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Molecular mechanics and dynamics study of DNA–furocoumarins complexes: Effect of methylation of the angular derivatives on the intercalation geometry

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SUMMARY

Results of molecular mechanics calculations on intercalation complexes between DNA and angelicin derivatives: angelicin, 4'-methylangelicin, 5'-methylangelicin, 4,4'-dimethylangelicin, 4,5'-dimethylangelicin, 4,6,4'-trimethylangelicin and 4,6,5'-trimethylangelicin, are presented. The correlation between the presence of methyl groups and an increase in DNA photobinding affinity is discussed on the basis of the molecular structures. The influence of the orientation of the angelicins within the intercalation cavity is also discussed. Finally, the consequences of the dynamical behaviour of angelicin in the intercalation site are studied.

INTRODUCTION

Furocoumarins are heterocyclic compounds resulting from the fusion of a furan ring to a coumarin molecule. They are widely studied for their use in the photochemotherapy of skin diseases [1] and as molecular probes in molecular biology [2]. Figure 1 represents the general skeleton of the linear furocoumarin or psoralen. The biological activity of furocoumarins relies upon their capacity to bind covalently to DNA pyrimidine bases under influence of UV-A irradiation, in a three-step reaction [3]. The first step consists of a noncovalent intercalation of the drug between two base pairs. Upon UV-A irradiation, monophotoadducts are formed by C_4 photoaddition between the 5,6 bond of a pyrimidine and either the 4',5' ethylenic bond (furan-side

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Abbreviations. CNDO: complete neglect of differential overlap; NMR: nuclear magnetic resonance; rms: root mean square; UV-A: ultraviolet light of class A ($320 < \lambda < 400$ nm).

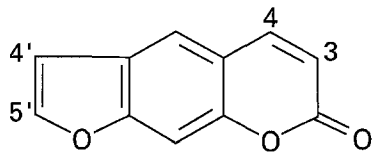

Fig. 1. Chemical structure of the psoralen.

adduct) or the 3,4 double bond (pyrone-side adduct) of the furocoumarin. In the last step, the furocoumarins linked by the furan side can absorb a second photon and, if the geometrical configuration is favourable, can form an interstrand cross-link by reacting with a pyrimidine belonging to the opposite strand of the DNA.

The most important application of furocoumarins, and of psoralen in particular, is their use in PUVA therapy (i.e. psoralen plus UV-A). In such treatments, psoralen derivatives are given orally or per os and the skin region to be treated is irradiated by UV light of class A ($320 < \lambda < 400$ nm). This treatment generally induces an efficient regression of skin cell proliferation, which has been largely connected to the above-quoted DNA photomodifications (monoadducts and/or interstrand cross-links), although the involvement of other macromolecular photomodifications is also suspected [4]. An undesired side-effect of PUVA therapy is the potential mutagenic and carcinogenic character of the treatment and several attempts have been made this last decade to look for new psoralen derivatives that would show less pronounced mutagenic and carcinogenic side-effects. The general guideline which has been proposed to search for new derivatives was to modify the chemical structure of the psoralen skeleton in order to avoid the formation of interstrand cross-links which are thought to be responsible for the mutagenicity and carcinogenicity of psoralens.

Among the ways to hinder the formation of cross-links is the use of angular furocoumarins, or angelicins, which have been proposed and largely studied by a group in Padova [5–7]. Figure 2a gives the chemical structure of angelicin. Although angelicins can photoreact through their 4',5' and 3,4 double bonds in solution [8–10], they are supposed to be unable to induce interstrand cross-links within DNA, for geometrical reasons. Indeed, the chemical structure of the various photoadducts formed within DNA by angelicins is progressively elucidated [8,11–14], and no biphotoadduct formed within DNA has been isolated to date. It is now clear that the geometrical arrangements allowed in the first step of the noncovalent angelicin–DNA intercalation determine the subsequent photoreactions. Therefore, a structural survey of the various intercalation complexes may be of help in the understanding of the angelicin photoreaction mechanism and especially in the explanation of the specific behaviour of the molecules, induced by the variations in design from the original angelicin.

Since no crystallographic or NMR data concerning these intercalation complexes are available, a theoretical approach can be of help. Indeed, model building and molecular mechanics (energy minimization) techniques are suitable to study intercalation complexes [15–18]. It has already been shown that these techniques yield molecular models which, although not as accurate as X-ray or NMR structures, are nevertheless precise enough to study intercalation complexes [15–21]. In particular, a good account of the experimental data has been obtained with molecular models of linear furocoumarins [19,20].

We have therefore built, energy-minimized and studied the intercalation complexes formed by

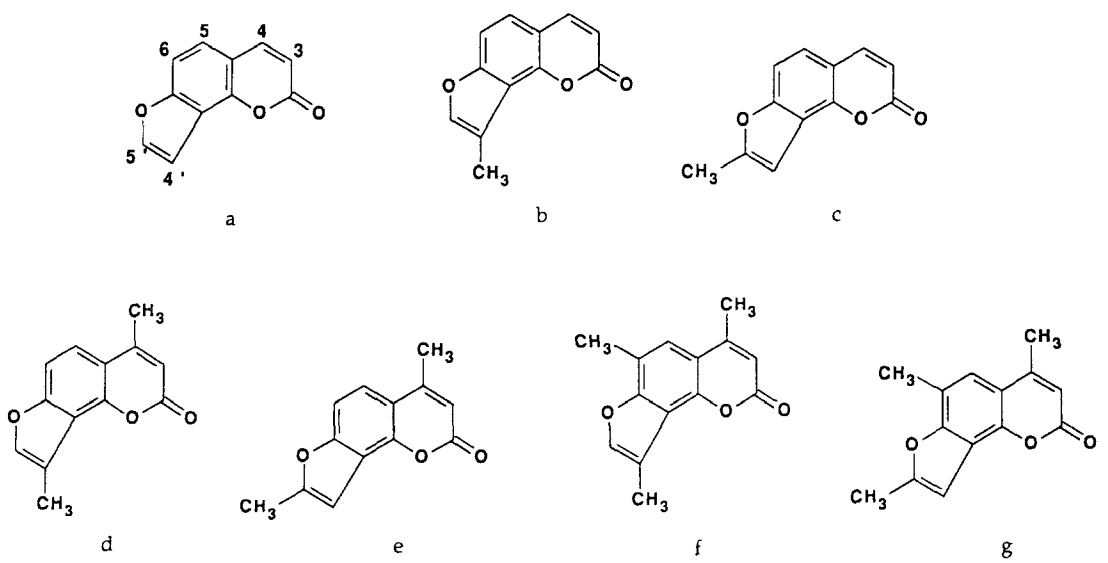

Fig. 2. Chemical structures of the angelicin derivatives. (a) angelicin; (b) 4'-methylangelicin; (c) 5'-methylangelicin; (d) 4,4'-dimethylangelicin; (e) 4,5'-dimethylangelicin; (f) 4,6,4'-trimethylangelicin; (g) 4,6,5'-trimethylangelicin.

a B-form d(CGCGATATCGCG)$_2$ duodecanucleotide and various angelicin derivatives recently proposed by the Padova group and exhibiting, for some of them, very interesting photobiological properties [5–7]: angelicin, 4'-methylangelicin, 5'-methylangelicin, 4,4'-dimethylangelicin, 4,5'-dimethylangelicin, 4,6,4'-trimethylangelicin and 4,6,5'-trimethylangelicin. Figure 2 shows the chemical structure of these molecules.

MATERIALS AND METHODS

Interactive model building and molecular dynamics trajectory visualization were performed on an Evans & Sutherland PS-390 interactive graphics display using the programs FRODO [22] and HYDRA (written by R. Hubbard). Molecular mechanics and dynamics calculations were performed on a MicroVAX II minicomputer and on a STAR ST-100 array processor (connected to a VAX 11/780 minicomputer), using CHARMm version 21 [23]. The STRANGE rigid-body energy minimization routine [19] was used in the molecular mechanics calculations.

Molecular structures

A canonical B-form d(CGCGATATCGCG)$_2$ duodecanucleotide [24] was used as the starting structure for the model building; the base-numbering convention is:

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A1...                 ...A12
5'-CGCGATATCGCG-3'
3'-GCGCTATAGCGC-5'
B12...                ...B1
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The angelicin atomic coordinates are from Bravic et al. [25]; the atomic coordinates of the methyl groups in the methylated derivatives were determined using standard stereochemical data [26].

Model building and energy minimization

The model building and energy minimization procedure has been described previously [19]. We have built models with the two possible orientations of the angelicins in the intercalation site (in fact, four orientations reduced to two by the symmetry of the sequence of the oligonucleotide). It is noteworthy that only the orientation which leads to the *cis-syn** furan-side and *cis-anti* pyrone-side adducts has been experimentally observed [14]. Nevertheless, we have also tried to model complexes with the alternative orientation of the angelicin, which would lead to the *cis-anti* furan-side and *cis-syn* pyrone-side adducts, in an attempt to determine the reasons why such adducts have never been observed experimentally.

Approximations were made to simulate the effect of counterions, solvent and shielding, since the calculations were performed in vacuo. Phosphate group charges were therefore reduced so as to give a slightly negative charge on each nucleotide (–0.32 e) [27] and a distance-dependent dielectric was chosen [23,28]. The parameter set [29] has been tailored to specific cutoff: van der Waals energy terms were shifted down to yield a zero value at 9.5 Å, while electrostatic and H-bond energy functions were zeroed by a sigmoid cutoff function in the intervals 9.5–10.5 Å and 4.0–7.0 Å, respectively. The additional parameters for angelicins were derived from a previous paper [19]; partial atomic charges have been obtained from CNDO calculations.

Molecular dynamics

A molecular dynamics simulation has been performed on the angelicin–oligonucleotide complex in a *cis-syn* furan-side and *cis-anti* pyrone-side conformation. Since the current STAR implementation of CHARMm does not allow the simulation of an 'infinite' DNA chain by using periodic boundary conditions, harmonic constraints were applied on the first and last base pairs of the oligonucleotides, in order to model the constraints due to the interactions with the adjacent base pairs in an 'infinite' chain. This technique, which would be unsuitable for the study of the oligonucleotide dynamics, has no effect on the dynamics of the intercalation site which is considered in the current study. Indeed, the value of the harmonic constraint (1.0 kcal/mol) has been fitted so that the rms fluctuations of the six central base pairs are similar in amplitude to those computed in a completely unconstrained dynamics run. Newtonian equations of motion were integrated with the Verlet algorithm [30]. A 0.25-fs integration step was chosen as no constraint was applied on internal coordinates; particularly, the SHAKE [31,32] algorithm was not used.

The procedure for the molecular dynamics calculations was:

– a 15-ps thermalization from 0 to 300 K in 2-K steps — a given temperature being obtained by assigning velocities from a Gaussian distribution with a variance corresponding to this temperature;

– a 20-ps equilibration during which the velocities were randomly reassigned every 0.4 ps to provide a homogeneous velocity distribution;

**cis* and *trans* refer to the position of the psoralen and pyrimidine moieties with respect to the cyclobutane rings, the two moieties being located at the same side of the cyclobutane ring in the *cis* conformation, and at opposite sides in the *trans* conformation. In the furan-side adducts, *syn* (head-to-head) defines adducts in which the cyclobutane ring involves covalent bonds between pyrimidine C6- and furan C5'-atoms (see Fig. 4), while *anti* (head-to-tail) defines adducts in which these carbons are located diagonally opposite each other within the cyclobutane ring. In the pyrone-side adducts, *syn* defines adducts in which C2 of the pyrone and N1 of the pyrimidine are bonded to adjacent corners of the cyclobutane ring while *anti* is applied when these atoms are bound diagonally to opposite corners.

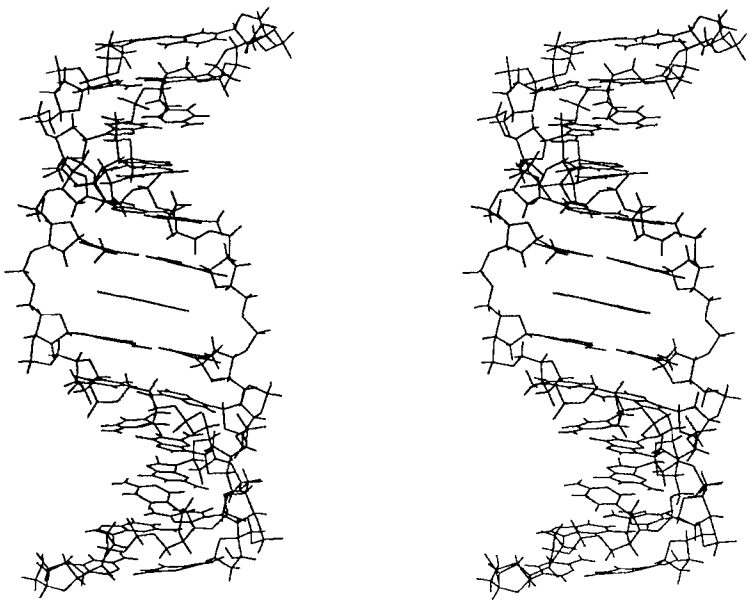

Fig. 3. Stereodrawing of the computed structure of the angelicin–d(CGCGATATCGCG)$_2$ complex; the angelicin is viewed from the major groove.

– a 30-ps equilibration during which the velocities were rescaled if the temperature fell outside a +/– 10K window, the temperature being checked every 0.4 ps (a subsequent 'productive' dynamics calculation is performed only if rescaling occurs once maximally);

– a 50-ps 'productive' (i.e. neither thermalization nor equilibration) dynamics calculation. A time-averaged structure (over the 'productive' dynamics) was then computed and energy-minimized.

RESULTS

Geometrical aspects

Angular vs. linear furocoumarins

Figure 3 is a stereoview of the final computed structure of the complex between d(CGCGATATCGCG)$_2$ and angelicin. As observed before with linear furocoumarins [19], the intercalation does not perturbate much the DNA geometry outside the intercalation cavity. Concerning angelicin itself, the result of the energy minimization is a configuration with a full intercalation, the drug being parallel to the adjacent base pairs.

Figure 4 represents a detailed view of the complex formed by the oligonucleotide and angelicin, after energy minimization. Figure 4a represents the molecules, with all their hydrogen atoms, viewed from the minor groove, the axis of the helix being parallel to the projection plane. The two dotted lines joining carbons 5 and 6 of the adjacent thymine and carbons 4' and 5' of angelicin indicate the chemical bonds which are formed experimentally in a predominant manner when irradiating such a complex (furan-side monoadduct). Figure 4b represents the molecules,

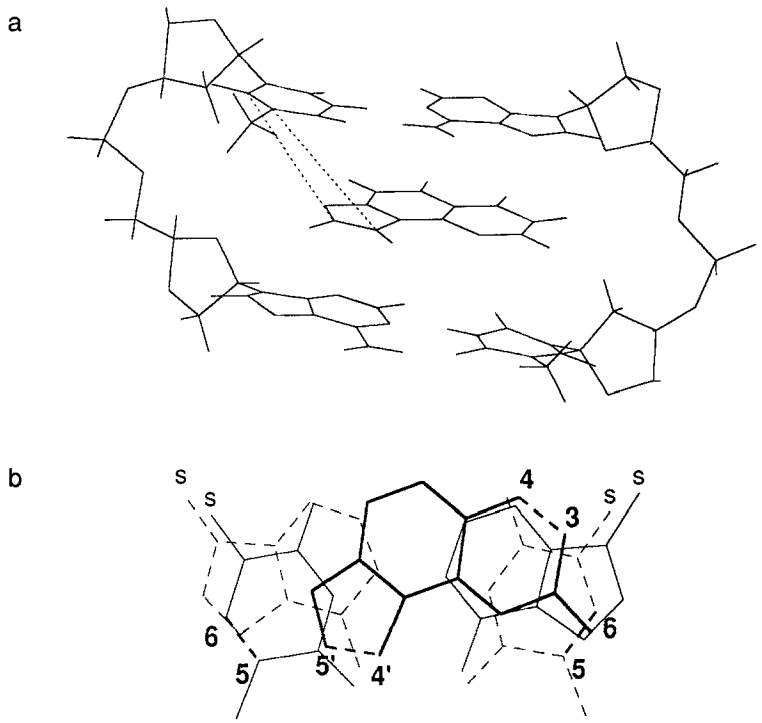

Fig. 4. (a) Angelicin viewed from the major groove, in a projection plane parallel to the helix axis. The vertical dotted lines which join the drug to the adjacent thymines indicate the covalent bonds which can be formed by irradiation of such a complex with UV-A light through the formation of C_4 cyclobutane bridges. (b) Angelicin and its two adjacent base pairs in a projection plane orthogonal to the helix axis. The left thymine and the right adenine are above the psoralen, the left adenine and right thymine (in dashed lines) are below the drug. S indicates the position of the sugar moiety.

without the hydrogens, in a projection plane perpendicular to the helix axis, the left thymine and right adenine being above the angelicin, and the left adenine and right thymine (in dashed lines) being under the angelicin, respectively. In order to visualize the differences observed in the geometry of the complexes formed between DNA and either angular derivatives (angelicins) or linear derivatives (psoralens), Fig. 5 shows the corresponding detailed view of the complex formed by the oligonucleotide and one of the most often used psoralen derivatives, namely 8-methoxypsoralen. The comparison of the two figures clearly shows that, whereas in the case of the linear derivative the geometrical arrangement appears to be favourable to the formation of both a furan-side and a pyrone-side photoadduct and therefore of an interstrand DNA cross-link, only the formation of monoadducts can be envisaged in the case of angular derivatives.

Effect of methylation of the angular furocoumarins

Figures 6 and 7 represent detailed views of the calculated energy-minimized geometries of various methylated angelicin derivatives, intercalated between the two central alternating AT base pairs of the duodecanucleotide, in a projection plane orthogonal to the helix axis. The molecules are represented without the hydrogen atoms, the left thymine and the right adenine (in

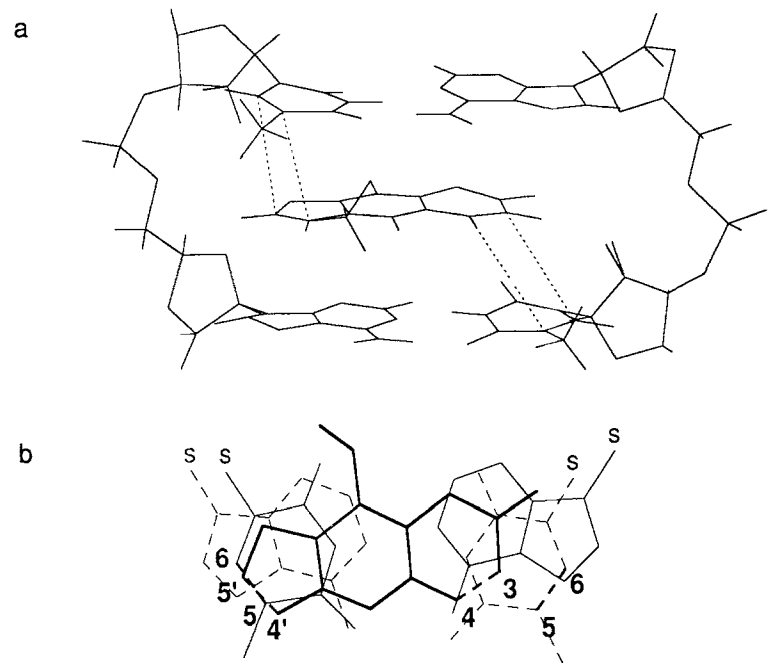

Fig. 5. (a) 8-MOP viewed from the major groove, in a projection plane parallel to the helix axis. The vertical dotted lines which join the drug to the adjacent thymines indicate the covalent bonds which can be formed by irradiation of such a complex with UV-A light through the formation of C_4 cyclobutane bridges. (b) 8-MOP and its two adjacent base pairs in a projection plane orthogonal to the helix axis. The left thymine and the right adenine are above the psoralen, the left adenine and right thymine (in dashed lines) are below the drug. S indicates the position of the sugar moiety.

bold line) being above the drug, the left adenine and the right thymine (in dashed lines) being under the drug. In bold dashed lines are shown the photoreactive double bonds of the angelicin (furan 4',5' and pyrone 3,4) and of the thymine (5,6), whose relative position gives an indication of the ability of the intercalating molecule to yield formation of photocycloadducts upon UV-A irradiation of the complex.

The two possible intercalation complexes of each angelicin are shown. Figure 6 represents the complexes with the orientation of the angelicin which leads to furan *cis-syn* and pyrone *cis-anti* adducts after photoreaction (which are experimentally observed and hereafter referred to as 'Orientation 1'), while Fig. 7 represents the complexes with the orientation of the angelicin which leads to furan *cis-anti* and pyrone *cis-syn* adducts after photoreaction (hereafter referred to as 'Orientation 2'). It is noteworthy that the structural features deduced from experimental data (i.e. the complete intercalation of the angelicins and therefore the orthogonality between the angelicins and the helix axis) are the main characteristics of the energy-minimized complexes.

Various geometrical parameters are reported in Tables 1 and 2 (for Orientations 1 and 2, respectively). These tables give the distances between the two carbon atoms of the angelicin photoreactive double bonds (furan and pyrone) and the corresponding carbon atoms of the thymines of strands A and B, respectively. They also contain the calculated values of two opposite angles of the virtual C_4 rings which prefigure the cyclobutanes, formed in some cases by UV-A irradiation of such complexes.

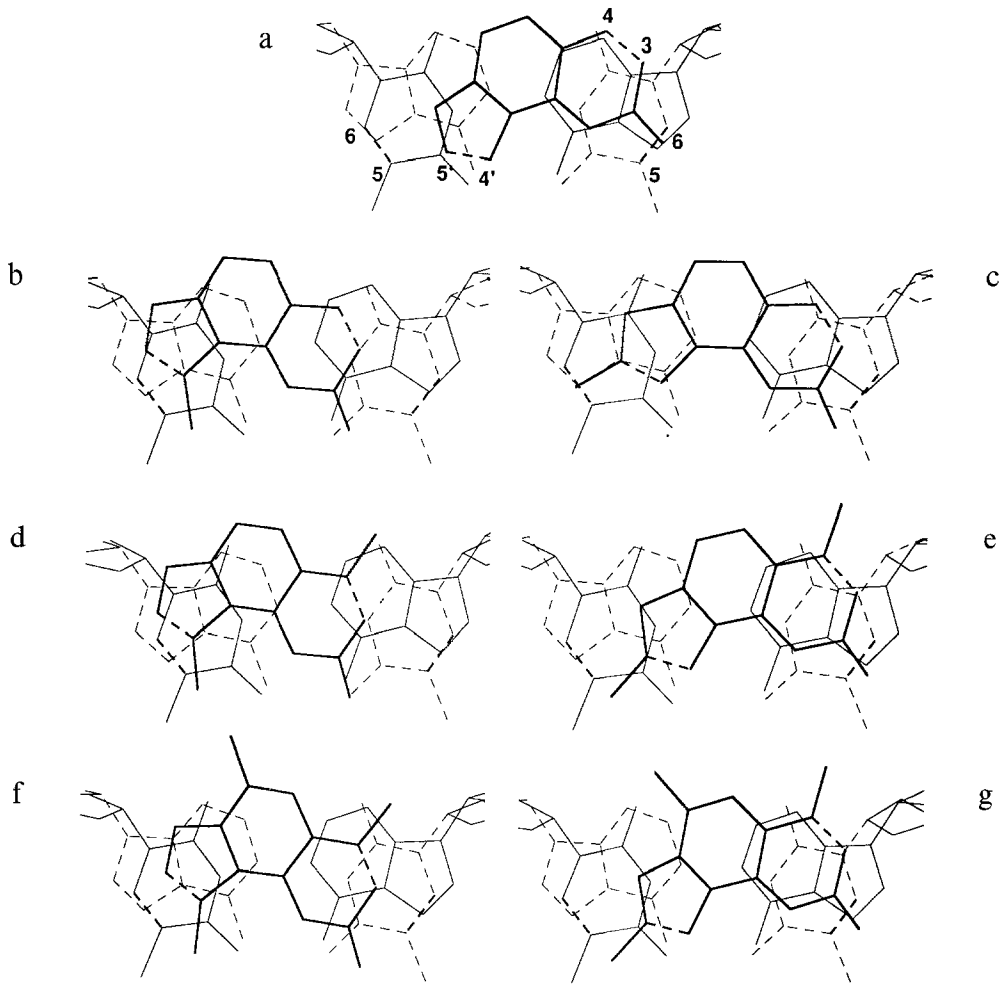

Fig. 6. Calculated intercalation geometries of the angelicins in the furan *cis-syn* and pyrone *cis-anti* conformations between the two central AT base pairs, in a projection plane orthogonal to the helix axis. The left thymine and the right adenine, in solid lines, are above the drug whereas the left adenine and the right thymine, in dashed lines, are below the drug; the photoreactive double bonds are represented by bold dashed lines. (a) angelicin; (b) 4'-methylangelicin; (c) 5'-methylangelicin; (d) 4,4'-dimethylangelicin: (e) 4,5'-dimethylangelicin; (f) 4,6,4'-trimethylangelicin; (g) 4,6,5'-trimethylangelicin.

Energetical aspects

Various potential energy terms which can be deduced from the molecular mechanics calculations are reported in kcal/mol in Tables 3 and 4 (for Orientations 1 and 2, respectively). The potential energy terms are given for the complex (E-tot) and for its DNA and angelicin moieties (E-oligo and E-drug, respectively). The interaction energy between DNA and angelicin (E-inter), as well as its van der Waals and electrostatic contributions (E-inter-vdW and E-inter-elec, respectively) are also given. It should be noted that E-tot = E-oligo + E-drug + E-inter.

It has to be recalled that the calculated values have no absolute meaning and thus should only be considered for comparative purposes. Amongst the energy terms which are listed in Tables 3

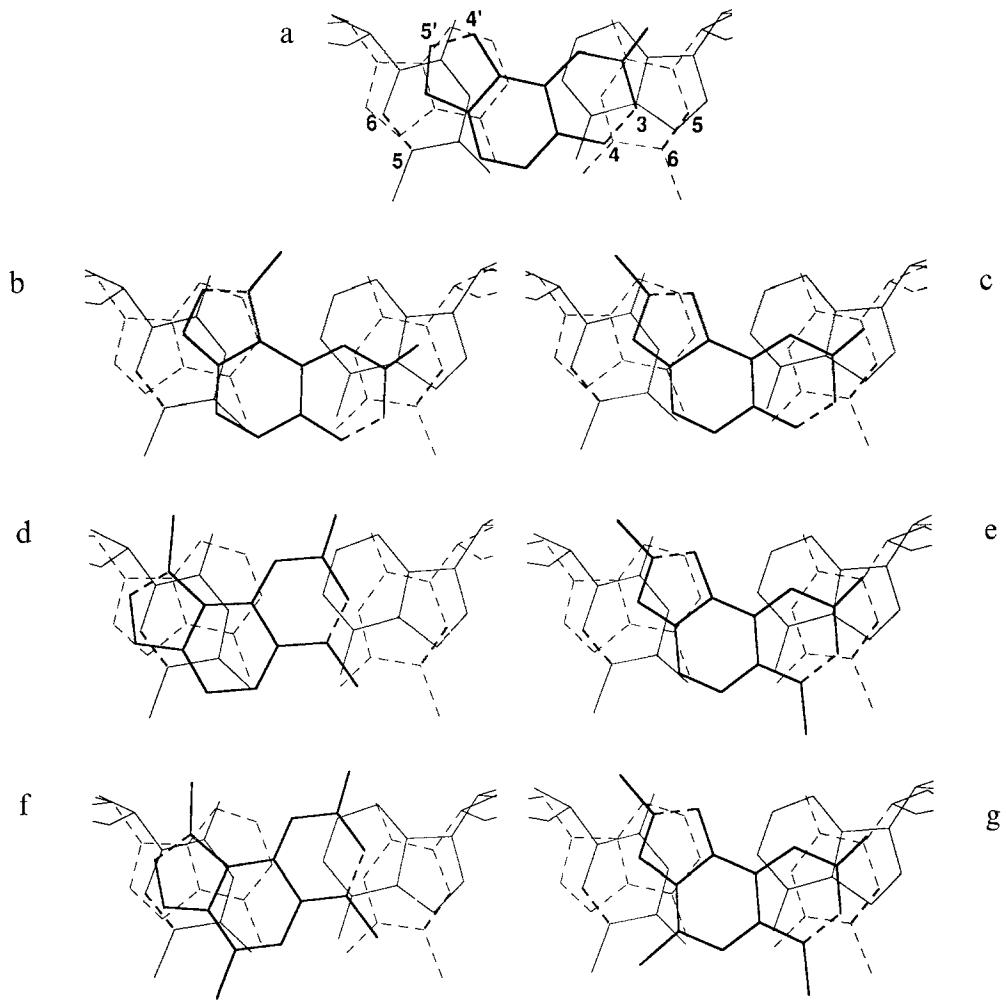

Fig. 7. Calculated intercalation geometries of the angelicins in the furan *cis-anti* and pyrone *cis-syn* conformations between the two central AT base pairs, in a projection plane orthogonal to the helix axis. The left thymine and the right adenine, in solid lines, are above the drug whereas the left adenine and the right thymine, in dashed lines, are below the drug; the photoreactive double bonds are represented by bold dashed lines. (a) angelicin; (b) 4'-methylangelicin; (c) 5'-methylangelicin; (d) 4,4'-dimethylangelicin; (e) 4.5'-dimethylangelicin; (f) 4,6,4'-trimethylangelicin; (g) 4,6,5'-trimethylangelicin.

and 4, the most interesting one is the interaction energy (E-inter), which is independent on the zero-energy reference level. Furthermore, this term is directly related to the experimentally measurable intercalation affinities. The E-tot value is the parameter which is minimized during the calculations. However, it gives no indication about the stabilities of the complexes with respect to each other, since E-tot is also dependent on the potential energy of the drug, E-drug.

Dynamical aspects

The various general conformational aspects pointed out in the section on Geometrical aspects (i.e. the complete intercalation, the parallelism between the angelicin and the adjacent base pairs) are preserved during the dynamics and, therefore, in the time-averaged structures. In fact, this

TABLE 1
DISTANCES (Å) AND ANGLES (DEGREES) BETWEEN THE REACTIVE DOUBLE BONDS OF THE ANGELICINS (INTERCALATED IN A FURAN *cis-syn* AND PYRONE *cis-anti* CONFORMATION) AND THE ADJACENT THYMINES

Compound	4'-5	5'-6	5-4'-5'	5'-6-5	3-6	4-5	4-3-6	4-5-6
Angelicin	4.75	4.50	40.6	53.0	4.30	4.94	41.4	67.8
4'-Methylangelicin	3.63	3.59	84.3	85.8	4.14	5.21	46.9	90.5
5'-Methylangelicin	4.14	3.96	61.2	68.7	3.77	4.54	48.1	78.9
4,4'-Dimethylangelicin	3.46	3.43	85.0	86.2	4.18	5.31	49.9	96.5
4,5'-Dimethylangelicin	4.61	4.34	45.0	57.3	4.56	3.97	45.6	69.1
4,6,4'-Trimethylangelicin	3.89	3.85	77.1	78.5	3.76	4.61	57.0	91.1
4,6,5'-Trimethylangelicin	4.74	4.47	40.0	52.9	4.01	4.60	46.0	69.7

[4'-5], [5'-6], [3-6] and [4-5] represent the distances between atoms [ang(C4')-thy(C5)], [ang(C5')-thy(C6)], [ang(C3)-thy(C5)] and [ang(C4)-thy(C6)], respectively.
[5-4'-5'], [5'-6-5], [4-3-6] and [4-5-6] represent the angles between atoms [thy(C5)-ang(C4')-ang(C5')], [ang(C5')-thy(C6)-thy(C5)], [ang(C3)-ang(C4)-thy(C6)] and [ang(C3)-thy(C5)-thy(C6)], respectively.

structure is quite similar to the one deduced from our molecular mechanics calculations, as can be seen in the data reported in Table 5. This point will be discussed later.

Figure 8 shows the evolution, during 50 ps (1-ps steps) and in Orientation 1 of the DNA, of the distance between the 4',5' double bond of the angelicin and the 5,6 double bond of thymine A6 (full line) and of the distance between the 3,4 double bond of the angelicin and the 5,6 double bond of thymine B6 (dotted line).

DISCUSSION

As mentioned earlier, angular furocoumarins such as angelicins have been introduced as DNA

TABLE 2
DISTANCES (Å) AND ANGLES (DEGREES) BETWEEN THE REACTIVE DOUBLE BONDS OF THE ANGELICINS (INTERCALATED IN A FURAN *cis-anti* AND PYRONE *cis-syn* CONFORMATION) AND THE ADJACENT THYMINES

Compound	4'-5	5'-6	5-4'-5'	5'-6-5	3-6	4-5	4-3-6	4-5-6
Angelicin	5.21	4.29	49.6	88.5	3.86	3.89	111.8	112.3
4'-Methylangelicin	5.50	4.75	56.7	88.0	4.21	4.31	130.9	127.5
5'-Methylangelicin	5.63	4.82	49.4	84.0	3.90	4.00	125.7	122.3
4,4'-Dimethylangelicin	4.15	3.44	63.1	92.2	4.72	4.47	101.7	115.3
4,5'-Dimethylangelicin	5.48	4.67	49.4	83.9	3.79	3.88	122.5	120.2
4,6,4'-Trimethylangelicin	4.40	3.58	55.3	88.9	4.69	4.45	93.8	105.7
4,6,5'-Trimethylangelicin	5.56	4.79	51.2	84.1	3.68	3.73	118.0	117.6

[4'-5], [5'-6], [3-6] and [4-5] represent the distances between atoms [ang(C4')-thy(C5)], [ang(C5')-thy(C6)], [ang(C3)-thy(C6)] and [ang(C4)-thy(C5)], respectively.
[5-4'-5'], [5'-6-5], [4-3-6] and [4-5-6] represent the angles between atoms [thy(C5)-ang(C4')-ang(C5')], [ang(C5')-thy(C6)-thy(C5)], [ang(C4)-ang(C3)-thy(C6)] and [ang(C4)-thy(C5)-thy(C6)], respectively.

TABLE 3
ENERGY VALUES (kcal/mol) DEDUCED FROM MOLECULAR MECHANICS STUDIES OF THE ORIENTATION 1 TYPE DNA–ANGELICIN COMPLEXES (ANGELICINS INTERCALATED IN A FURAN *cis-syn* AND PYRONE *cis-anti* CONFORMATION)

Compound	E-tot[a]	E-oligo[b]	E-drug[c]	E-inter[d]	E-int-elec[e]	E-int-vdW[f]
Angelicin	−461.6	−431.6	0.3	−30.3	−29.5	−0.7
4'-Methylangelicin	−464.6	−432.4	−0.7	−31.4	−31.3	−0.1
5'-Methylangelicin	−460.5	−429.5	−0.5	−30.5	−30.5	0.0
4,4'-Dimethylangelicin	−464.8	−430.4	−0.6	−33.8	−33.1	−0.7
4,5'-Dimethylangelicin	−464.0	−429.6	−0.3	−34.1	−33.8	−0.3
4,6,4'-Trimethylangelicin	−468.0	−432.3	0.2	−35.9	−34.7	−1.2
4,6,5'-Trimethylangelicin	−463.6	−429.4	0.3	−34.5	−33.9	−0.6

[a] Potential energy of the intercalation complex.
[b] Potential energy of the unwound oligonucleotide.
[c] Potential energy of the intercalated angelicin.
[d] Interaction energy between oligonucleotide and angelicin.
[e] Electrostatic contribution to the interaction energy.
[f] van der Waals contribution to the interaction energy.

photosensitizers analogous to psoralens, with the aim that their angular geometry would inhibit the formation of interstrand cross-links with adjacent pyrimidines, through the formation of C_4 cycloadducts under UV-A irradiation. This goal has been demonstrated experimentally and angelicins have been shown to be able to form only two types of monoadducts within DNA (furan-side and pyrone-side monoadducts), but no interstrand cross-link. The past two decades, a number of methylated angelicins have been chemically synthesized and studied by the Padova group. The goal was to obtain an increase in the ability of angelicins to photobind to DNA and to increase their photobiological activity [33–35]. It was the aim of our present study to check whether molecular mechanics and dynamics could predict the methylation effects observed experimentally, could help in the understanding of the photobiological activity of such angelicin deriva-

TABLE 4
ENERGY VALUES (kcal/mol) DEDUCED FROM MOLECULAR MECHANICS STUDIES OF THE ORIENTATION 2 TYPE DNA–ANGELICIN COMPLEXES (ANGELICINS INTERCALATED IN A FURAN *cis-anti* AND PYRONE *cis-syn* CONFORMATION)

Compound	E-tot[a]	E-oligo[b]	E-drug[c]	E-inter[d]	E-int-elec[e]	E-int-vdW[f]
Angelicin	−457.5	−430.0	0.2	−27.7	−29.7	2.0
4'-Methylangelicin	−462.8	−432.5	−0.6	−29.7	−30.7	1.0
5'-Methylangelicin	−460.5	−429.5	−0.7	−30.3	−31.3	1.0
4,4'-Dimethylangelicin	−465.0	−432.3	−0.5	−32.2	−33.0	0.8
4,5'-Dimethylangelicin	−463.0	−430.9	−0.4	−31.7	−33.0	1.3
4,6,4'-Trimethylangelicin	−461.7	−429.5	0.2	−32.4	−33.6	1.2
4,6,5'-Trimethylangelicin	−462.9	−430.6	0.4	−32.7	−34.2	1.5

[a] Potential energy of the intercalation complex.
[b] Potential energy of the unwound oligonucleotide.
[c] Potential energy of the intercalated angelicin.
[d] Interaction energy between oligonucleotide and angelicin.
[e] Electrostatic contribution to the interaction energy.
[f] van der Waals contribution to the interaction energy

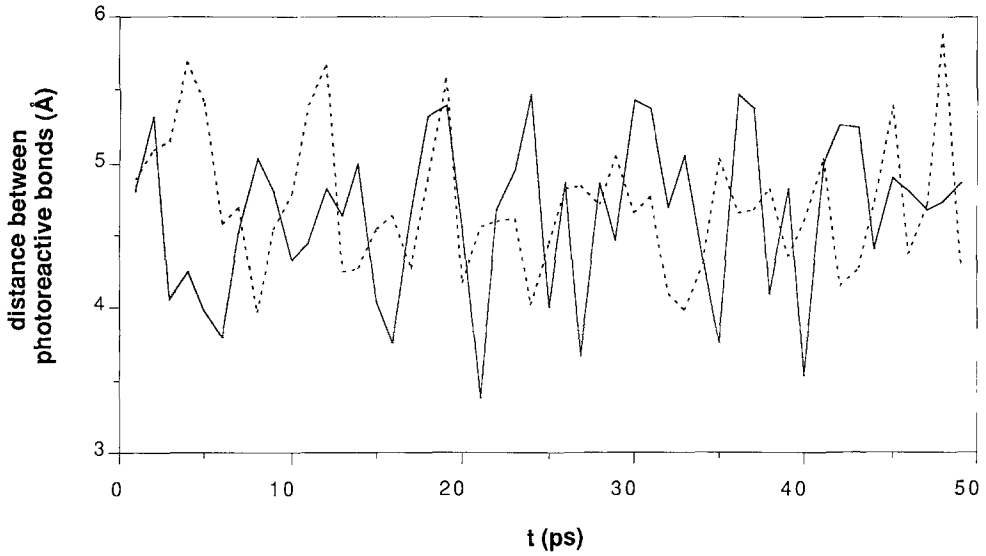

Fig. 8. Molecular dynamics of DNA–angelicin complex. Evolution, during the molecular dynamics simulation, of the distance between the 5,6 double bond of the thymine A6 and the 4',5' double bond of the angelicin (solid line) and between the 5,6 double bond of the thymine B6 and the 3,4 double bond of the angelicin (dotted line).

tives and therefore could help as a guideline in the design and synthesis of new furocoumarin derivatives with enhanced photobiological activities.

Orientation 1: Furan-side vs. pyrone-side monoadducts

It appears from our calculations that the geometry is always more favourable to the formation of an adduct at the furan side than at the pyrone side, under the conditions defined for the linear furocoumarins [19,20]. Indeed, the relative position of the photoreactive bonds foreshadows the cyclobutane ring formed after photoreaction, with the interatomic distances close to the sum of the van der Waals radii, and the angles between the atoms around 90°. This is particularly true for the angelicins methylated in the 4' position (configurations shown in the left part of Fig. 6, derivatives b, d and f). Indeed, as can also be seen in Table 1, the three considered 4'-methylated angelicins present have distances between reactive double bonds in the range 3.4–3.8 Å, at the furan side. Furthermore, the angular values are close to those observed in the C_4 ring formed after

TABLE 5
DISTANCES (Å) AND ANGLES (DEGREES) BETWEEN THE REACTIVE DOUBLE BONDS OF THE ANGELICIN (INTERCALATED IN A FURAN *cis-syn* AND PYRONE *cis-anti* CONFORMATION) AND THE ADJACENT THYMINES, IN THE TIME-AVERAGED STRUCTURE OVER THE PRODUCTIVE DYNAMICS AND ENERGY-MINIMIZED COMPLEX

Compound	4'-5	5'-6	5-4'-5'	5'-6-5	3-5	4-6	3-4-6	3-5-6
Angelicin	4.62	4.38	42.4	55.45	4.42	4.79	45.5	64.2

Bond and angle labels are those from Table 1.

photoreaction [36]: they range from 77° to 89°, which is in reasonable agreement with the value of 88° measured by Peckler et al.

For all the angelicins studied, the calculated energy-minimized conformation does not appear to be favourable to a pyrone-side monoadduct. In order to obtain such adducts, which are experimentally observed with a lower yield and which correspond to a *cis-syn* conformation, an important rotation of the angelicin has to be carried out for the linkage of angelicin carbons 3 and 4 to the thymine carbons 5 and 6, respectively.

These results are in accordance with the experimental data which indicate a far more important yield at the furan side than at the pyrone side [14].

Orientation 1: Influence of the methyl groups

The influence of the methyl groups on the DNA photobinding affinity of angelicin derivatives has been studied experimentally [12,37–41]. It appears that the role of methyls in terms of increase in DNA photobinding affinity has the following order of importance: 4' > 6 > 5' > 4, the methyl in the 4'-position giving the greatest increase, at the furan side.

As already pointed out, a methyl in 4'-position favours the formation of furan-side adducts. The reasons for this can be easily analysed with the aid of Fig. 6. Diagrams b, d and f indeed indicate that the 4'-methyl is positioned in the major groove, between thymine oxygen O4 and thymine methyl C5M, thus taking the angelicin 4',5' reactive double bond closer to the thymine 5,6 photoreactive double bond. From an energetical point of view (Table 3), this situation is less clear, but the 4'-methylated angelicins generally present interaction energies (E-inter) lower than those calculated for the other compounds. Therefore, our models appear to be in accordance with the experimental data concerning the preeminent influence of 4'-methylation on the photobinding affinity.

The role of the methyl in the 6-position is less clear, from both geometrical and energetical points of view. The addition of a methyl in position 6 to 4,4'-dimethylangelicin and to 4,5'-dimethylangelicin does not result in a more favourable intercalation geometry for photoreaction (Table 1) and the interaction energy of the trimethylated compounds is only slightly lower than that computed for dimethylated ones (Table 3). In fact, 6-methyl does not appear to directly interact with the adjacent bases (Figs. 6f and g).

The methyl group in position 5', in the 4,5'-dimethylangelicin and the 4,6,5'-trimethylangelicin, is positioned in the major groove, between thymine oxygen O4 and thymine methyl C5M (Figs. 6e and g), similar to the compounds methylated in the 4'-position. However, this configuration has an opposite effect, as it pushes the angelicin 4',5' reactive double bond away from the thymine 5,6 photoreactive double bond, thus creating intercalation geometries rather unfavourable for a subsequent photoreaction. The position of the 5'-methyl between thymine methyl C5M and thymine hydrogen H6, which would favour the formation of furan-side adducts (just like in the 5'-methylangelicin described above), does not occur in the 4,5'-dimethylangelicin and the 4,6,5'-trimethylangelicin due to sterical hindrance of the methyl in the 4-position: there is no room to accommodate the methyl in position 4 between the central thymine and adenine of strand B.

It has to be pointed out that, in the 4'-methylated angelicins, addition of a methyl group in the 4-position does not lead to bad contacts and may also induce a closer distance between photoreactive bonds at the furan side (Table 1). In fact, this improvement depends on its relative position with respect to the carbon C6 of adenine B7 and the oxygen O2 of thymine B6: Table 1

and Fig. 6f indicate slightly longer distances between photoreactive bonds at the furan side for the 4,6,4'-trimethylangelicin, due to the position of the 4-methyl group.

In conclusion, our models are globally in accordance with the experimental data concerning DNA photobinding affinity. The effects on the intercalation geometry and on the interaction energy (E-inter) of 4'-methylation and, to a lesser extent, of 5'-methylation are clearly seen and give a coherent explanation for the methyl dependence observed in the DNA photobinding affinity. The influence of 4-methylation and of 6-methylation on the intercalation geometry is less clear. It has nevertheless to be recalled that intercalation geometry and interaction energy are only a part of the complex ensemble of geometrical, photophysical and excited-states properties which rule the DNA photobinding affinity.

Orientation 1 vs. Orientation 2

No photoadduct confirming the possibility of Orientation 2, which would give rise to either a hypothetical *cis-anti* furan-side or to a *cis-syn* pyrone-side cyclobutane adduct, has been isolated to date [8,11–14]. From our calculations (Fig. 7), this is somewhat surprising. Indeed, although this orientation appears to be geometrically unsuitable to yield a furan-side photoadduct, the intercalation geometry is not unfavourable for the formation of a pyrone-side photoadduct. It also appears possible from an energetical point of view. Indeed, the distances between the photoreactive bonds of the angelicins and of the adjacent thymines (Table 3) or the interaction energies (Table 4) are rather similar to the values obtained with Orientation 1 (Tables 2 and 3).

Some important features have, however, to be pointed out. Firstly, short distances between photoreactive bonds are never obtained with near-90° angles between the atoms: for instance, the shortest distance (3.83 Å at the pyrone side, for 4,5'-dimethylangelicin) is combined with angles around 120°. In fact, the mean geometries are never very favourable to the formation of pyrone-side *cis-syn* adducts and always unfavourable to the formation of *cis-anti* furan-side adducts. Nevertheless, obtaining mean geometries which are not favourable to a subsequent photoreaction does not completely hinder the formation of adducts, as will be discussed in the section on Molecular dynamics.

Energetical reasons for the supposed nonexistence of Orientation 2 have also to be taken into account (Table 4). The interaction energy is systematically higher for the complexes with Orientation 2 than for those with Orientation 1 (Table 3). In fact, the electrostatic interactions appear to be less energetically favourable in Orientation 2 than in Orientation 1. These differences appear to be mainly due to the orientation of the furan ring in the intercalation site: the position with the furan oxygen pointing towards the minor groove, as in Orientation 1, is energetically more favourable than the alternative one. This is in accordance with the *cis-syn* structure of all the furan-side adducts isolated up to now in DNA irradiated in the presence of angular, and also linear [42–45], furocoumarins.

Molecular dynamics

An important feature of the calculated fluctuations of the distances between the photoreactive bonds in the DNA–angelicin intercalation complex, which are around 1.5–2.0 Å (Fig. 8), is that they appear to be significantly larger than those calculated for psoralens [20]. This is probably due to the smaller volume occupied by the angelicin, which allows larger displacements within the intercalation site: fluctuations around the mean structure of up to 1 Å occur. As, during the

molecular dynamics simulation, the structure is supposed to fluctuate around its lowest energy conformation, the time-averaged (over the 'productive' dynamics) and energy-minimized structure should be very close to this global minimum. We have calculated the time-averaged structure, which is very similar to the model obtained by molecular mechanics calculations and exhibits very similar distances between the photoreactive double bonds (Tables 1 and 5). Similar results have been observed for the methylpyridopsoralens [20] and confirm the closeness to the global minimum of the models obtained by molecular mechanics.

The plot of the fluctuations of the distances between photoreactive bonds also indicates that, even if the average values are too large to allow a photoreaction, these distances can decrease to values compatible with a photoreaction. For example, although the distance between reactive bonds is 4.50 Å at the furan side in the time-averaged and energy-minimized model (4.62 Å in the model obtained with molecular mechanics), this distance is six times (over 50) less than 3.70 Å in our plot. Photoreactions are therefore possible, even for molecules which exhibit unfavourable distances between photoreactive bonds in their time-averaged structure; short distances simply indicate a much greater probability of a photoreaction.

Finally, note that although the distances between the 4',5' bond and the 5,6 bond of the adjacent thymine are sometimes compatible with a photoreaction, the distance between the 3,4 bond and the adjacent thymine does hardly decrease below 4.0 Å. This observation is in accordance with the experimental data, which show a marked preference for the formation of furan-side adducts. It further confirms the validity of the models obtained by molecular mechanics, in which the intercalation geometry is more favourable for the formation of furan-side *cis-syn* photoadducts than for the formation of pyrone-side *cis-anti* adducts.

In conclusion, our theoretical approach of the geometry of DNA–angelicin complexes, using molecular mechanics and dynamics, can partly explain the photomodifications of DNA observed experimentally when such complexes are irradiated by UV-A. Such an approach could be helpful in the design of new DNA photosensitizing drugs with the objective of biomedical applications.

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