Theor Chem Acc (2003) 110: 466–472
DOI 10.1007/s00214-003-0503-x

Theoretical
Chemistry Accounts

*Regular article*

# Caffeine interactions with nucleic acids. Molecular mechanics calculations of model systems for explanation of mechanisms of biological actions

**V.I. Poltev**[1,2], **T.I. Grokhlina**[3], **A. Deriabina**[1], **E. González**[1]

[1] Fac. Ciencias Físico-Matemáticas, BUAP, Ciudad Universitaria, San Manuel, C.P. 72570 Puebla Pue., México
[2] Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia
[3] Institute of Mathematical Problems of Biology, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia

Received: 8 September 2002 / Accepted: 15 May 2003 / Published online: 25 November 2003


**Abstract.** To understand the molecular mechanisms of the influence of caffeine (CAF) on DNA functioning, molecular mechanics calculations of the interaction energy of CAF with nucleic acid bases and base pairs have been performed. The calculations reveal three types of mutual CAF–base (and CAF–base pair) arrangements corresponding to minima of the interaction energy. Besides well-known stacking mutual positions of the molecules, two other types of arrangements are revealed and studied. One of these arrangements corresponds to the nearly in-plane position of CAF and base (or base pair) and the formation of a single hydrogen bond. Another type of minimum corresponds to nearly perpendicular arrangements of the molecular planes and the formation of intermolecular hydrogen bonds. These two arrangements are possible both for individual nucleic acid monomers and for DNA duplexes. The calculations suggest the molecular mechanisms of the influence of CAF on DNA interactions with other biologically active molecules.

**Keywords:** Caffeine – DNA interactions – Molecular mechanics – Energy calculations

## Introduction

Caffeine (CAF) is one of the most widely and regularly consumed biologically active substances [1]. The main action of CAF is mood altering. It acts as a stimulant of the central nervous system [2]. This is the reason for consumption by most humans of various of CAF-containing beverages, such as coffee, tea, chocolates and colas. Another action of CAF is cardiovascular [3]: it influences blood pressure. At the same time CAF has an effect on various other biological processes, including DNA functioning; for example, it has been shown that CAF is capable of reducing the toxicity of a typical DNA intercalator such as ethidium bromide [4, 5], and the efficacy of a number of aromatic anticancer drugs, such as doxorubicin and its analogues, and of mitoxantrone, ellipticine etc. [6, 7, 8]. There is evidence of inhibition of DNA repair by CAF [9, 10, 11].

Some aspects of the biological activity of CAF result from interactions with nucleic acids. Addition of CAF affects the binding of aromatic drugs with DNA [12, 13, 14], and the changes in binding of drugs and other aromatic molecules with DNA are due both to the competition of drug and CAF for DNA binding sites and to the formation of CAF–ligand complexes [13, 14]. Thus, complexation of CAF with nucleic acid components and with biologically active substances is of great importance from the viewpoint of both fundamental and applied science.

In this paper we describe the results of molecular mechanics computations of interactions of CAF with nucleic acid bases and base pairs in relation to CAF effects on DNA functioning.

CAF is a purine derivative, 1,3,7-trimethylxantine (Fig. 1). It contains three hydrogen-bond acceptors (atoms O2, O6 and N9), three methyl groups, but has no proton–donor groups; hence CAF cannot form complexes containing two hydrogen bonds either with other CAF molecules or with any of the DNA bases. By searching the minima of interaction energies between CAF and nucleic acid bases or base pairs, the various possibilities of CAF binding to nucleic acids and their fragments have been examined. To the best of our knowledge, there are no such systematic computations on these systems either by molecular mechanics or by quantum mechanical methods.

From the Proceedings of the 28th Congreso de Químicos Teóricos de Expresión Latina (QUITEL 20002).

*Correspondence to:* E. González
e-mail: gonzalez@fcfm.buap.mx

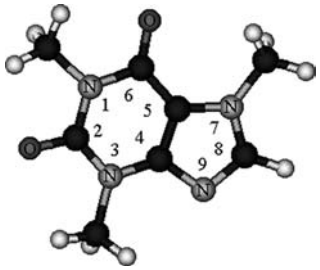

**Fig. 1.** The structure and atom numbering of the caffeine (*CAF*) molecule

Three types of minima of interaction energies between CAF and nucleic acid bases and base pairs have been revealed. The first type corresponds to stacking or a nearly parallel arrangement of the aromatic rings of the molecules, and resembles a base–base stacking minimum. The second type of minimum refers to a nearly in-plane position of the two aromatic molecules. These minimum-energy arrangements are stabilized by hydrogen bonds between one of the CAF hydrogen-bond acceptors (O2, O6, N9) and a hydrogen-bond donor group of the base. One more type of energy minimum corresponds to a substantially nonplanar, nearly perpendicular arrangement of the aromatic rings of the two molecules. These minima are the most important ones from our viewpoint. The last two types of minima may be due to interaction of CAF with both bases of monomeric DNA units and base pairs in undisturbed DNA duplexes.

## Methods of computation

The methods of computation of interaction energies for CAF–CAF, CAF–base and CAF–base pair complexation resemble those for base–base interactions [15]. The energy of the base–base interactions was calculated as a sum of pairwise interactions of all atoms constituting the molecules. Each atom–atom interaction consists of a Coulomb term and a Lennard-Jones 6–12 term, commonly used in molecular mechanics calculations (Eq. 1). For descriptions of interactions of hydrogen atoms capable of forming hydrogen bonds, the 6–12 term is substituted by a 10–12 term (Eq. 2).

$$E_{ij} = \frac{e_i e_j}{r_{ij}} - \frac{A_{ij}}{r_{ij}^{6}} + \frac{B_{ij}}{r_{ij}^{12}}, \tag{1}$$

$$E_{ij} = \frac{e_i e_j}{r_{ij}} - \frac{A_{ij}^{(10)}}{r_{ij}^{10}} + \frac{B_{ij}^{(10)}}{r_{ij}^{12}}. \tag{2}$$

In these equations $r_{ij}$ is the distance between atoms $i$ and $j$, and $e_i$ and $e_j$ are charges on atoms $i$ and $j$ (calculated by the semiempirical methods of quantum chemistry and reproducing the experimentally determined dipole moments of molecules). The coefficients $A_{ij}$, $B_{ij}$, $A_{ij}^{(10)}$ and $B_{ij}^{(10)}$ are adjustable parameters, which were somewhat recently changed [16] compared to our previous studies (e.g. Ref. [15]). The adjustment of these parameters results in better agreement with experimental data for the interaction energies between bases in a vacuum, for interatomic distances in hydrogen-bonded base pairs and for the distances between base planes in crystals of nucleic acid monomers [16]. These coefficients are listed in Table 1. The main changes in the refinement of the potentials refer to interactions of atoms participating in hydrogen bonds (H1, N3 and O) with each other and with other atoms. These changes do not disturb other correlations between the results of calculations and the experimental data mentioned in our earlier paper [15].

The model molecules of 1-methylpyrymidines and 9-methylpurines were considered to avoid hydrogen bonding for the atoms that participate in glycoside bonds of nucleic acids. The energy was calculated and minimized as a function of variables corresponding to the displacements of one molecule with respect to another along $x$-, $y$- and $z$-axes, to rotations around these axes and to intramolecular rotations around the single bonds connecting methyl groups to the aromatic rings. The geometry of the nucleic acid bases cor-

**Table 1.** Coefficients for 6–12 and 10–12terms of atom–atom potential functions. The first row for each atom pair corresponds to the 6th-power term ($A_{ij}$, Å$^6$ kcal/mol) or to the10th-power term ($A_{ij}^{(10)}$, Å$^{10}$ kcal/mol). The second row corresponds to the 12th-power term ($B_{ij}$, Å$^{12}$ kcal/mol). Hydrogen atoms H1, H2 and H3 are capable of forming hydrogen bonds, aromatic and aliphatichydrogens, respectively; C1 and C2 are aliphatic and aromatic carbons,respectively; N1 is the nitrogen of the amino group; N2 and N3 are pyrrolo and pyridino nitrogens, respectively; O is the carbonyl oxygen

| Atom | H1 | H2 | H3 | C1 | C2 | N1 | N2 | N3 | O |
|---|---|---|---|---|---|---|---|---|---|
| H1 | 35 | 35 | 40 | 76 | 100 | 80 | 126 | 9,070[a] | 10,670[a] |
| | 5,914 | 5,914 | 7,740 | 53,940 | 25,700 | 29,200 | 24,150 | 27,000 | 31,100 |
| H2 | 35 | 40 | 40 | 100 | 126 | 105 | 126 | 146 | 121 |
| | 5,914 | 7,740 | 7,740 | 70,600 | 61,700 | 53,700 | 53,700 | 58,700 | 17,800 |
| H3 | 40 | 40 | 40 | 100 | 126 | 105 | 126 | 146 | 121 |
| | 7,740 | 7,740 | 7,740 | 70,600 | 81,600 | 62,000 | 71,400 | 78,300 | 42,200 |
| C1 | 76 | 100 | 100 | 250 | 316 | 264 | 316 | 367 | 305 |
| | 53,940 | 70,600 | 70,600 | 512,000 | 601,000 | 464,000 | 538,000 | 598,000 | 349,000 |
| C2 | 100 | 126 | 126 | 316 | 400 | 477 | 400 | 464 | 385 |
| | 25,700 | 61,700 | 81,600 | 601,000 | 704,000 | 947,900 | 630,000 | 699,000 | 406,000 |
| N1 | 80 | 105 | 105 | 264 | 477 | 280 | 334 | 391 | 455 |
| | 29,200 | 53,700 | 62,000 | 464,000 | 947,900 | 421,000 | 488,000 | 544,000 | 664,000 |
| N2 | 126 | 126 | 126 | 316 | 400 | 334 | 400 | 440 | 616 |
| | 24,150 | 53,700 | 71,400 | 538,000 | 630,000 | 488,000 | 565,000 | 537,300 | 816,000 |
| N3 | 9,070[a] | 146 | 146 | 367 | 464 | 391 | 440 | 550 | 465 |
| | 27,000 | 58,700 | 78,300 | 598,000 | 699,000 | 544,000 | 537,300 | 705,000 | 413,000 |
| O | 10,670[a] | 121 | 121 | 305 | 385 | 455 | 616 | 465 | 400 |
| | 31,100 | 17,800 | 42,200 | 349,000 | 406,000 | 664,000 | 816,000 | 413,000 | 240,000 |

[a] $A_{ij}^{(10)}$

responds to the averaged crystal structure; it was not changed as compared to in our previous works [15, 16]. The geometry of the CAF molecule is that from the CAF monohydrate crystal [17]. Heterocyclic ring geometries were fixed during minimization. Standard gradient minimization techniques were used throughout the work. Minimizations were started from various mutual molecule positions, corresponding both to suggested complex configurations (e.g. stacking or hydrogen-bonded ones) and to arbitrarily chosen configurations obtained by random displacements and rotations of one molecule with respect to another one.

## Self-association of CAF molecules and CAF–base stacking

Like many aromatic heterocyclic molecules CAF has energy minima corresponding to stacking self-association. This process has been investigated using both experimental [14, 18, 19, 20, 21] and computational [21, 22] techniques. According to an NMR study [14, 18, 20] CAF molecules in aqueous solutions form stacks with substantial overlap of the aromatic rings, with about 90° rotation of one molecule with respect to another, and with an interplane distance of about 3.4 Å.

A search for minima in the interaction energies for two CAF molecules was performed starting from the position of one molecule above another and various angles of rotations around the axis perpendicular to the plane of the heterocyclic ring. Both parallel (face-to-back) and antiparallel (face-to-face) arrangements were considered. These computations reveal seven local minima, all of them corresponding to nearly parallel arrangements of the rings, to interplane distances from 3.37 to 3.42 Å and to energy values between 10.9 and 11.9 kcal/mol. Similar to the minima of the energy of interaction between two stacked DNA bases, these minima are rather flat, i.e. substantial consistent mutual shifts and rotations are possible without an increase of the interaction energy by more than 0.5 kcal/mol.

Two of the minima found are displayed in Table 2 and Fig. 2, one for face-to-back and one for face-to-face arrangements of molecules; they are considered to correspond most closely to the experimental data. In addition with the general features mentioned previously, there is qualitative correlation between the interproton distances in the calculated structures and published experimental data on intermolecular nuclear Overhauser enhancement (NOE) measurements [21]. NOE

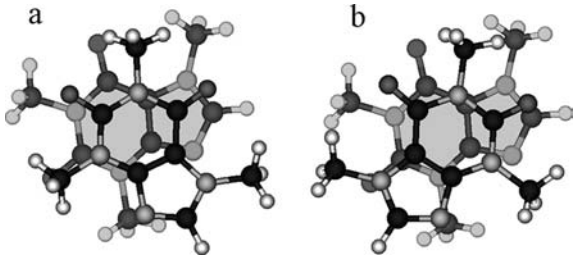


**Fig. 2a,b.** Stacking arrangements of two CAF molecules in two calculated local energy minima of CAF–CAF interactions. **a** Face-to-back and **b** face-to-face arrangements

**Table 2.** Energy values, inter plane distances and angles for two calculated local energy minima of caffeine (*CAF*)–CAF interactions. Mutual arrangements of the molecules are shown in Fig. 2

| Arrangement | *E* (kcal/mol) | Interplane distance (Å) | Interplane angle |
|---|---|---|---|
| Face-to-back | −11.1 | 3.40 | 3.1 |
| Face-to face | −11.1 | 3.42 | 0.6 |

techniques allow pairs of hydrogen atoms rather close to each other to be detected and the distance between them to be estimated. It appears that the distances between H8 and C7 hydrogens and between C1 and C3 hydrogens are larger than 6 Å, consistent with the absence of NOE contacts between these nuclei in 1-D NMR experiments [21].

The closest interproton distance in the calculated structures is for hydrogen atoms of methyl groups in the first and in the seventh positions of the ring, which corresponds to the most intensive NOE peak in the NMR experiment. As the minima are flat and several minima have rather similar CAF–CAF interaction energies, one might expect superposition of a few types of rather different configurations for molecules in aqueous solutions. The most probable configurations are selected by interactions with the surrounding water.

There are several stacking minima for interactions of CAF with each of the bases of DNA. Only one minimum for parallel (↑↑) and one for antiparallel (↑↓) CAF–base arrangements is displayed (Table 3, Fig. 3). Other minima may correspond to considerably different mutual arrangements of molecules, and all the comments on the influence of surrounding water for CAF–CAF minima are valid for CAF–base minima as well.

## In-plane association of CAF with nucleic acid bases. Energy minima with a single N–H...N or N–H...O hydrogen bond

There are a few minima of such type for each DNA base, corresponding to all pairwise combinations of CAF acceptor atoms and base hydrogens capable of forming hydrogen bonds. For some such combinations, two or more energy minima with rather different CAF–base arrangements may exist. A rather close (but not shortened) contact between the methyl group of CAF and a negatively charged atom of the base arises in most of the situations corresponding to such minima. One minimum is considered here for each CAF–base pair. The mutual arrangements of the molecules in these minima are presented in Fig. 4 and the values of the energy in these

**Table 3.** Energy values (kcal/mol) for some minima of caffeine-base stacking interaction energies

| Base | Adenine | Thymine | Guanine | Cytosine |
|---|---|---|---|---|
| *E* (↑↑) | −10.5 | −9.5 | −13.1 | −11.4 |
| *E* (↑↓) | −10.8 | −9.6 | −13.1 | −10.8 |

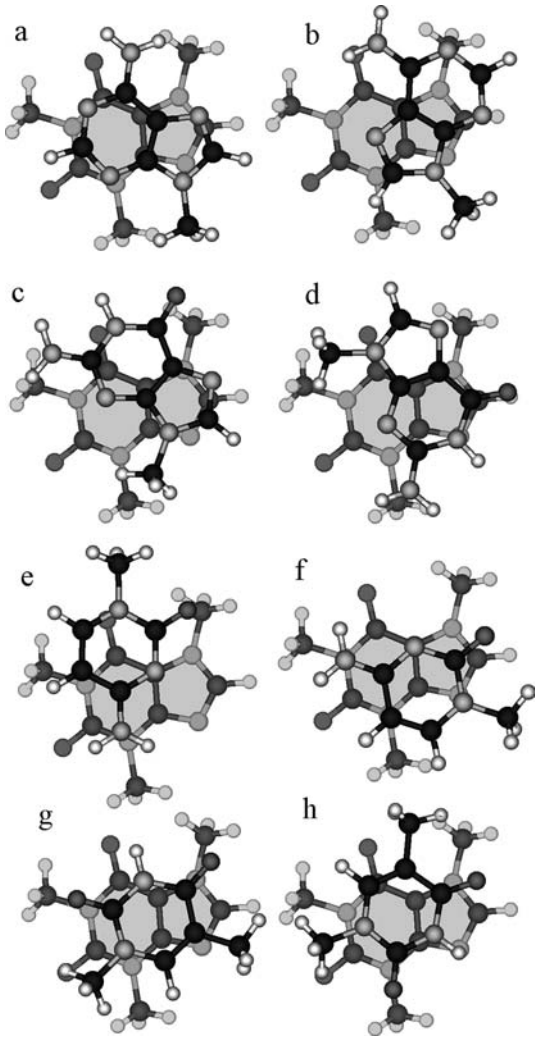


**Fig. 3a–h.** Some stacking arrangements of CAF and bases: **a**,**b** adenine; **c**,**d** guanine; **e**,**f** cytosine; **g**,**h** thymine. *Left* and *right* positions correspond to parallel and antiparallel minima, respectively. The energy values are listed in Table 3

minima and the atoms involved in the hydrogen bonds are shown in Table 4.

The majority of the deepest minima of this type for each base have more negative energy values when compared to similar minima for interaction energies between the natural bases. For guanine–CAF interactions, minima with bifurcated hydrogen bonds, i.e. with two hydrogen bonds formed by the same acceptor atom, are found. The guanine molecule contains two hydrogen-bond donor groups (N1–H and N2–H21) rather close to each other, which enables such minima to occur with both natural and modified nucleic acid bases. The minimum presented here is an example of a minimum with greater (by absolute value) energy compared to the minimum with a single hydrogen bond.

The minima listed in Table 4 and Fig. 4 correspond to the interaction of CAF with base ring hydrogens which can also be involved in complementary base pairing; thus, these CAF–base arrangements are only possible for separate bases or singled-stranded nucleic acid fragments. There are other minima of such a type when base hydrogens exposed in helix grooves are involved. These minima are considered in Sect. 6. It is practically impossible to observe these minima experimentally in simple model systems (such as mixed solutions of CAF and base) because there are other, more favorable positions, corresponding to the minima of the other types of interactions (Sects. 3, 5). Nevertheless, mutual arrangements similar to those considered in this section are possible for more complex systems, such as DNA fragments and their complexes with intercalated ligands.

### Minima corresponding to perpendicular arrangements of CAF–base planes

Analogous to the minima considered in the previous section, there are corresponding minima for each combination of CAF hydrogen-bond acceptors and base

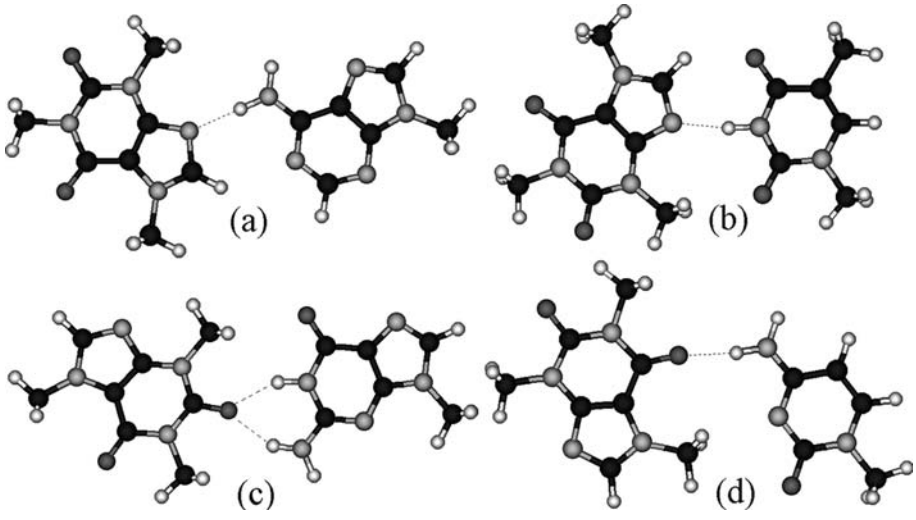


**Fig. 4a–d.** Some mutual in-plane arrangements of CAF and nucleic acid bases in the local minima of interaction energies. **a** CAF–adenine, **b** CAF–thymine, **c** CAF–guanine, **d** CAF–cytosine. The energy values as well as the atoms of CAF and the base involved in hydrogen bonding are listed in Table 4

**Table 4.** Energy values for minima of interaction energy corresponding to some nearly in-plane arrangements of CAF–base. The atoms of CAF and the base involved in hydrogen bonding are listed

| Bases | Adenine | Thymine | Guanine | Cytosine |
|---|---|---|---|---|
| Hydrogen-bonded atoms | N9–H61 | N9–H3 | O2–H1, H21 | O6–H41 |
| *E* (kcal/mol) | −8.0 | −9.2 | −11.6 | −9.2 |

**Table 5.** Values of energy and interplane angle for some nearly perpendicular arrangements of CAF and base at the minima of the interaction energy

| Bases | Adenine | Thymine | Guanine | Cytosine |
|---|---|---|---|---|
| Hydrogen-bonded atoms | N9–H62 | N9–H3 | N9–H1, H21 | N9–H41 |
| Angle | 69.8 | 77.4 | 87.1 | 89.9 |
| *E* (kcal/mol) | −10.7 | −9.7 | −14.2 | −12.0 |

hydrogen-bond donor groups. For guanine, the minima correspond to formation of bifurcated hydrogen bonds between the pair of neighboring hydrogens, H1 and H21, and CAF hydrogen-bond acceptors. All the other minima of such a type are stabilized by a single hydrogen bond that is practically linear.

The quantitative results are presented for one example of such minima for each type of DNA base (Fig. 5, Table 5) in which the minima with nearly perpendicular arrangements of the planes of the interacting molecules are considered to be the most important ones. For each base the calculations have shown that the minima of this type are the deepest ones. However, similar to the minima with two molecules in a plane, these CAF–base arrangements are practically impossible to detect experimentally in aqueous solutions of mixtures of CAF and a base. Although the interaction energy in these minima is more favorable than for stacking minima, interactions with water molecules make stacking association complexes more favorable in aqueous solutions. We suggest that the minima of such a type may be revealed in experimental NMR studies of mixed solutions of CAF and nucleic acid bases. To the best of our knowledge, no such study has been reported in the literature yet. Considering various relative CAF–base concentrations and using the results of molecular mechanics calculations, it would be possible to characterize the most favorable CAF–base arrangements in a nonpolar environment as a model of interactions of CAF with DNA bases in a cell.

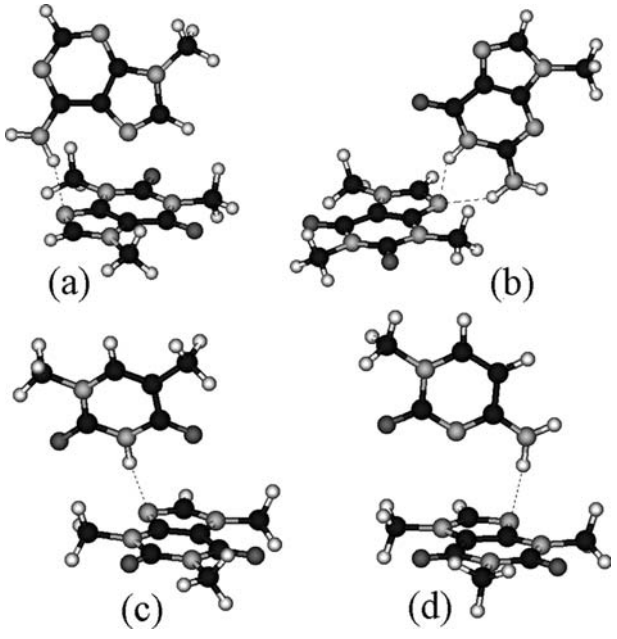


**Fig. 5a–d.** Examples of energy minima for nearly perpendicular arrangements of CAF and base. Energy values and interplane angles are listed in Table 5

## Interactions of CAF with complementary base pairs. Possible patterns of major groove and minor groove CAF–DNA complexation

All three types of minima considered in the previous sections are possible for interactions between CAF and both mispairs and complementary base pairs. The minima corresponding to a stacking arrangement of CAF above (and below) base pairs will not be considered in detail here. The energy of the deepest minimum for the interaction of CAF with the adenine:thymine base pair is −14 kcal/mol, the angle between CAF and the base pair plane being about 4° and the approximate interplane distance being about 3.4 Å. The energy values for these minima are more negative when compared with the two other types of minima, but intercalation of CAF into the DNA duplex requires a substantial change of the helix structure, resulting in the weakening of base stacking. Such changes are possible when intercalation occurs for molecules with three or four conjugated rings (like acridine dyes or the heterocyclic part of daunomycin or actinomycin D), where the interaction energies with base pairs of such aromatic molecules are 2–3 times greater than that with CAF.

More interesting minima are those corresponding to nearly coplanar or perpendicular CAF–base pair arrangements. The adenine:thymine base pair has only one hydrogen, H62, capable of forming a hydrogen bond with CAF. The deepest minimum, corresponding to a nearly perpendicular arrangement of the CAF–base pair and formation of an N9...H62–N2 hydrogen bond has an energy value of −10.6 kcal/mol. Structures close to this minimum position can be realized in many conformations of B-DNA duplexes.

The guanine:cytosine base pair has two hydrogens, H42 of cytosine and H22 of guanine, capable of forming hydrogen bonds with CAF. The first case corresponds to the CAF molecule situated in the major groove of the

**Table 6.** Values of energy and interplane angles for some minima of the CAF-guanine:cytosine base pair interaction energies

| Base | Base atom | CAF atom | *E* (kcal/mol) | Angle |
|---|---|---|---|---|
| Guanine | H22 | N9 | −11.5 | 80.9 |
| | | O6 | −11.4 | 57.1 |
| | | O2 | −10.2 | 62.4 |
| Cytosine | H42 | N9 | −11.2 | 89.7 |
| | | O6 | −10.8 | 88.7 |
| | | O2 | −8.0 | 84.2 |
| Cytosine | H42 | N9 | −9.1 | 0.5 |
| | | O6 | −10.0 | 0.8 |
| | | O2 | −7.8 | 18.4 |

duplex and the second case to the CAF molecule in the minor groove.

Formation of a hydrogen bond between CAF and H42 of cytosine is possible for many duplex conformations and nucleotide sequences, whilst binding to guanine is only possible for certain conformations of defined sequences. The deepest energy minima of the interaction between the guanine:cytosine pair and CAF are shown in Table 6 and Fig. 6.

## Conclusions

1. Molecular mechanics calculations of CAF interactions with nucleic acid bases and base pairs have been performed.
2. The calculations enable three types of interaction energy minima to be revealed. Besides the well-known stacking arrangement of aromatic molecules, the energy minima correspond to nearly in-plane and nearly perpendicular arrangements.
3. The existence of these minima enables the influence of CAF on DNA functioning via complexation with both biologically active molecules and double helical DNA fragments to be explained.

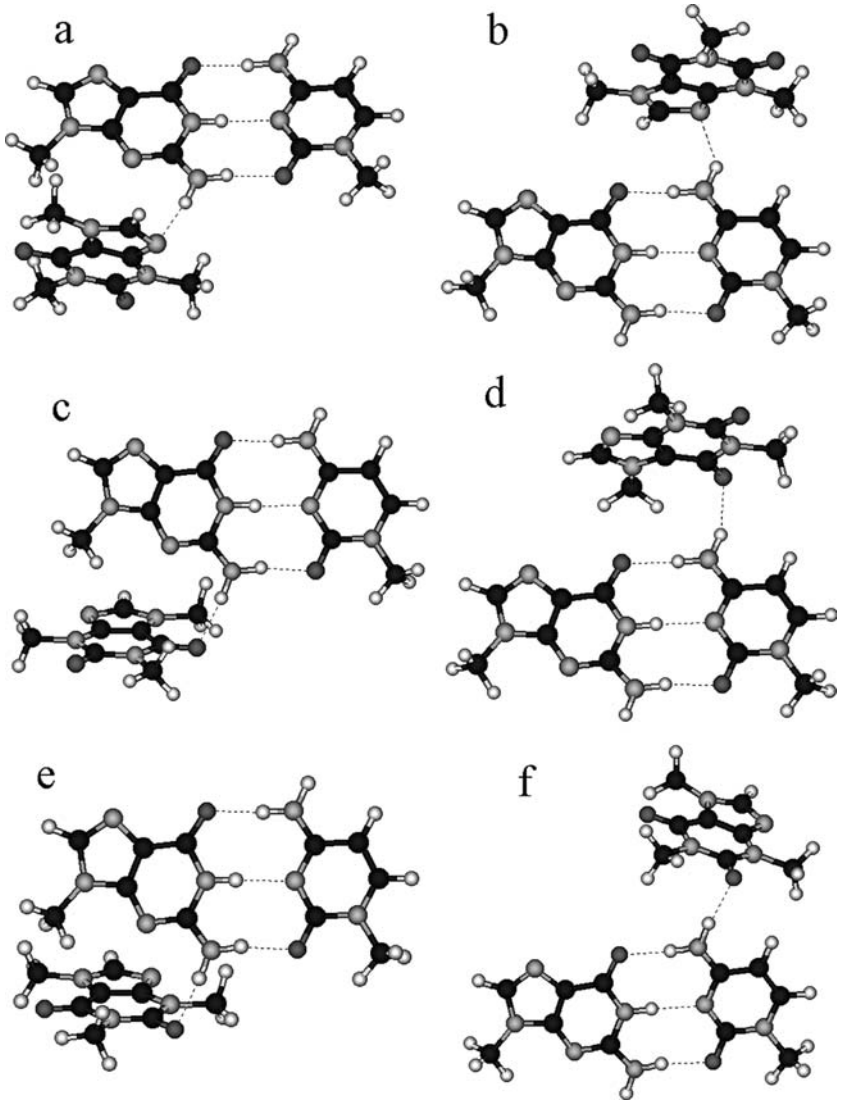


**Fig. 6a–f.** Examples of nearly perpendicular mutual positions of CAF and the guanine:cytosine base pair for the local minima of the interaction energies. For clarity, the base pairs are somewhat inclined with respect to the figure plane and oriented upwards by its major (non-glycosidic) groove and downwards by its minor (glycosidic) groove. Energy values and interplane angles are listed in Table 6

*Acknowledgements*. We thank D. Davies and A.N. Veselkov for helpful discussion. This work was partially supported by CONACyT, Mexico, project no.35239-E, by VIEPBUAP-CONACyT, project no. II28G02, and by INTAS, project no. 97-31753.

## References

1. Brice CF, Smith AP (2002) Int J Food Sci Nutr 53:55
2. Brauer LH, Buican B, De Wit H (2002) Behav Pharmacol 5:111
3. Nurminen ML, Niittynen L, Korpela R, Vapaatalo H (1999) Eur J Clin Nutr 53:831
4. Hixon SC, Yielding KL (1976) Mutat Res 34:195
5. Wolf K, Kaudewitz F (1976) Mol Gen Genet 146:89
6. Eberhard C, Herrmann RL (1972) J Bacteriol 112:224
7. Selby CP, Sancar A (1991) Biochemistry 30:3841
8. Traganos F, Kapuscinsky J, Darzynkiewicz Z (1991) Cancer Res 51:3682
9. Selby CP, Sancar A (1990) Proc Natl Acad Sci USA 87:3522
10. Tempel K, von Zallinger C (1997) Z Naturforsch C 52:466
11. Piosik J, Zdunek M, Kapuscinski J (2002) Biochem Pharmacol 63:635
12. Larsen RW, Jasuja R, Hetzler RK, Muraoka PT, Andrada VG, Jameson DM (1996) Biophys J 70:443
13. Davies DB, Veselkov DA, Evstigneev MP, Veselkov AN (2001) J Chem Soc Perk Trans 2:61
14. Davies DB, Veselkov DA, Djimant LN, Veselkov AN (2001) Eur Biophys J 30:354
15. Poltev VI, Shulyupina NV (1986) J Biomol Struct Dyn 4:739
16. Poltev VI, Deriabina AS, Gonzalez E, Grokhlina TI (2002) Biophysics 47:972
17. Sutor DG (1958) Acta Crystallogr 11:453
18. Low-Sing Kan, Borer PhN, Cheng DM, Ts'o POP (1980) Biopolymers 19:1641
19. Fritzsche H, Petri I, Schutz H, Weller K, Sedmera R, Lang H (1980) Biophys Chem 11:109
20. Fritzsche H, Lang H, Sprinz H, Pohle W (1980) Biophys Chem 11:121
21. Falk M, Chew W, Walter JA, Kwiatkowski W, Barclay KD, and Klassen GA (1998) Can J Chem 76:48
22. Danilov VI, Slyusarchuk ON, Poltev VI, Alderfer JL, Wollman RM, Brickmann JA, Lautenschlager P (1992) J Biomol Struct Dyn 9:1239