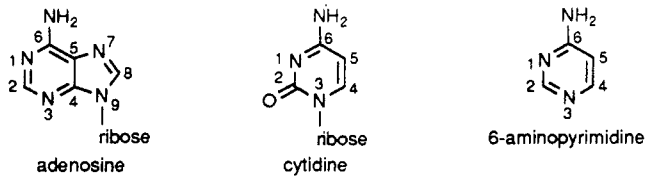
A quantum chemical study of hydroxyl attack on a reduced model of adenosine and cytidine has been performed by using both semiempirical MNDO and ab initio 4-31G methodologies. Because the studies on the reaction pathways were carried out by using semiempirical methods, the validity of MNDO for the study of such reactions was tested first. For this purpose, hydroxyl attack on the formaldehyde molecule (a well-known and documented reaction similar to the reaction of interest) was considered. Results obtained from the study of this reaction at the MNDO level were consistent with both ab initio 6-31+G* results and experimental data. The study of hydroxyl attack on the 6-aminopyrimidine molecule reveals the existence of two reactions: the first consists of proton capture by the hydroxyl of the amine hydrogen trans to N1, while the second consists of the formation of a Meisenheimer complex by means of hydroxyl attack at C6. Ab initio and semiempirical "static" reactivity parameters point to the first reaction as being favored over the second if no restrictions are imposed on the hydroxyl attack pathway. The imposition of orientation restrictions on the hydroxyl attack results in feasible "reactive pathways" that are almost perpendicular to the molecular plane and that lead to the Meisenheimer complex formation with a very low energy barrier. The biochemical implications of the results obtained on the mechanism of the reaction of both adenosine and cytidine deaminases are extensively discussed in the context of previous theoretical and experimental data. Finally, several possible "microscopic" reaction mechanisms for these enzymes are suggested.

Introduction

Adenosine deaminase (ADA EC 3.5.4.4) and cytidine deaminase (CDA EC 3.5.4.5), which catalyze the conversion of adenosine to inosine and of cytidine to uridine, respectively, are probably the most important and well-known deaminative enzymes. In recent years, the reaction mechanisms of both ADA and CDA have been extensively studied, not only because of the biological relevance of these enzymes but also from a pharmacological point of view, as their substrates and inhibitors are currently used as antineoplasic, antihypertensive, antimetabolic, antiviral, and antibiotic agents.[1-9]

Scheme I



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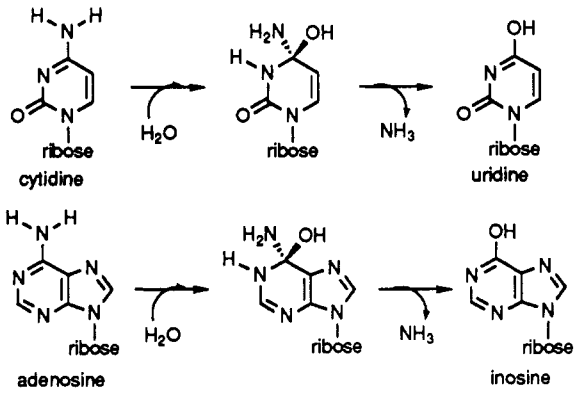
0022-3263/90/1955-2630$02.50/0

Scheme II



Several authors[10-17] have suggested that the two enzymes have a similar reaction mechanism. This hypothesis, which is consistent with divergent evolution from a common ancestor, is also supported from a mechanistic viewpoint as the chemical structures of the two substrates are similar (see Scheme I).

The proposed mechanism[10-17] of reaction (see Scheme II) would consist of two different processes. First, the formation of a tetrahedral intermediate by means of protonation at N1 and hydroxylation at C6 (for atom numbering, see Scheme I), and second, the formation of product (inosine or uridine and ammonia). It is assumed that it is during the formation of the tetrahedral intermediate that the rate-determining step of the enzymatic reaction occurs. Experimental results such as the extremely low inhibition constant of nucleosides resembling the tetrahedral intermediate (e.g., deoxycoformycin or tetrahydrouridine[10,12,18-21]), the existence of a sulfhydryl group in the active site of adenosine[16,17,22] and cytidine[23,24] deaminases, as well as the change from sp^2 to sp^3 in the C6 atom during the rate-limiting step in the ADA reaction mechanism[25] support the proposed mechanism.

Recent theoretical studies performed in our laboratory based on both semiempirical (MNDO and AM1) and ab initio methodologies concerning the mechanism of action of adenosine deaminase[26,27] suggested that the protonation of N1 rather than the hydroxyl attack on C6 is the rate-determining step. These theoretical studies were surprising in light of the fact that our chemical intuition suggests that the protonation of a purine or pyrimidine nitrogen would be easier than nucleophilic attack at an aromatic carbon atom.

The aim of the current work was to study the hydroxyl attack on the C6 atom of adenosine and cytidine in order to gain insight into the characteristics of such a reaction, specifically as it occurs at the active site of deaminative enzymes.

Methods

6-Aminopyrimidine was chosen as a model to study the attack of a hydroxyl group. This molecule was selected as it embodies the greater common structure shared by adenosine and cytidine (see Scheme I) and consequently the general conclusions obtained from the study of such a model compound could be extrapolated to both nucleosides.

The study of hydroxyl attack on 6-aminopyrimidine was performed by using the semiempirical MNDO method[28] as well as ab initio computations using the split valence 4-31G basis set.[29] The geometries of all molecules were fully optimized by using the semiempirical MNDO method.

The molecular electrostatic potential (MEP) above the aromatic ring was calculated at the 4-31G level. MEPs were calculated at 2 and 4 Å above the aromatic plane according to Pullman's suggestions,[30,31] as this author indicates that useful information concerning nucleophilic attack can be obtained from MEPs only if they are calculated beyond the Van der Waals radius of the molecule.

The entropies of reactants and products for both proton transfer and nucleophilic substitution reactions were calculated at the MNDO level for thermodynamic calculations.

Semiempirical calculations were performed with a locally modified version[32] of the MOPAC package.[33] Ab initio calculations were performed with a locally modified version (F. Illas and J. Rubio, unpublished results) of the HONDO-76 computer program.[34] All calculations were carried out on the IBM-3090 computer of the Centre de Calcul de la Universitat de Barcelona.

Results

Since to our knowledge, there is no reference in the literature to either experimental or theoretical calculations

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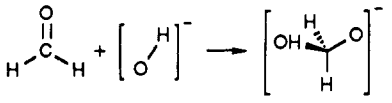
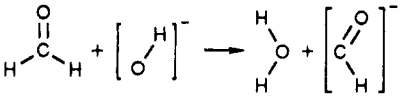
Scheme III

reaction A: nucleophilic substitution



ΔH_A = −53.32 kcal/mol
ΔS_A = −31.45 cal/K mol
ΔG_A = (T = 300 K) = −43.92 kcal/mol

reaction B: proton transfer



ΔH_B = −17.34 kcal/mol
ΔS_B = 8.01 cal/K mol
ΔG_B = (T = 300 K) = −19.77 kcal/mol

concerning hydroxyl attack on the 6-aminopyrimidine molecule, the assessment of the reliability of the MNDO method was performed by studying a similar reaction: hydroxyl attack on carbonyl groups, for which both experimental[35-37] and ab initio theoretical results are reported.[38-43] Particularly, the usefulness of this semiempirical method was tested by comparing MNDO results with experimental[35,37] and ab initio quantum chemical data[38,42] for hydroxyl attack on the formaldehyde molecule.

1. Hydroxyl Attack on the Formaldehyde Molecule. This reaction has been well studied.[35,37,38,42] One of the most relevant theoretical studies was performed by Madura and Jorgensen at the ab initio level with the large 6-31+G* basis set.[38] In the present paper we have simulated Madura's calculation at the MNDO level, and our results are compared with those of Madura as well as with other experimental[35,37] and theoretical results.[42]

Several experimental[35,36] and theoretical results[39,43] suggest that the approach of a hydroxyl group to a carbonyl molecule can follow two different paths: the first leads to the capture of a proton by the hydroxyl group, while the second process leads to the formation of a Csp^3 tetrahedral compound. Since it has been well established through both experimental[35,37] and theoretical evidence[38,42] that the second pathway is favored for formaldehyde, our first objective was to determine if the MNDO method correctly reproduces that finding. For this purpose, the reaction enthalpy, entropy, and free enthalpy variation for both addition and proton transfer processes (see Scheme III) as well as the energetic (enthalpic) profile of the hydroxyl attack (see Figure 1A) were calculated by using the MNDO method.

The results displayed in Scheme III show that the nucleophilic addition is thermodynamically favored over the proton transfer process, the enthalpic term being about −53 kcal/mol for the nucleophilic reaction and about −17 kcal/mol for the proton transfer. These results are in agreement with the experimentally[35,37] and theoretically[38,42] demonstrated preference for the nucleophilic addition over the proton transfer reaction process. The values of the reaction enthalpies for both reactions are similar to the experimental[36,44] and ab initio[39] determined enthalpies of similar reactions. Moreover, the MNDO reaction enthalpy for the nucleophilic addition is similar to Madura's 6-31+G* results (in fact, the MNDO results are closer to 6-31+G* than the ab initio STO-3G results of Williams.[42]) On the other hand, the entropy favors the proton transfer

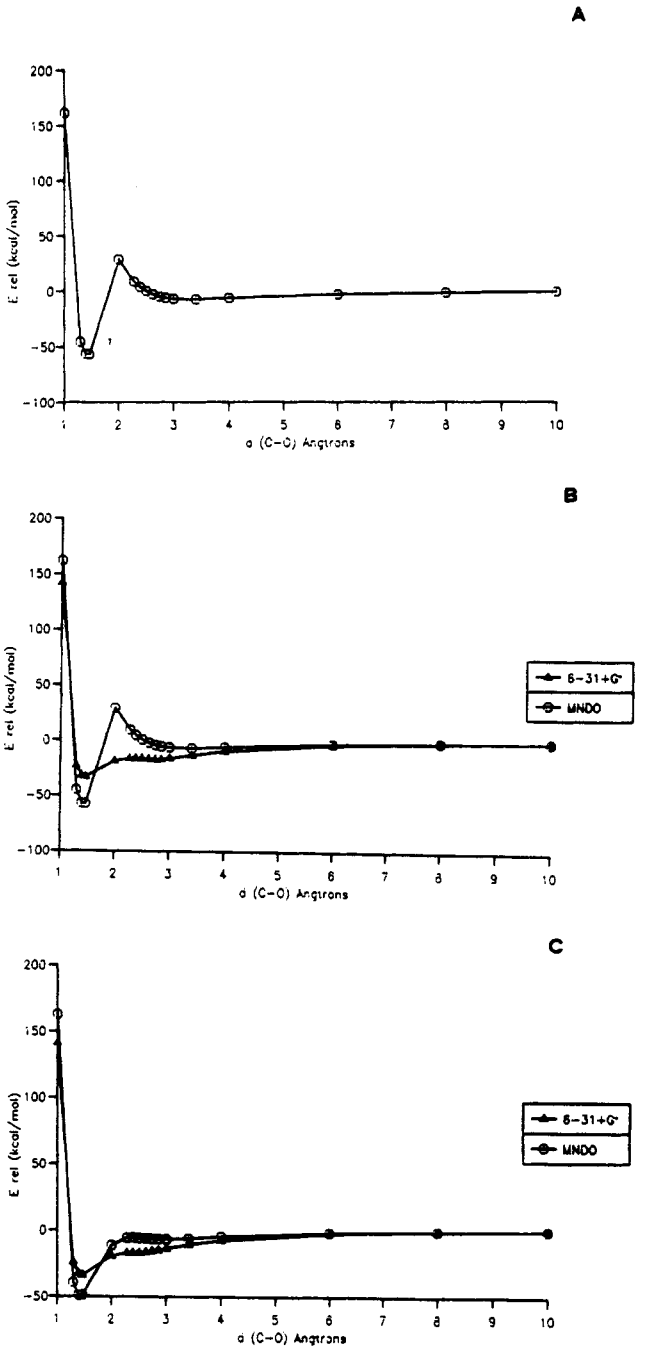


Figure 1. Energetic profiles for hydroxyl attack on the formaldehyde molecule: (a) free attack; (b) comparison of MNDO and 6-31+G* energetic profiles with the supermolecule forced to have C_s symmetry; (c) comparison of MNDO and 6-31+G* energetic profiles with the supermolecule forced to have C_s symmetry and with the O-C-O angle equal to 126.9°.

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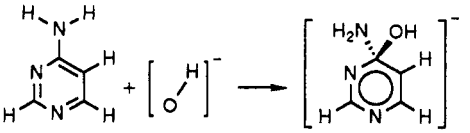
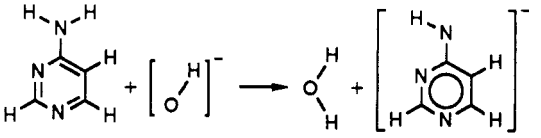
Scheme IV

reaction A: aromatic nucleophilic substitution



ΔH_A = –67.87 kcal/mol (MNDO)
–34.51 kcal/mol (ab initio 4-31G)
ΔS_A = –33.11 cal/K mol (MNDO)
ΔG_A (T = 300 K) = –57.98 (MNDO)

reaction B: proton transfer



ΔH_B = –76.87 kcal/mol (MNDO)
–45.25 kcal/mol (ab initio 4-31G)
ΔS_B = 6.6 cal/K mol (MNDO)
ΔG_B (T = 300 K) = –78.77 (MNDO)

pathway over Csp^3 formation. The entropic term $T\Delta S$ is about –9 kcal/mol for the nucleophilic addition and 1 kcal/mol for the proton transfer reaction at T = 300 K. These results, which are in agreement with experimental and entropic data determined by both semiempirical[45] and ab initio methodologies[39] for similar reactions, clearly show that consideration of the entropic term does not introduce qualitative changes. Therefore entropy has not been taken into consideration for all subsequent calculations of reaction pathways.

The MNDO energetic profile of hydroxyl attack on the formaldehyde molecule, taking the C–O distance as the reaction coordinate and without any geometrical restrictions is shown in Figure 1a. An energetic profile such as this points to the Csp^3 tetrahedral adduct as being the final product, in good accordance with the above-noted static thermodynamic data. An energetic barrier appears for values of the C6–O distance around 2 Å. This barrier seems to be overestimated in the light of experimental[35] and ab initio theoretical data[38,42] and would arise from hydrogen bond interactions between the C-hydrogen atoms and the hydroxyl group, which divert the hydroxyl group from the reactive pathway.[45-47]

The relevance of the orientation factors have been taken into consideration by different authors,[38,39,41-43] who introduced several geometrical restrictions on the hydroxyl attack. Thus, in Madura's work two different restrictions were introduced: first, C_s symmetry was imposed on the supermolecule and second, a value of 126.9° for the O–C–O angle was kept frozen. In order to further test the validity of our calculations, MNDO energetic profiles calculated with these restrictions are compared with Madura's 6-31+G* results (see Figure 1B,C). The results show that when only the C_s symmetry on the supermolecule is forced, the energetic profile that is obtained is almost identical with that observed when no restrictions are imposed. Thus, an overestimation of the activation barrier for the nucleophilic addition is obtained (see Figure 1b). In contrast, when in addition to the imposition of the C_s symmetry the O–C–O angle is kept constant along the reaction path, the MNDO energetic profile obtained is similar to that reported by Madura and Jorgensen (see Figure 1c).

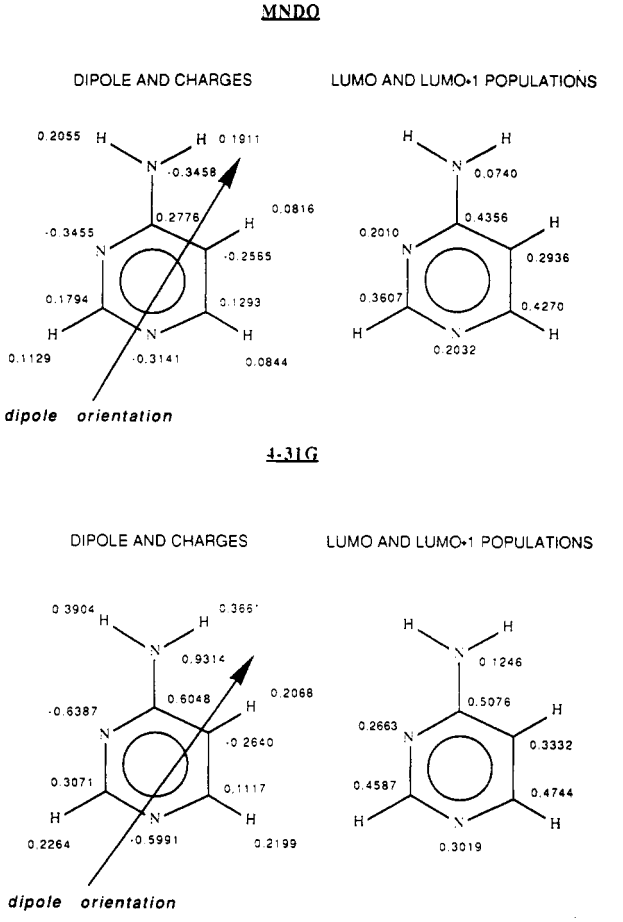


Figure 2. Mulliken net charges, dipole orientation, and LUMO and LUMO+1 orbital populations for the 6-aminopyrimidine molecule calculated at both MNDO and ab initio 4-31G levels.

2. Hydroxyl Attack on the 6-Aminopyrimidine Molecule. Inspection of the chemical structure of 6-aminopyrimidine (see Scheme I) suggests that the attack of a hydroxyl group can follow two different pathways, similar to those described for the formaldehyde molecule (see Schemes III and IV). The first reaction, which is the proposed process at the active site of adenosine and cytidine deaminases, consists of nucleophilic attack of the hydroxyl group at the C6 atom (see Scheme IVA), leading to a Meisenheimer complex (for a review of Meisenheimer's complexes see refs 48 and 49); the second consists of the capture of an amine proton by the hydroxyl group and leads to a dead-end product that is not detected in the enzymatic deamination.

The first approach for studying such reactions is provided by the static reactivity indices. Thus, Mulliken charges and the LUMO and LUMO+1 orbital distributions over all the atoms of the molecule, as well as the dipole moment and the ab initio molecular electrostatic potential at 2 and 4 Å over the molecule's plane, have been calculated and are shown in Figures 2 and 3. Ab initio and semiempirical net charges provide quantitatively different results, which are in part due to the orthogonality of the MNDO wavefunction;[50] from a qualitative point of view, however, both MNDO and ab initio 4-31G methods in-

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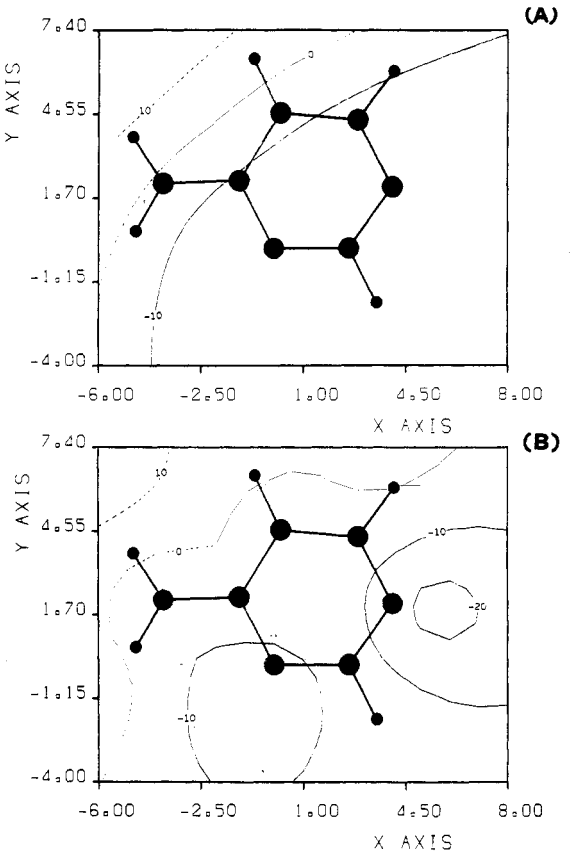

Figure 3. Ab initio 4-31G molecular electrostatic potential maps calculated at 4 (A) and 2 (B) Å above the aromatic ring.

dicate that C6 is the atom most likely to suffer a nucleophilic attack. In contrast, the dipole orientation (see Figure 2) shows that the dipole–charge complexes between the hydroxyl group and the 6-aminopyrimidine will be formed in the ring plane and close to the amino hydrogen group trans to N1. The ab initio 4-31G molecular electrostatic potential maps (see Figure 3), show that, at large distances from C6 (where the electrostatic term gives the strongest interaction[43,44] the hydroxyl group will be placed near the amino hydrogen group trans to N1 and consequently the hydroxyl approach following the electrostatic path will lead to the proton transfer process. On the other hand, considering the electronic distribution of LUMO and LUMO+1 (orbitals with II symmetry), it is clear (see Figure 2) that nucleophilic addition at C6 is favored by the orbital interactions between the hydroxyl's HOMOs and the 6-aminopyrimidine LUMOs.

The MNDO and ab initio reaction enthalpy, entropy, and free enthalpy changes for both processes (see Scheme IV) as well as the MNDO energetic (enthalpic) and charge profiles (see Figure 4) of the hydroxyl attack (calculated without any geometric restrictions) have been calculated to determine the most favorable reaction.

Enthalpy terms determined at the MNDO level are about −68 kcal/mol for Meisenheimer complex formation and about −77 kcal/mol for proton transfer. MNDO determined entropy terms ($T\Delta S$) are about −10 kcal/mol for Meisenheimer complex formation and about 2 kcal/mol for proton transfer and consequently favor proton transfer over Meisenheimer complex formation. The results demonstrate that entropic terms are of minor relevance for qualitative studies, not only with respect to their small magnitude compared with the enthalpy, but also because the variation in the entropic terms parallels the enthalpy variation; thus, no significant changes in the conclusions

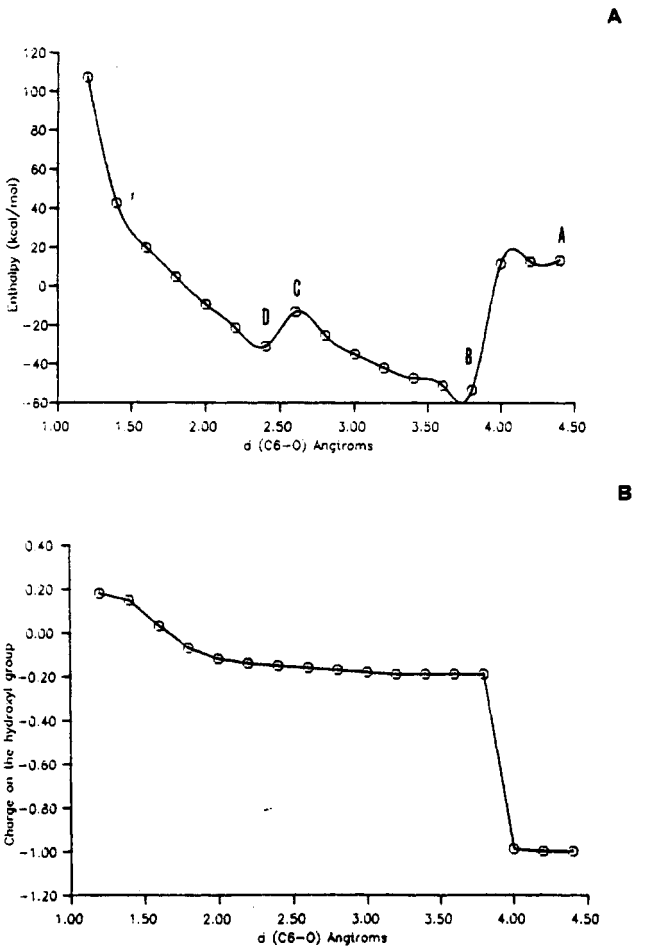

Figure 4. MNDO energetic (A) and hydroxyl charge profiles (B) for free hydroxyl attack on the 6-aminopyrimidine molecule. Key points of the reactive path are noted in the energetic profile (see Figure 5).

derived from enthalpy considerations emerge when entropic terms are included. Therefore, all calculations of reaction pathways have been carried out without including the entropic terms.

The results obtained from the study of the reaction paths (see Figures 4 and 5) agree with the static thermodynamic data discussed above, demonstrating that the proton transfer is the most likely reaction and that this reaction occurs via formation of a dipole–charge complex and subsequent capture of an amino proton by the hydroxyl group. The proton transfer occurs at an early stage in the approximation of the OH group to C6 (at a C6–O distance of about 3.8 Å); at this distance the hydroxyl group is close to one of the amine hydrogen atoms and thus the proton transfer reaction occurs easily. Subsequent approach of the water molecule to C6 occurs following the dipole orientation until 2.5 Å, when the water molecule moves above the C6 atom (see Figure 5) in order to avoid steric hindrance with the 6-NH^- group. It must be stressed that approach of the water molecule to C6 is strongly unfavored.

The static reactivity indices (see Figures 2 and 3) described above suggest that both reactions are possible, depending upon the orientation of the hydroxyl attack.

The approach of OH to the 6-aminopyrimidine molecule has been studied with the introduction of orientation restrictions at two different levels to test this possibility: (i) fixing the O–C6–N1–C2 dihedral angle and (ii) fixing the O–C6–N1 angle at different values and restricting the O–C6–N1–C2 dihedral angle to its more favored values.

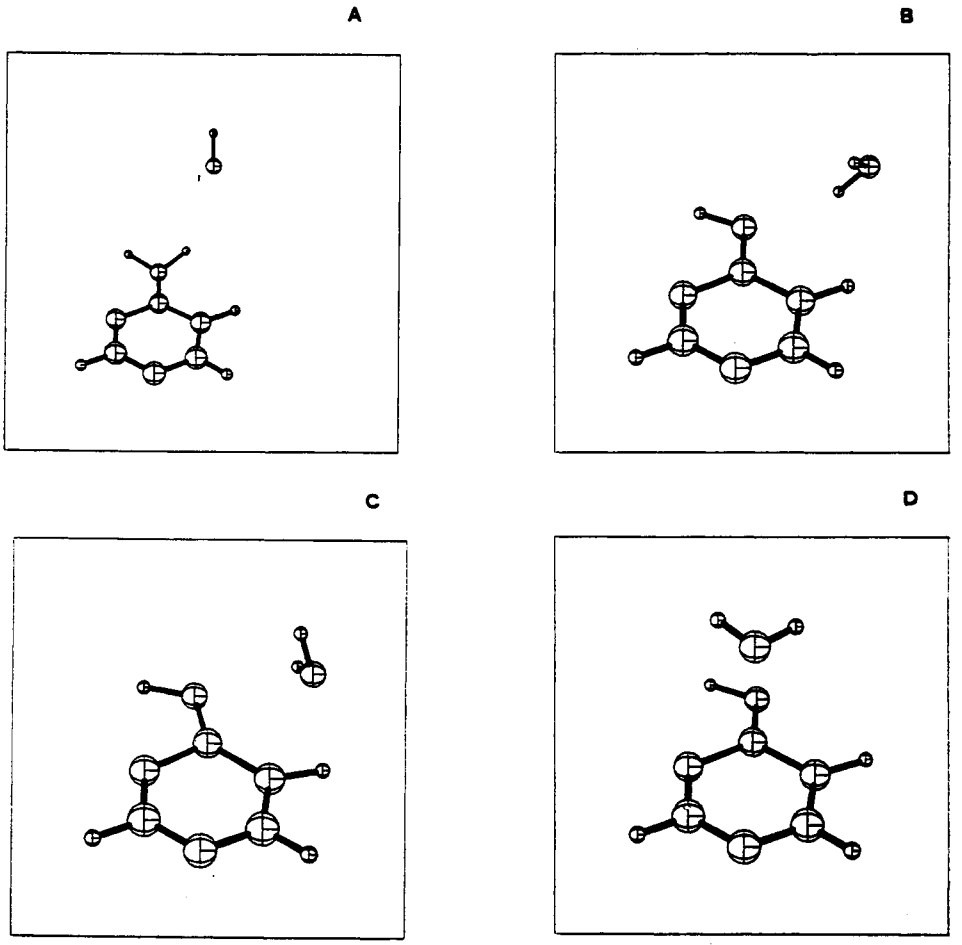

Figure 5. Significant structures (see Figure 4) detected during the free approach of the hydroxyl to the 6-aminopyrimidine molecule.

Energetic profiles for hydroxyl attack on the molecule at different O–C6–N1–C2 dihedral angles are shown in Figure 6. The results demonstrate that both Meisenheimer complex formation and proton transfer reactions are possible, depending upon the orientation of the OH group. Nucleophilic addition at C6 occurs (with very small energetic barriers) only for values of the O–C6–N1–C2 dihedral angle between −100° and −70°. The most favored orientation of the OH group for the addition reaction is determined by dihedral angles in the range −100° to −90° (see Figures 6 and 7). Dihedral angles outside this range lead to the proton transfer reaction (see Figure 6). It should be noted (see Figure 7B) that the charge transfer process occurs at short C6–O distances (smaller than 2.3 Å).

Energetic profiles obtained by fixing the O–C6–N1 angle at values of 90° and 109.5° (for values of the O–C6–N1–C2 dihedral angle of −90° and −100°), compared with those obtained when the O–C6–N1 angle was not fixed are displayed in Figure 8. These results clearly indicate that 109.5° is the most favorable angle for the hydroxyl attack, in good accordance with our chemical intuition.

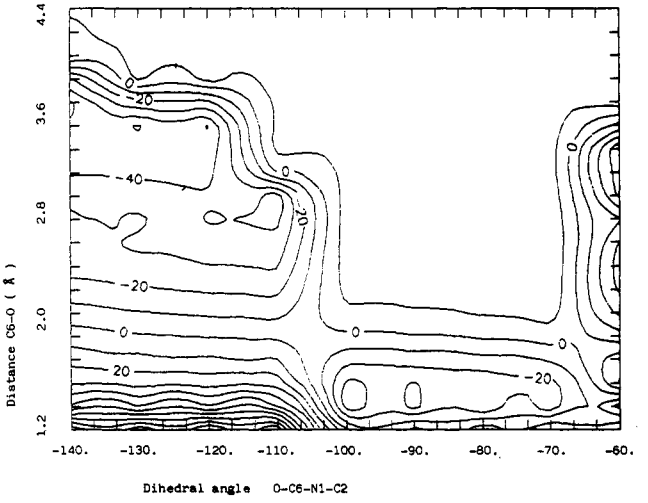

Figure 6. MNDO energy map for hydroxyl attack on the 6-aminopyrimidine molecule, taking the distance between the oxygen and the C6 atoms and the O–C6–N1–C2 dihedral angle as "reaction coordinates".

Discussion

The theoretical study of nucleophilic reactions that take place inside the active sites of enzymes whose tridimensional structures are unknown can prove quite challenging. ADA and CDA catalyze the hydrolytic deamination of molecules with up to 30 atoms, increasing the difficulties involved in such theoretical studies. This fact renders nearly impossible the ab initio study of such reactions and makes the use of simulative reduced models of adenosine and cytidine for semiempirical calculations desirable.

The study of hydroxyl attack on the formaldehyde molecule not only provides information concerning the reliability of the MNDO method for qualitatively representing the nucleophilic attack of the hydroxyl group at Csp^2 centers but also yields information about the different processes that can occur when the OH approaches the target molecule. The results summarized in Scheme III and Figure 1 clearly show the ability of the MNDO method to give qualitatively correct results close to experimental data and to Madura's nearly Hartree–Fock results, if orientation restrictions are taken into consideration. It

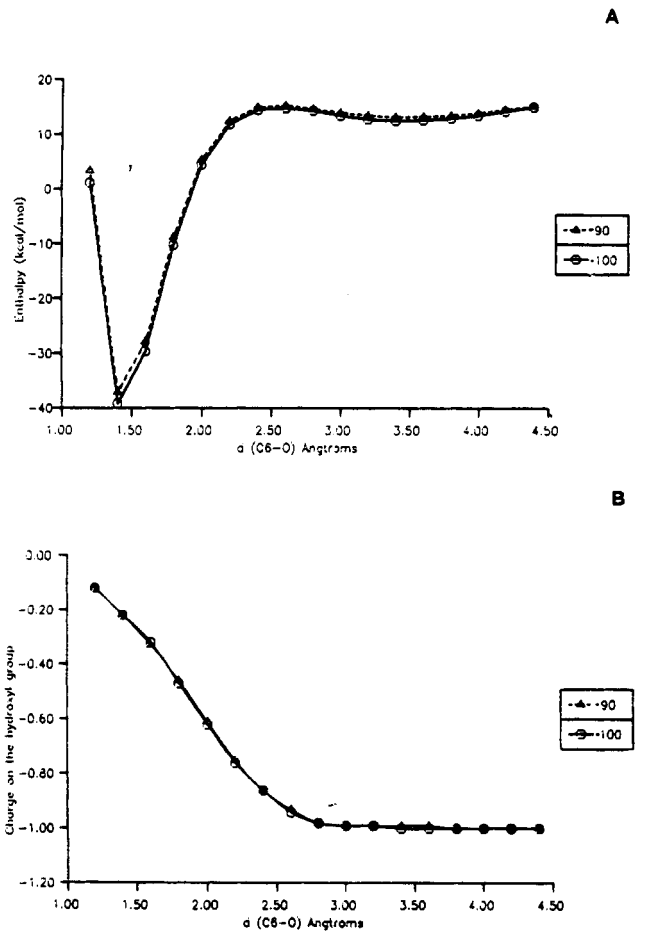

Figure 7. MNDO energetic (A) and hydroxyl charge (B) profiles for hydroxyl attack on the 6-aminopyrimidine molecule at values of the O–C6–N1–C2 dihedral angle of −90° and −100°.

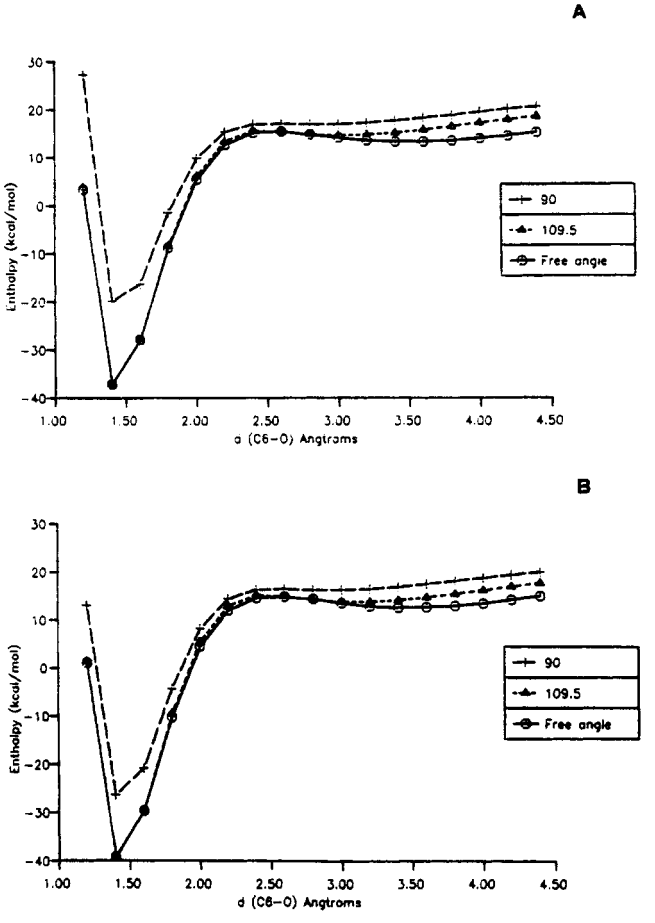

Figure 8. MNDO energetic profiles for hydroxyl attack on the 6-aminopyrimidine molecule depending upon the attack angle O–C6–N1 (free, fixed at 90°, and fixed at 109.5°), with the dihedral angle O–C6–N1–C2 fixed at −90° (A) and −100° (B).

should be stressed that the MNDO method is quite capable of correctly determining which reaction (proton transfer or nucleophilic addition) is the most favored. Thermodynamic results (see Scheme III) emphasize the enthalpy-guided nature of these reactions, as evidenced by the fact that it is possible to neglect the entropic effect without introducing major changes in the qualitative discussion of both processes.

The thermodynamic study of the reaction between 6-aminopyrimidine and a hydroxyl group points out that the proton transfer is favored over the nucleophilic substitution by both entropic and enthalpic terms (the latter being the most important factor). The analysis of static reactivity indices of 6-aminopyrimidine demonstrates that the electrostatic interaction facilitates the proton transfer, while the orbital interaction favors the nucleophilic attack at C6. Since the electrostatic interaction predominates at large distances, it is not surprising that the free attack of a hydroxyl group on 6-aminopyrimidine leads to the proton transfer reaction via a charge–dipole complex (see Figures 4 and 5).

It is well-known that many water molecules are removed from the active site of an enzyme when the substrate enters. Very few water molecules remain[51] and, hence, the environment is similar to the gas phase.[52] It therefore seems logical to assume that the reaction likely to occur in the enzyme is the one most favored in the gas phase. However, orientation factors must be taken into consideration, since in the enzymes, the reaction paths were presumably fixed along the evolutive process in the orientation most conducive to the biologically relevant reaction (hydroxylation in ADA and CDA). Thus, it is interesting to note that although proton transfer is the most favored reaction, when the direction of the reaction path is partially or totally fixed, pathways (near the perpendicular route) that lead to formation of the Meisenheimer complex might exist (see Figure 6–8).

Energetic profiles (see Figures 6–8) demonstrate the existence of a small energy barrier for the hydroxyl attack at C6 in the gas phase, in accordance with results obtained for formaldehyde in the first phase of this work and with several other studies of hydroxylic attack at Csp^2 atoms.[35-43] At this point it should be noted that some studies,[35,38-40] wherein the solvent (water) effect has been taken into consideration, have demonstrated that the presence of water introduces an activation barrier to the reaction that is due not to the intrinsic characteristics of nucleophilic reactions but rather to the disturbing effect of solvent molecules (which are not present in the catalytic site of the enzyme).

The set of results obtained strongly suggest that the hydroxyl attack at the C6 atom of nucleosides is a highly exothermic process with a very low activation barrier and can easily occur if (i) the reaction path is fixed in the proper orientation and (ii) no water is present. It must be stressed that both conditions are expected to be met in the catalytic site of an enzyme, and therefore a reaction that is difficult in water (due to the disturbing effect of

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the solvent) and in the gas phase (due to the existence of a more feasible competitive reaction) can easily occur inside the active site of an enzyme.

The present results indicate that hydroxyl attack is a strongly favored process, which occurs at a high rate due to the small activation barrier. Therefore, it seems obvious that the hydroxyl attack at the C6 atom of nucleosides cannot be the rate-determining step of the deamination reactions catalyzed by ADA and CDA.

The ease of hydroxyl attack on the neutral 6-aminopyrimidine molecule introduces one intriguing question: Why is the protonation of N1 necessary[6,12,26,27,53,54] for the deamination reaction? Stated another way, what is the biochemical role of the protonation of N1? Three possible explanations follow.

(i) Protonation at N1 creates a net charge over the pyrimidine ring and consequently the hydroxyl attack on C6 is presumably favored (for both orbital and electrostatic reasons) over the proton transfer. This charge could open new reactive pathways such that a hydroxyl group located in a "nonreactive" orientation becomes "reactive".

(ii) Protonation at N1 could be responsible for a conformational change occurring in the enzyme during the formation of the tetrahedral intermediate. This change is well documented for ADA,[55-57] whereas several indirect experimental indications of this change as the slow binding of transition state inhibitors[11] exist for CDA. As a consequence, a hydroxyl group initially in a nonreactive position could become reactive.

(iii) This work is focused on the study of hydroxyl attack on nucleosides. However, the great reactivity of the hydroxyl group in the gas phase (clearly demonstrated in this work) makes it difficult to envision its existence in free form at the active site of an enzyme. Therefore, the hydroxyl group must be generated, presumably from a water molecule, by the action of a basic residue of the enzyme (probably a histidine group[16,26]). In this context, a new reaction, the proton transfer from a water molecule to a residue of the active site, appears. It could be suggested that the protonation at N1 and consequent increase of the electrophilicity at C6 facilitates this hydroxyl generation.

The results discussed above answer certain questions concerning the mechanism of reaction of nucleoside deaminatives enzymes but introduce new ones such as the mechanistic role of the protonation step. Three hypothesis are postulated; no experimental evidence supports any one of them over the others, and perhaps the true role of the protonation step within the whole deaminative reaction is a mixture of all of them.

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Registry No. ADA, 9026-93-1; CDA, 9025-06-3; 6-aminopyrimidine, 591-54-8; hydroxyl, 14280-30-9; formaldehyde, 50-00-0.

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Conformational Analysis of Bridged Biphenyls and 2,2'-Bipyridines. Empirical Force Field Calculations (MM2-V4)

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The conformational analysis of bridged biphenyls and 2,2'-bipyridines has been undertaken with MM2-V4 force field (a modification of original MM2 force field containing the V4 torsional term). The known conformational properties of these compounds have been correctly reproduced. Ethano- and propano-bridged compounds have low interconversion barriers (ca. 4.5 and 10 kcal/mol, respectively), while butano-bridged derivatives present highly energetic barriers (ca. 25 kcal/mol).

Biaryls, mainly 2,2'-bipyridines, are extensively used as effective ligands to coordinate a large diversity of metals, and this effect is extended to annulated biaryls. E.g., 2,2'-bipyridine ruthenium complexes are important photosensitizers,[1] a bridged 2,2'-bipyridine (the 1,10-phenanthroline-cuprous complex) is an oxidative correactant in B DNA single-stranded break,[2] and some annulated 2,2'-bipyridine diquaternary salts have potent herbicide properties.[3]

In spite of the great interest of the chemistry of biaryls, these molecules have not been deeply studied from a theoretical point of view although some MO calculations on the conformations of biphenyl[4] have been carried out. Several articles concerned with experimental determinations on ground-state conformation and/or rotational barriers in biphenyl have been published.[5] Theoretical

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