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Determination of the Conformational Preferences of Adenosine at the Active Site of Adenosine Deaminase

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Abstract: The conformational flexibility of adenosine in the gas phase is examined by molecular mechanic procedures. Results show that adenosine is found mainly in anti conformation (O1'C1'N9C4 dihedral angle around 200–210°); a less stable conformation appears in the syn zone (glycosidic torsion angle around 50–60°). Two stable puckerings of the ribose are in the N and S areas, the S puckerings being more stable in both the anti and syn zones. The energy barriers for the anti–syn conversion are ~4–5 kcal/mol, whereas the energy barriers for the pseudorotational movement S–N of the ribose are ~2 kcal/mol through the east zone. Bidimensional studies have permitted us to determine three "preferred" conformational areas for adenosine (S anti, N anti, and S syn), as well as the existence of "forbidden" conformational areas for this molecule (E high syn and high anti). Molecular modeling studies, where up to 69000 conformations were generated and tested, have permitted us to propose a set of active conformations of adenosine at the active site of adenosine deaminase, which are consistent not only with energy data shown in this paper, but also with substrate specificity obtained experimentally and reported in the literature.

Introduction

Adenosine (Figure 1) and purine ribosides are not only structural units of nucleic acids but also serve as neuromodulators, neurohormones, and intermediaries in secondary metabolism. One of the most significant characteristics of these molecules is their high conformational flexibility, which has been studied mainly in relation to the topology of DNA.[1-5] Several authors have pointed out the importance of extending these studies to the interaction of nucleosides with metabolizing enzymes or specific receptors.[2,3,6,7]

There is a great deal of experimental data, mainly from X-ray and NMR studies (see below), on the stable conformations of nucleosides. Nevertheless, these results must be considered with caution, since the measuring conditions for both NMR and X-ray studies are different from those at the active sites of enzymes or receptors.[7] Interactions between ligands and active sites have been described as similar to those of the gas phase.[7-9] Thus, as theoretical methods permit a good description of the behavior of a given compound in the gas phase, they are suitable tools for the study of compounds interacting with enzymes or receptors.[7,9]

In the present paper the conformational flexibility of adenosine and the conformational requirements necessary for its interaction with the active site of adenosine deaminase are studied from a theoretical point of view.

Adenosine deaminase (ADA, EC 3.5.4.4) is a key enzyme of purine metabolism, which catalyzes the conversion from adenosine

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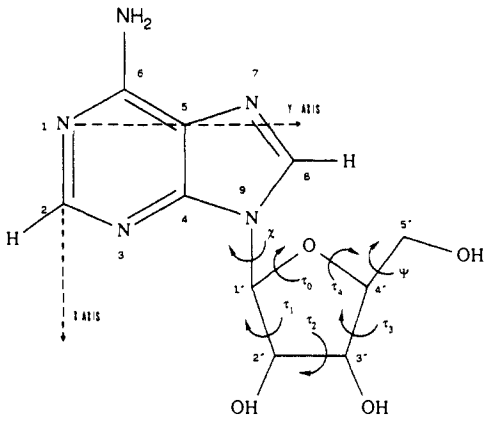

Figure 1. Schematic representation of adenosine, showing its more relevant conformational movements, as well as the reference system used.

to inosine. The importance of this deamination is demonstrated by its relationship to cellular disfunctions such as leukemias and AIDS,[10,11] in which the level of the enzyme is altered. Further, its genetic deficiency has been related to severe combined immunodeficiency.[12] The catalyzed reaction (see Figure 2) consists of an addition–elimination process with direct water attack on the substrate,[13,14] resulting in the formation of a tetrahedral intermediary.[15,16] Experimental evidence suggests that the "rate-limiting step" occurs during the formation of this intermediary.[15,16] The formation of such a tetrahedral intermediary can be divided into at least two elemental steps:[17-19] (i) a protonation on N1, probably by the action of a sulfhydryl group,[20-23] and (ii) a hydroxylation on C6 (see Figure 2). Theoretical studies performed in our laboratory support the hypothesis that the protonation is at least partially responsible for the rate-limiting step.[17-19]

The interaction between the active site of adenosine deaminase and its substrates is notably influenced by the conformation of the nucleosides.[3,24-26] Therefore, knowledge of the conformation that adenosine adopts at the active site of the enzyme is of great importance in the design of new drugs interacting with adenosine deaminase. At this point, it must be noted that not only are substrates and inhibitors of adenosine deaminase used as chemotherapeutic agents, but adenosine deaminase is also an important "interfering" agent in the action of several drugs that are active in the amino forms but do not have any pharmacological effect in their deaminated forms.

Experimental evidence indicates that adenosine can be deaminated by the enzyme only in its anti (or high anti) conformation around the glycosidic bond.[3,25,26] On the other hand, the similar K_m values for adenosine and adenine,[27] along with their very different rates of deamination, suggest that the enzyme does not interact with ribose during the enzyme–substrate recognition step, but this interaction should occur during the formation of the tetrahedral intermediary, stabilizing it, and consequently increasing the rate of deamination. Other experimental results, such as the enzymatic susceptibility of several intercyclated and ribose-modified substrates of adenosine deaminase,[24,25,28,29] agree with this hypothesis. The interaction between the ribose and the active site of the enzyme is achieved[24,26,30] by means of a specific interaction between the O5′ group of ribose (O3′ if the O5′ group is not accessible[31-33]) and an amino acid side chain of the active site.

The present study was undertaken to establish the conformational requirements of adenosine at the active site of adenosine deaminase. Two convergent strategies were used. First, the potential energy of adenosine as a function of (i) the conformation around the glycosidic bond, (ii) the ribose puckering, and (iii) the conformation around the exocyclic bond C4′–C5′ was studied theoretically (see Figures 1 and 3–6). Second, a spatial fitting procedure was performed in order to determine a possible relationship between the position of the O5′ (or occasionally O3′) and the logarithm of the maximum rate of deamination of several rigid substrates of adenosine deaminase. The combination of both strategies has led us to suggest a range of active conformations of adenosine, consistent with both energy and "pharmacophoric" requirements.

Methods

Nucleosides show high conformational flexibility, due to the rotations around single bonds (which can be defined by the values of the corresponding dihedral angles, also named torsion angles). The most important factors contributing to the conformational flexibility of adenosine are the following: (i) rotation around the glycosidic N9–C1′ bond, (ii) ribose puckering, and (iii) rotation around the exocyclic C4′–C5′ bond (see Figure 1). Accordingly, the conformational flexibility of adenosine was studied as follows:

1. Rotation around the Glycosidic Bond. Rotation around the glycosidic bond was represented by the dihedral angle O1′C1′N9C4 (named χ). Although the dihedral angle O1′C1′N9C8 was used in previous studies, we selected O1′C1′N9C4, according to recent recommendations.[34,35] Note that $\chi_{new} \approx \chi_{old} + 180°$.

Depending upon the conformation around the glycosidic bond, four zones can be defined (see Figure 3) following Paul's criterion:[36] high syn zone, for values of χ from 75 to 165°; anti zone, for values of χ from 165 to 255°; high anti one, for values of χ from 255 to 345°; syn zone, for values of χ from 345 to 75°.

2. Ribose Puckering. An accurate description of the conformations of the ribose would need five torsion angles names τ_1, τ_2, τ_3, τ_4, and τ_0, corresponding to the following dihedral angles: τ_1, dihedral angle O1′C1′C2′C3′; τ_2, dihedral angle C1′C2′C3′C4′; τ_3, dihedral angle O1′C4′C3′C2′; τ_4, dihedral angle C1′O1′C4′C3′; τ_0, dihedral angle C2′C1′O1′C4′.

The pseudorotational model[37-39] permits the description of one con-

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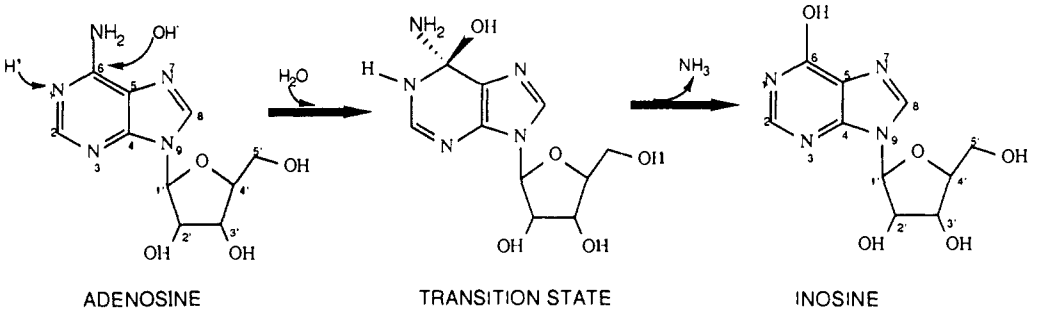

Figure 2. Schematic representation of the proposed reaction mechanism of adenosine deaminase.

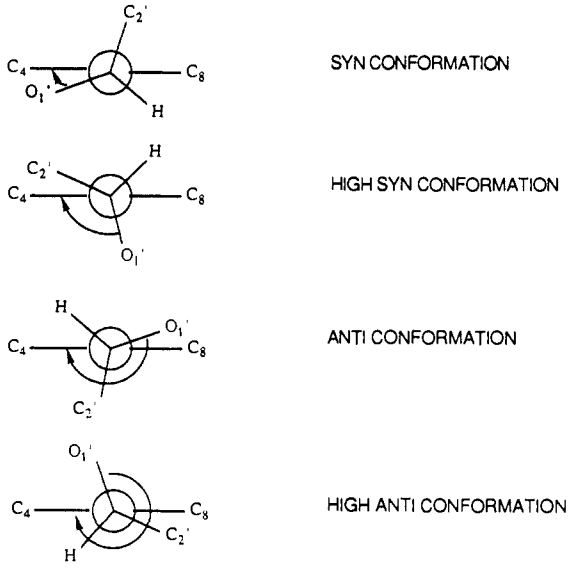

Figure 3. Newman projection along the C1′–N9 bond of the four conformational areas (syn, high syn, anti, and high anti) around the glycosidic bond. The dihedral angle O1′C1′N9C4 (χ) is indicated by means of an arrow.

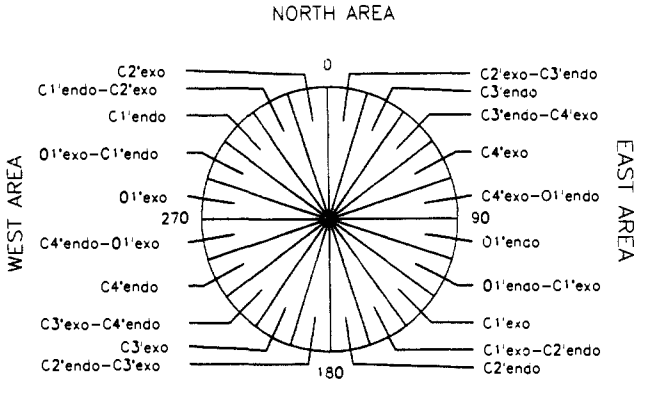

Figure 4. Representation of the pseudorotational circle.

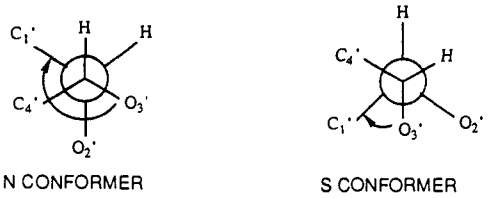

Figure 5. Newman projection along the C3′–C2′ bond, showing the value of the $\sum$3′C3′C2′C1′ dihedral angle (ϕ') in both north (N) and south (S) puckerings.

formation of the ribose by means of only two parameters. These parameters, in the model proposed by Altona and Sundaralingam,[2,37] are

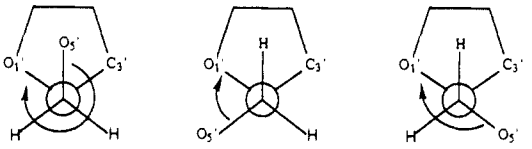

Figure 6. Newman projection along the C5′–C4′ exocyclic bond, showing the three classical conformations gg, gt, and tg around the exocyclic bond. The dihedral angle O5′C5′C4′O1′ (Ψ) is indicated by means of an arrow.

τ_m (the amplitude puckering) and P (the phase angle), which are defined according to

$$P = \text{ATAN}\,[(\tau_1 + \tau_4 - \tau_0 - \tau_3)/(b_1\tau_2)] \quad (1)$$

$$\tau_m = \tau_2/(\cos P) \quad (2)$$

where b_1 = 3.077 683 537, and ATAN is the arctangent function.

With this model (see Figure 4) all the ribose puckerings are classified in four "areas", named north, east, south, and west (N, E, S, and W).

Several authors have studied ribose conformational flexibility.[37-52] In most of these studies the ribose movement was followed by the variation of the phase angle P from 0 to 360° at a fixed value of T_m (usually between 38 and 40°), the dihedral angles τ_1, τ_2, τ_3, τ_4, and τ_0 being fixed for each value of P according to eq 3:

$$\tau_i = \tau_m \cos\,[P + c_1(i - 1)] \quad (3)$$

where i = 0, 1, 2, 3, 4 and c_1 = 144.

Studies based on the direct application of the pseudorotational model permit the examination of all the conformations of the ribose around the pseudorotational circle. Nevertheless, such studies have been criticized by several authors because (i) the amplitude of puckering (τ_m) is not strictly constant around the pseudorotational circle[40,46,50] as such studies suppose, and (ii) if five dihedral angles are fixed, the ribose loses three degrees of freedom, which causes the geometry optimization procedure to give wrong results.[50] Both problems lead to an artifactual overestimation of the pseudorotational barriers in most of these studies.[40,46,50] Levitt and Warsell[46] demonstrated that a better representation of the ribose movement around the pseudorotational pathway can be obtained by varying only one dihedral angle and optimizing the rest of the molecule. In the present study, following the Levitt and Warsell criterion, ϕ' (dihedral angle O3′C3′C2′C1′) has been chosen to follow the pseudorotational movement of the ribose (see Figure 5). Note that $\phi' \approx \tau_3 + 120$.

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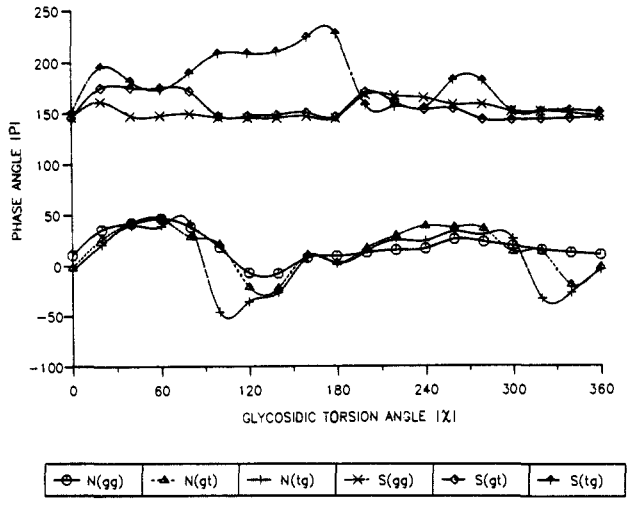

Figure 7. Representation of the variation of the phase angle (P) during the rotation around the glycosidic bond for Ngg, Ngt, Ntg, Sgg, Sgt, and Stg conformers.

3. Rotation around the Exocyclic C4′–C5′ Bond. The dihedral angle O5′C5′C4′O1′ (named Ψ) was chosen for following the rotation around the exocyclic C4′–C5′ bond (see Figures 1 and 6). There are three stable conformations with respect to this bond, which are usually called the following: gauche–gauche (gg), for values of Ψ around −60°; gauche–trans (gt), for values of Ψ around 60°; trans–gauche (tg), for values of Ψ around 180°.

All the dihedral angles are considered as positive counterclockwise.

Molecular mechanical methods provide, with a reasonable computational time, a good description of the conformational flexibility of molecules, sometimes better than the more expensive quantum chemical methods (see Discussion). Therefore, accurate and detailed conformational studies of adenosine can be performed by means of molecular mechanical methods.

The MM2 method based on Allinger's force field[52-54] is used. Parameters for the 6-aminopurine ring were taken from the values given by Kollman[55,56] and Allinger.[57] Mulliken's net charges over all the atoms of the molecule, calculated from the semiempirical AM1 wave function,[58] were included for the calculation of the electrostatic term of the total potential energy (the set of steric parameters and net charges used is available upon request).

Semiempirical AM1 calculations were performed with a locally modified version of the QCPE MOPAC package.[59,60]

Results

1. Rotation around the Glycosidic Bond. The dihedral angle O1′C1′N9C4 (χ) was rotated at 20° intervals from 0 to 360°. The three classical conformers around the exocyclic bond C4′–C5′ (gg, gt, and tg) and the two main conformations of the furanosic ring, N (C3′ endo) and S (C2′ endo), were considered. During the rotation, the entire molecule was fully optimized for every value of the dihedral angle O1′C1′N9C4 (χ).

The dependence between the glycosidic torsion angle (χ) and the phase angle (P) for gg, gt, and tg conformers in both N and S conformations is shown in Figure 7. No relevant conformational changes in the ribose puckering occur during the glycosidic rotation. For the "north" puckerings of ribose, the most favored value of P is in general in the range of 30–40°. Nevertheless, for the high syn zone, P values between −40 and 0° (C2′ exo) were obtained. Moreoever, in anti and high anti zones the phase angle oscillated between 10 and 30°. On the other hand, for the "south" puckerings, values of P between 140 and 160° were obtained in all zones except for the tg conformation, which gave values of P around 200° (C3′ exo) in the high syn zone.

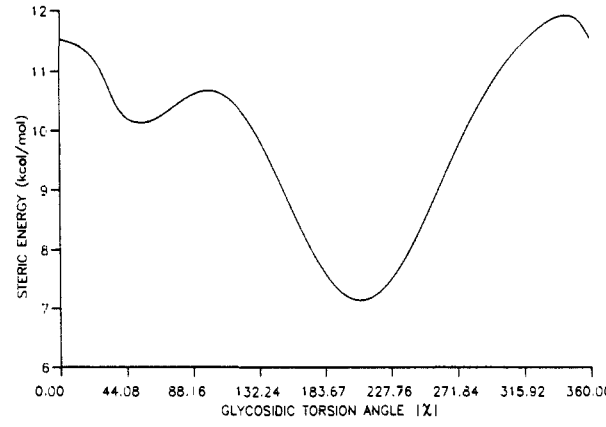

Figure 8. Representation of the variation of the steric energy of adenosine during the rotation around the glycosidic bond.

Table I. Conformations (in Both Syn and Anti Areas) Corresponding to the Minima Found during Rotation around the Glycosidic N9–C1′ Bond for All the Ngg, Ngt, Ntg, Sgg, Sgt, and Stg Conformers of Adenosine

conformer	P, deg	τ_m, deg	χ, deg	steric energy, kcal/mol
N gg anti	13.0	38.8	200	7.2
N gg syn	46.9	38.0	60	11.6
S gg anti	168.5	34.7	200	6.3
S gg syn	148.3	39.9	60	9.0
N gt anti	17.3	40.0	200	7.3
N gt syn	46.8	40.2	60	9.9
S gt anti	170.8	37.4	200	6.5
S gt syn	175.7	34.0	60	10.8
N tg anti	14.29	39.7	200	7.7
N tg syn	45.6	39.1	60	10.1
S tg anti	157.9	38.8	200	5.9
S tg syn	182.1	33.2	40	9.7

The puckering amplitude τ_m underwent small variations during the glycosidic rotation (mean value 39.1 with a standard deviation of 2.8); the minimum value of τ_m was 32.2 and the maximum 43.6.

The energy profiles obtained for the different puckerings of the ribose and for the different conformations around the exocyclic bond (Ngt, Sgt, Ngg, Sgg, Ntg, and Stg) were similar. Thus, in order to provide a more general representation of the energy profile of the glycosidic rotation and to avoid anomalous results due to local minima,[51] the mean values of the energy of the six conformations for every value of χ were chosen and a polynomial function (accounting for up to 99% of the variance) was fitted. The obtained function (Figure 8) presents two minima: one in the syn zone (values of χ around 50°), and the other in the anti zone (values of χ around 200°). The anti conformation is ~3 kcal/mol more stable than the syn. Rotation from anti to syn requires ~4 kcal/mol through the high syn zone and 5 kcal/mol through the high anti zone.

The energy minima obtained for all the Ngt, Sgt, Ngg, Sgg, Ntg, and Stg conformers are shown in Table I. The S conformation is usually more stable than the N in both syn and anti zones (differences of ~1 kcal/mol are shown). In the anti zone, tg and gg were the most stable conformers around the C4′–C5′ exocyclic bond, whereas gg was the most stable in the syn zone.

2. Ribose Puckering. The dihedral angle O3′C3′C2′C1′ (φ′) was rotated at intervals of 10° from 170 to 60° for gg, gt, and tg conformers of the exocyclic bond. The glycosidic torsion angle (χ) was initially set at 200° (anti zone). The whole molecule was fully optimized for every value of φ′.

The mean variations of the puckering amplitude (τ_m), phase angle (P), and glycosidic torsion angle (χ) detected for the gt, tg, and gg conformers during the pseudorotational movement of the ribose are represented in Figure 9 (the three conformers show

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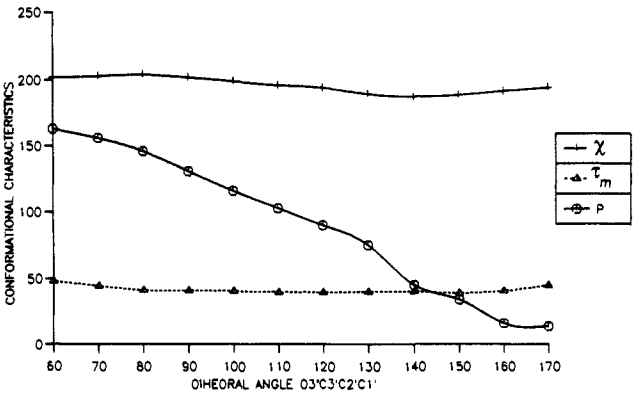

Figure 9. Representation of the variation of the phase angle (P), torsion amplitude (τ_m), and glycosidic torsion angle (χ) during rotation around the C3′–C2′ bond measured by the O3′C3′C2′C1′ dihedral angle (ϕ').

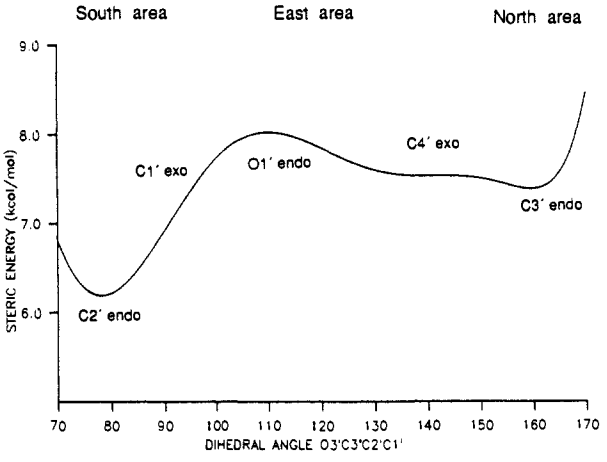

Figure 10. Representation of the variation of the steric energy of adenosine during rotation around the C3′–C2′ bond. Ribose puckerings achieved for some significant values of the dihedral angle O3′C3′C2′C1′ (ϕ') are indicated.

very similar values here, and consequently, only the means are represented). No significant variations from the starting value were found for the torsion angle χ. The puckering amplitude τ_m also exhibited small variations except for extreme values (ϕ' equal to 60, 70, and 170°). On the other hand, the phase angle varied from values greater than 150° (S area) to values around 15° (N area). The change N ↔ S occurs for $P \approx 90°$ (E area). Values of P around 270° (W area) are not detected in our study; see Figures 4 and 9.

Since all the gg, gt, and tg conformers show equivalent energy profiles, the mean value of the energy for every vaue of ϕ' was used to fit a polynomial function (explaining up to the 99% of the variance), which is represented in Figure 10. Two minima at values of ϕ' around 80° (C2′ endo) and around 160° (C3′ endo) are shown. C2′ endo is 1 kcal/mol more stable than C3′ endo. The pseudorotational barrier for conversion from south (C2′ endo) to north (C3′ endo) areas appeared at values of ϕ' around 110° (O1′ endo conformation) and was approximately 2 kcal/mol. Low-energy forms are also found for values of ϕ' centered at 140° (C4′ exo).

3. Bidimensional Study. In order to complete the conformational study of adenosine, a bidimensional study, where the pseudorotation and glycosidic rotation were simultaneously considered, was performed. The puckering of the ribose was followed by using the dihedral angle O3′C3′C2′C1′ (ϕ') and varying it from 50 to 170° in intervals of 15°; the rotation around the glycosidic bond was followed by employing the dihedral angle O1′C1′N9C4 (χ), varying it from 0 to 330° in intervals of 30°. The three classical conformers around the C4′–C5′ exocyclic bond (gg, gt, and tg) were considered. At every point of the potential surface, the whole molecule was fully optimized.

Bidimensional results (Figure 11 A–C) show the existence of a deep minimum in the south anti zone (χ around 200°) for all the gg, gt, and tg conformers (the widest and deepest minima are presented by the gg and tg conformers). Another minimum (~1 kcal/mol less stable than the first) appeared in the north anti zone. A less defined minimum (this minimum is especially clear for the gg conformer) appears in south syn zone (χ around 40–60°). Plots also show the existence of high-energy zones like the high anti zone and the east high syn zone, as well as the absence of minima in the north syn zone.

4. Molecular Modeling. A QSAR strategy was used in order to determine the conformation of adenosine in the active site of adenosine deaminase. For this purpose, the enzymatic susceptibility of several "rigid" substrates of adenosine deaminase was compared with their conformational requirements.

As noted above (see Introduction), several authors have suggested that adenosine deaminase interacts with the O5′ group of the substrate during the formation of the transition state, stabilizing the rate-limiting step of the reaction. This hypothesis is in complete agreement with the enzymatic behavior of compounds I ((*S*)-8,5′-cycloadenosine), II (8,2′-anhydro-8-hydroxy-9-β-D-xylofuranosyladenine), and III ((*R*)-8,5′-cycloadenosine), which in contrast with their close structural similarity (see Figure 12) exhibit very different V_{max} [10.2 (I), 1.7 (II), and 0.1 (III) (values taken from ref 24, V_{max}(adenosine) = 100)]. Assuming that the relative enzymatic susceptibility of compounds I–III is only related to the location of their respective O5′ groups (or O3′ if the O5′ group is unaccesible to the recognition point of the enzyme; see below), the O5′ of compound I will be closer to the optimum position for interacting with the enzyme than the O3′ of compound II, the O5′ of compound III being placed in the most unfavored position. Since adenosine (the natural substrate of the enzyme) exhibits a great conformational flexibility and is the native substrate of the enzyme, we suppose that the O5′ group of adenosine takes the optimum position for interaction with the enzyme. Consequently, it can be assumed that for the active conformation of adenosine

$$\text{distance (O5′Ado–O5′(I))} < \text{distance (O5′Ado–O3′(II))} < \text{distance (O5′Ado–O5′(III))} \quad (4)$$

In order to determine the active conformation of adenosine, the first step should be the generation of all the possible conformations of such a molecule. For this purpose, we generated up to 69 000 conformers of adenosine, resulting from the rotation of the glycosidic torsion angle (χ) at intervals of 5° from 150 to 360°, for values of the exocyclic torsion angle (Ψ) varying from 0 to 360° in intervals of 5°, and considering all the ribose puckerings determined by phase angles (P) varying from 0 to 352° in intervals of 16° for a standard puckering amplitude of 38.8°.[42,43] The syn conformation has not been taken into consideration, since (i) several authors have demonstrated that the nucleoside cannot exist in this conformation at the active site of the enzyme,[3,24-26] and (ii) when values of the dihedral angle O1′C1′N9C4 below 150° are considered, the O5′ group of adenosine is too far from the position of the O5′ group of compound I.

The conformations obtained were then subjected to an elimination procedure. First, a simple energy calculation, based on van der Waals spheres, was performed in order to determine the sterically forbidden conformations. Second, the distance between the O5′ of adenosine and the O5′ of compound I was restricted to be less than 3 Å. Third, we imposed the restriction shown in eq 4. Only a small number of conformers of adenosine were able to overcome the elimination procedure (see Figures 13–16). The active conformations of adenosine appeared for a ribose puckering determined by a phase angle between 304 and 32° and 160 and 224°. None of the active conformations appeared in the east zone (values of P from 48 to 144°) of the pseudorotational pathway, whereas the west zone presented active conformations only for values of P greater than 304° (C1′ endo close to the north area). It is interesting to note that the greatest number of "active conformations" appeared near the most stable puckerings of the ribose. That is to say, the "north" and "south" areas.

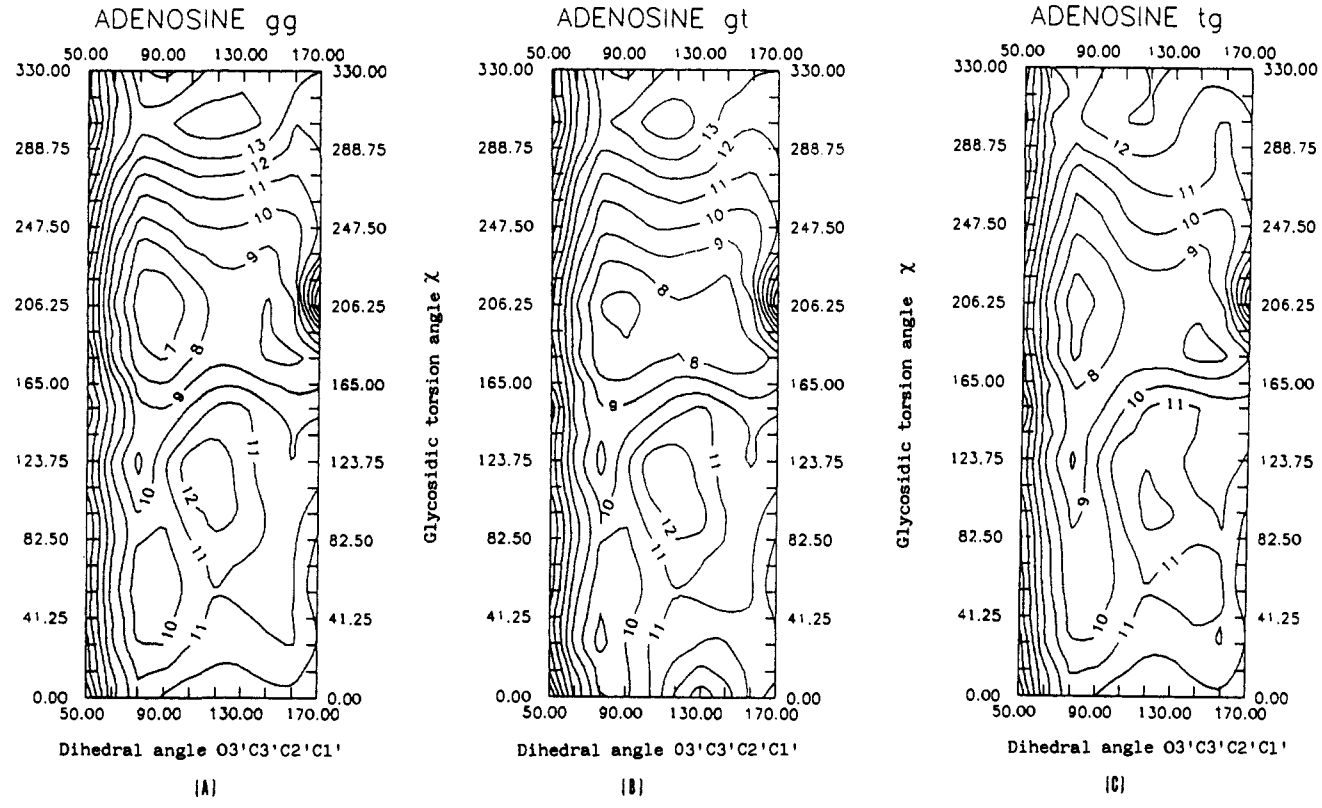

Figure 11. Steric energy surface for the simultaneous rotation around the C3′–C2′ and N9′–C1′ bonds for the three classical conformations around the exocyclic bond (gg, gt, and tg).

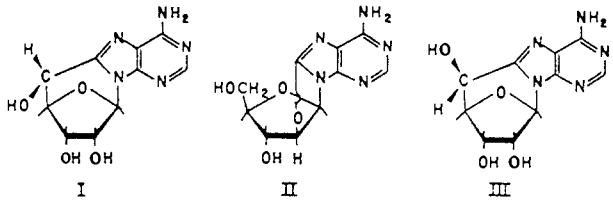

Figure 12. Schematic representation of the three intercyclated compounds chosen in the present study to define the "pharmacophoric" conditions to be achieved by the active conformation of adenosine.

With respect to the rotation around the glycosidic bond, "active" conformations were found for values of χ from 150 to 235°, the most probable values being in the range 160–200° (see Figures 13–16). It should be stressed that nearly all the conformations around the glycosidic bond detected as active were in the anti zone, close to the values detected as the most stable. Finally, when rotation about the exocyclic bond N9–C1′ was considered, only gg and gt conformations were detected as active (except for west puckerings), the gg conformation being present in a greater number of active conformers of adenosine (see Figures 15 and 16). Also, it is notable that the proportion of gg versus gt was always greater in low-energy puckerings (P between 16 and 32° and between 160 and 176°).

Discussion

1. Conformation of Adenosine around the Glycosidic Bond. Results point to the existence of two areas of minimum energy, one in the syn zone (χ = 50°) and the other in the anti zone ($\chi \approx 200°$). They also reveal the existence of unstable conformations in the high syn and high anti zones. These facts are in excellent agreement with experimental data. Thus, crystal data reported by Rao[39] suggest that the mean value of the glycosidic torsion angle for syn nucleosides is around 48° (with a standard deviation equal to 12), and data compiled by Sundaralingam[37] suggest that χ is around 50° (standard deviation equal to 13). Likewise, data for anti nucleosides compiled by Sundaralingam[37] suggest a mean value of χ around 210° (standard deviation equal to 23) in the anti zone. It should also be stressed that very few nucleosides appear in high anti and high syn zones,[37] as our theoretical studies predict. Finally, when our results are compared with the two X-ray structures of adenosine, the agreement is also excellent (χ = 190°[61] for neutral adenosine, and χ = 223°[62] for N1-protonated adenosine). The agreement is also excellent with the NMR data,[63] which indicate that the most stable glycosidic angle is around 190°.

Data obtained from X-ray studies of neutral (χ = 190°[61]) and protonated (χ = 223°[62]) adenosine fall near the minima detected by us. NMR data[63] suggest values of $\chi \approx 190°$. Finally, a few nucleosides appear in high anti and high syn zones,[37] in clear accordance again with our results.

The location of the minima are very similar to those obtained from recent[64] ab initio STO-3G calculations (χ = 30 and 210°) and the classical potential (CP) method[63] (χ = 40 and 200°). Both methods also predict the low stability of high syn and high anti zones. Discrepancies are found with the IEHT method,[36] which detects minima at χ = 340, 150, 200, and 250°. Thus, the minimum in the syn zone does not appear in IEHT calculations, whereas two minima in "unstable" high syn and high anti zones appear. Different results are obtained with respect to PCILO calculations; this method found minima in the syn zone around 90°[63] or 100°,[65] which are not in syn but rather in the high syn area. On the other hand, it is not clear whether another PCILO minimum is placed around 270°[41,63] or between 160 and 240°.[65]

The energy difference between syn and anti zones calculated in the present work is around 3 kcal/mol, whereas the barrier for anti–syn conversion is around 4 (high syn area) and 5 kcal/mol (high anti area). These results show that adenosine is most frequently found in the anti conformation, which is in clear accordance with the predominance of nucleosides in the anti zone,[37,39] and with all the X-ray and NMR data of adenosine.[1,61–63] Our

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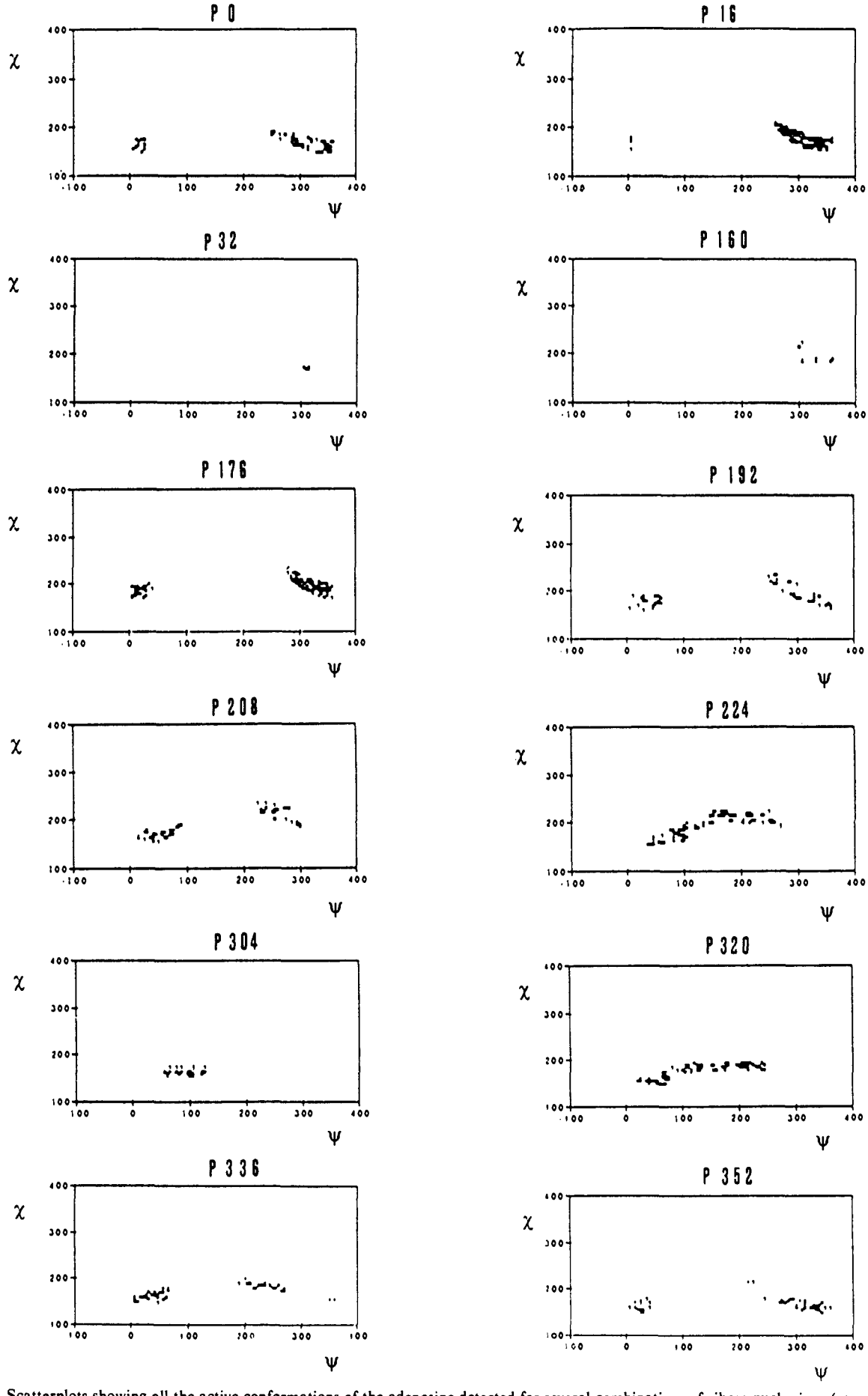

Figure 13. Scatterplots showing all the active conformations of the adenosine detected for several combinations of ribose puckerings (represented by means of the phase angle *P*), of conformations around the glycosidic bond (represented by the glycosidic torsion angle χ), and of conformations around the exocyclic bond (represented by the dihedral angle O5′C5′C4′O1′ Ψ).

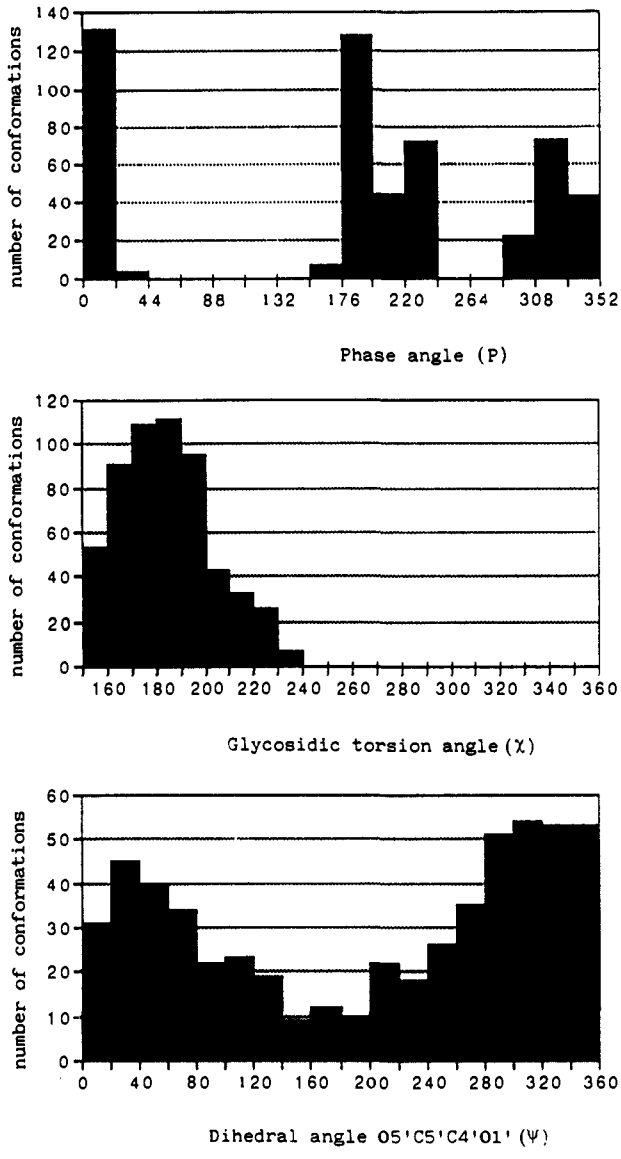

Figure 14. Representation of the number of active conformations of adenosine depending on the ribose puckering, glycosidic torsion angle, and O5′C5′C4′O1′ (Ψ) dihedral angle. The total number of conformations generated and tested was 69 552 (see text).

value of the energy difference syn–anti is greater than that suggested by NMR results.[63] On the other hand, our energy barrier for anti–syn conversion is in reasonable agreement with the value of 6.2 kcal/mol[66] reported in relaxation studies.

Our syn–anti differences are very close to those determined with ab initio methodology[64] (2.5 kcal/mol); whereas the CP method[63] suggests that both syn and anti conformations are nearly energetically equivalent. Since the PCILO method[41,63,65] does not detect minima in the anti and syn zones, it does not provide comparable results. On the other hand, our anti–syn conversion barriers seem more realistic than those detected by IEHT,[36] CP,[63] and ab initio STO-3G[64] methods (greater than 20 kcal/mol). Barriers detected by the PCILO method are in the range 2.5–4 kcal/mol,[41,63,65] but as was noted above, they correspond to incorrect values of χ.

No drastic changes in the ribose puckering during the rotation around the glycosidic bond are observed in our study, the calculated values of the phase angle (*P*) being in excellent agreement with the experimental data.[37] Nevertheless, a high flexibility is shown in both N and S areas and several minor conformational changes occur. Thus C3′ endo predominates in the N area in syn anti and high anti zones, whereas C2′ exo and C3′ endo coexist

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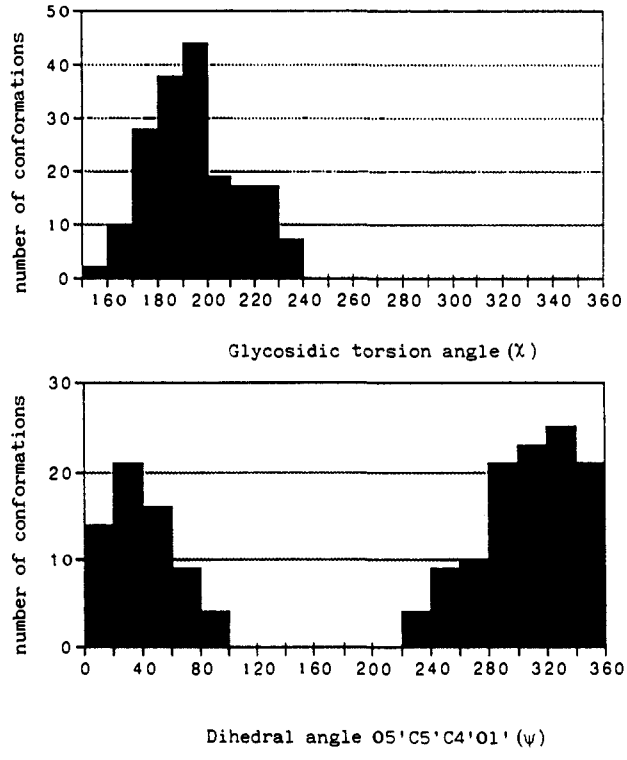

Figure 15. Representation of the number of active conformations of adenosine depending on the glycosidic torsion angle (χ) and O5′C5′C4′O1′ dihedral angle (Ψ) for puckering in the south area of ribose (values of phase angle (*P*) between 160 and 208°).

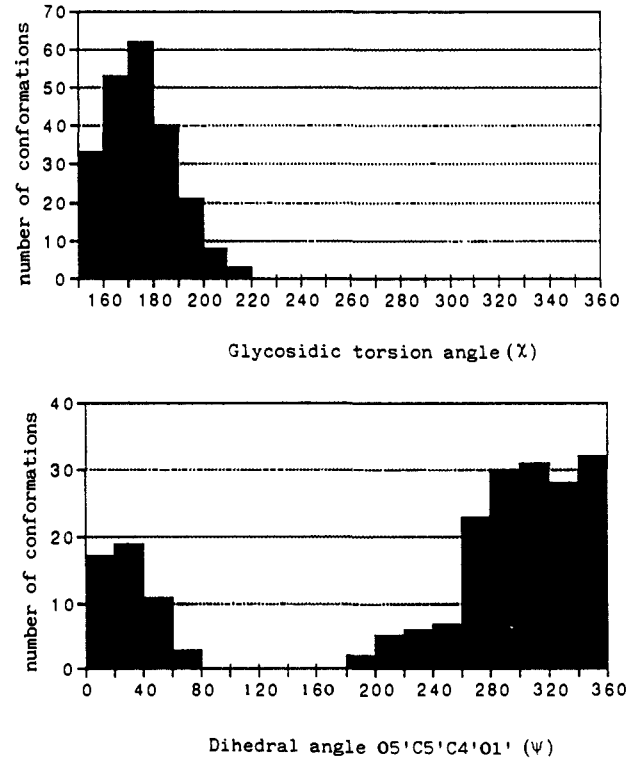

Figure 16. Representation of the number of active conformations of adenosine depending on the glycosidic torsion angle (χ) and the O5′C5′C4′O1′ dihedral angle (Ψ) for puckering in the north area of ribose (values of phase angle (*P*) between 336 and 32°).

in the high syn zone. In the S area, C2′ endo predominates for all the values of χ (except for the tg conformer in the high syn zone where C3′ exo puckering exists).

The puckering amplitude τ_m undergoes small variations upon glycosidic rotation. The mean value (39.1°) is similar to that commonly found for the puckering amplitude of nucleosides.[37]

Comparison with other theoretical studies is not feasible since, to our knowledge, no references appear in the literature about the variation of ribose puckering during the glycosidic rotation.

2. Ribose Puckering. Results presented in Figure 9 show that the dihedral angle O3′C3′C2′C1′ (ϕ') is able to fully represent the N to S conversion, which occurs through the east area of the pseudorotational pathway. This result agrees with all the available experimental and theoretical data (see refs 37–51), which state that the west area of the pseudorotational circle is energetically forbidden to the ribose. Two energy minima appear, one for values of ϕ' around 80° (P around 145–150° (C2′ endo)), and the other for ϕ' around 160° (P around 20° (C3′ endo)). A low-energy zone is detected for values of ϕ' around 140° (C4′ exo zone). All these results are in excellent accordance with available experimental data for N (P from 1 to 34°, with a mean value of 12°[37]) and S nucleosides (P from 139 to 213°, with a mean value of 164°[37]), as well as with the relative stability of C4′ exo conformers.[40,46]

Our results show that the S conformation (C2′ endo) is ~1 kcal/mol more stable than the N conformation (C3′ endo). The pseudorotational barrier from S to N conversion being ~2 kcal/mol in the east zone (O1′ endo). The preference suggested by our results for the S conformation has been demonstrated experimentally,[39,63,67] and differences between 0.12 and 0.42 kcal/mol[39,63,67,68] have been reported. The barrier for the S to N conversion was estimated by early relaxation studies[47] as lying between 4.2 and 5.2 kcal/mol. Nevertheless, this range has recently been criticized[48] since it seems to be overestimated; thus, a pseudorotational barrier of approximately 3 kcal/mol has been proposed by several authors.[42,43,48]

Previous theoretical works, such as PCILO studies, found differences between S and N in the range 0.4–0.7 kcal/mol[63] or around 1 kcal/mol[41] and barriers around 4–5 kcal/mol.[41] Levitt's force field calculations[46] give values of the pseudorotational barrier of 0.5 kcal/mol (N and S being of similar stability). Molecular dynamic studies of Gerlt and Youngblood[69] indicate differences of 0.4 kcal/mol between S and N conformations and barriers around 1.5 kcal/mol. Other molecular mechanical and dynamic calculations[51] suggest differences between S and N around 1 kcal/mol, and barriers between 1 and 2 kcal/mol. Weiner and Kollman[55,56] found differences between S and N from −0.68 to +1.46 kcal/mol. Olson's force field[42,43] provides barriers from 1.3[55] to 4 kcal/mol.[42,43] On the other hand, ab initio STO-3G//3-21G calculations[49] of several furanose rings led to unrealistic results because low puckering amplitudes were observed (τ_m around 20°), and no pseudorotational barriers in the east zone were present.

3. Bidimensional Study. Bidimensional studies (Figure 11) summarize a great part of the monodimensional results discussed above. In addition, these studies also provide other valuable information not evident in the monodimensional studies. Thus, three stable zones appear: two in the anti zone (for N and S puckerings) and only one in the syn zone (for S puckering). High anti and high syn zones (specially for east puckerings) are highly unstable. The predictive power of Figure 11 can easily be understood if it is considered that almost all the purine nucleosides listed in refs 37 and 39 fall in the preferred zones shown in these figures. Also, both X-ray[61,62] and NMR structures[1,63,67,68] of adenosine fall in these areas. To our knowledge, only one bidimensional study like the present is available in the literature. It was performed by Saran, Perahia, and Pullman in the 1970s using the PCILO method.[41] In that work, the pseudorotational phase angle (at a fixed value of τ_m) was chosen to follow the ribose puckering and the glycosidic torsion angle χ (following the old criteria; see Methods) was chosen to follow the rotation around the glycosidic bond. The study was performed without optimization of the angles and bond distances and only the gg conformer was taken into consideration. The results obtained by Saran et al. suggest the existence of minima in high anti and high syn areas in contrast with experimental evidence.[37m39]

4. Molecular Modeling. Our results make it evident that, if it is assumed that O5′ is the only group of the ribose interacting with the enzyme (as experimental evidence mentioned above suggest), adenosine can be recognized by the active site of adenosine deaminase in more than one conformation, since the high flexibility of the molecule makes it possible that the O5′ group can adopt the optimum position in different conformations of adenosine.

Our results suggest that adenosine could be recognized in either north or south puckerings, but it seems plausible that east or west puckering would be recognized by the enzyme. Results also show that adenosine is probably recognized in the anti conformation around the glycosidic bond in a conformation near the most stable one. Results suggest that it is possible to discard the high anti as the active conformation in opposition with previous suggestions.[3] With respect to the conformation around the exocyclic C4′–C5′ bond, our results allow the conclusion that the gg and gt can be active conformers.

It must be emphasized that results obtained from molecular modeling studies are in excellent accordance with the energy results mentioned above, since the areas with a higher number of active conformations (N and S, gg and anti with χ between 170 and 200°) are the most feasible conformational areas from an energy point of view.

In conclusion, both strategies used here (energy and molecular modeling studies) strongly suggest that adenosine is recognized by the active site of adenosine deaminase in the anti north or anti south areas, probably in the most stable conformation or in one that is very close to that.

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