*DNA Backbone Conformation in Cis–Syn Pyrimidine[ ]Pyrimidine Cyclobutane Dimers**

INTRODUCTION

Ultraviolet light has well-known deleterious effects on biological systems. It causes mutations in organisms at every level of development.[1] In humans, the mutations induced by uv light can cause skin cancer.[2] Of particular interest is the condition known as *Xeroderma pigmentosum*, a genetic disease marked by unusual sensitivity to sunlight that leads to malignancies, especially the rapidly growing melanomas.[2]

The target of the uv light is the genetic material,[3] and it damages the template activity of DNAs[1] and RNAs[4] (in viruses) and the messenger activity of RNAs.[4] The predominant lesion is a cyclobutane-type dimer produced at adjacent pyrimidines, particularly thymine and uracil, on the same strand,[5,6] which causes local denaturation of about 4.3 base pairs in duplexes.[7] Moreover, the quantum yield of the reaction is 30% higher in single strands.[6] Of the possible cyclobutane dimers, the *cis–syn* isomer (Fig. 1) is the most important product.[5,6] This is true for fragments as small as dinucleoside monophosphates and for larger polymers, including apurinic acid[8] and denatured[9] and native DNA.[5,6] *In vivo* the lesion is normally repaired by excision endonucleases, but this repair system is usually defective in *Xeroderma pigmentosum* patients.[12]

In order to understand how these dimers induce mutations that can lead to cancer if not repaired, it is necessary to have an understanding of the molecular distortion they impose on the nucleic acid helix. Crystallographic analyses have elucidated conformations of the dimerized bases themselves. Crystal structures for *cis–syn* dimers of U[ ]U,[10] T[ ]T,[11] 6MeU[ ]6MeU,[12] Me_2T[ ]Me_2T,[13] and T[$(CH_2)_3$]T[14] ([ ] denotes the dimer) are available. A *cis–syn* dimer moiety is also present in a thymine trimer whose structure has been solved.[15] A review of these structures has been presented by Karle.[16] The thymine dimers are prone to monomerization by x-rays, which has hampered investigation of these structures.

The -$(CH_2)_3$- group of T[$(CH_2)_3$]T models the constraints imposed on the bases by the nucleic acid backbone, since it links the two N1 atoms (Fig. 1). However, little is known about the influence of the *cis–syn* dimers on the backbone conformation. An nmr investigation of the dTpdT dimer, dTp[ ]dT, reveals alterations in sugar pucker, C4′-C5′ torsion, and glycosidic bond rotation.[17] No crystal structure giving information about the backbone is available to date, perhaps because the instability of thymine dimers under x-irradiation may be even greater in larger subunits.

In the present work, a theoretical calculation was made to determine the conformation of a deoxydinucleoside monophosphate pyrimidine[ ]pyrimidine *cis–syn* cyclobutane dimer. Such single-stranded moieties are useful models for the *in vivo* situation because single strands are more easily damaged,[6] and the damage inhibits the normal function of single-stranded replicating DNA.[18,19] The calculated minimum-energy conformer was incorporated in a model of B-DNA to assess the influence of this covalent lesion on the normal form of DNA.

METHODS

Potential energies were computed, as detailed previously,[20] considering van der Waals, electrostatic, torsional, and deoxyribose strain contributions. In addition, the following terms

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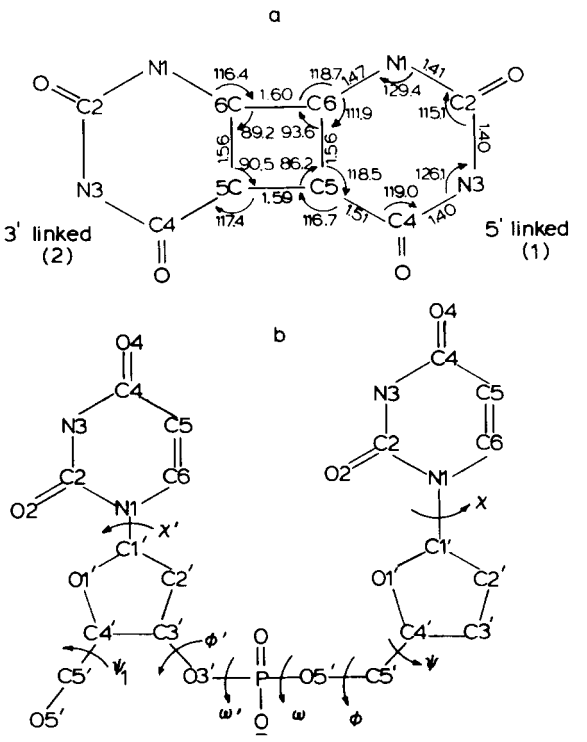

Fig. 1. (a) *Cis–syn* cyclobutane dimer of uracil. The pucker of the cyclobutane ring is described by α, the dihedral angle C6-(1)-C6(2)-C5(2)-C5(1), where (1) denotes the 5′-linked base. The dihedral angle C4(1)-C5(1)-C5(2)-C4(2), β, describes the twist of the bases. For the dihedral angle A-B-C-D, a clockwise rotation of D with respect to A, when looking down the B-C bond, defines the positive rotation sense. Bond lengths and angles of the cyclobutane ring are for the dUp[ ]dU minimum-energy conformation. Parameters for the bases were fixed close to the values observed for *cis–syn* pyrimidine[ ]pyrimidine dimers (Refs. 11, 16). Both uracils have the same geometry. (b) Structure, numbering scheme, and conformational angle designations for dUpdU. The torsion angles are defined as follows: χ',χ, O1′-C1′-N1-C6; ψ,ψ_1, C3′-C4′-C5′-O5′; ϕ', P-O3′-C3′-C4′; ϕ, C4′-C5′-O5′-P; ω', O5′-P-O3′-C3′; ω, C5′-O5′-P-O3′. The positive sense of rotation is defined in part (a).

were calculated, which forced the closure of the cyclobutane ring with the proper geometry:

$$E_b = k_b(b - b_0)^2 \tag{1}$$

$$E_a = k_a(a - a_0)^2 \tag{2}$$

$$E_\alpha = k_\alpha|\alpha - \alpha_0| \tag{3}$$

$$E_\beta = k_\beta|\beta - \beta_0| \tag{4}$$

Two terms of the form of Eq. (1) were employed. These force the C5(1)-C5(2) and C6(1)-C6(2) bonds to have nearly the desired lengths, b_0. [Base (1) is the 5′-linked moiety.] The instantaneous value of the bond length is denoted b; b_0 was assigned values of 1.60 and 1.55 Å for C5(1)-C5(2) and C6(1)-C6(2), respectively, corresponding to the observed[14] bond lengths in T[$(CH_2)_3$]T (the nucleic acid backbone model structure), and in T[ ]T.[11] Observed bond lengths are in the range of 1.55–1.60 Å for C5(1)-C5(2) and 1.55–1.59 Å for C6(1)-C6(2).[11,16] Eight terms of the form of Eq. (2) were used. These force the cyclobutane bond

TABLE I
Minimum-Energy Conformation of dUp[ ]dU[a]

χ'	ψ_1	ϕ'	ω'	ω	ϕ	ψ	χ	**P**	α	β
148	61	154	317	251	116	158	1	60	5	0

[a] All angles in degrees.

angles to take appropriate values, a_0. The instantaneous value of the calculated bond angle is given by a. Four of these terms were used to establish the internal cyclobutane bond angles, and a_0 was assigned a value of 90° for them; this is close to the observed quantity in pyrimidine[ ]pyrimidine *cis–syn* cyclobutane dimers. Two terms established the C4(1)-C5(1)-C5(2) and C4(2)-C5(2)-C5(1) bond angles, using the a_0's of 114° observed in T[$(CH_2)_3$]T.[14] The bond angles N1(1)-C6(1)-C6(2) and N1(2)-C6(2)-C6(1) were similarly determined, with a_0's of 114° and 115° of T[$(CH_2)_3$]T.[14] Equation (3) establishes α, the cyclobutane torsion angle defined by atoms C6(1)-C6(2)-C5(2)-C5(1), which is a measure of the ring's planarity. When $\alpha = 0°$, the ring is flat. Equation (4) determines β, the dihedral angle defined by atoms C4(1)-C5(1)-C5(2)-C4(2); this is a measure of the twist of the two pyrimidine rings with respect to each other. When $\beta = 0°$, the projections of the two pyrimidine rings overlap completely when viewed down the C5(1)-C5(2) bond. Both α_0 and β_0 are close to 0° in T[$(CH_2)_3$]T.[14] However, in other *cis–syn* cyclobutane dimers of thymines and uracils, values up to about 27° are observed.[16] The values of α_0 and β_0 were set to −2° and 0°, respectively, as in T[$(CH_2)_3$]T[14] in most trials. However, in the final stages of the calculation, values of +25° and −25° were also tried for each parameter, in the search for puckered and twisted conformations. The proper choice of k's in Eqs. (1)–(4) is critical to obtaining a closed structure, and some experimentation was needed to determine the magnitude of the constants that would permit closure. Initially, the k's of Eqs. (1) and (2) were set at 288 kcal/(mol Å^2) and 70 kcal/(mol rad^2), respectively, following Levitt[21] and Jack et al.[22] The k's of Eqs. (3) and (4) were assigned values of 1.0 kcal/(mol deg). However, these constants were individually varied over a considerable range in the search for a solution with a proper cyclobutane geometry (see below).

The calculations were made for dUpdU (Fig. 1) rather than dTpdT because the thymine methyls are in close contact in the *cis–syn* crystal structures.[16] However, thymine and uracil *cis–syn* cyclobutane dimers have similar crystalline conformations.[10,11] H5, H6, H1′, and H2′ were not included in the calculations since their positions are uncertain. Bond lengths and angles employed for uracil are in the ranges observed for *cis–syn* dimers[16] (Fig. 1). Both bases were fixed planar.

In the process of energy minimization, the dinucleoside monophosphate dUpdU was forced to become the corresponding photodimer. Starting conformations were the A- and B-form minimum-energy conformations previously calculated for dTpdT,[20] and also the conformation observed in the crystal structure of pdTpdT,[23] which has the O3′-P and O5′-P torsions *trans* (~180° region) and *gauche*$^-$ (~300° region), respectively. Other minimum-energy conformations of dTpdT[20] were also employed as starting conformers, but with only the initial set of constants. The energy was minimized, using the Powell algorithm,[24] with the eight DNA backbone torsion angles and the deoxyribose pseudorotation parameter, **P**,[25] flexible. Once a form was obtained that was nearly closed, it was used as a starting conformation in minimizations with a range of k's, as well as with α_0 and β_0 values of +25° and −25°. Criteria for determining whether a given calculated minimum was acceptable were that bond lengths and bond angles of the cyclobutane ring, and α and β, fall within the range of values observed in crystals of pyrimidine[ ]pyrimidine *cis–syn* cyclobutane dimers.[11,16]

RESULTS AND DISCUSSION

Only one acceptable minimum-energy conformation was found; the A-form of dTpdT[20] was the starting conformation. It has the following unique conformational features: the glycosidic torsion χ' at the 3′-linked base is high-*anti* (or at the *anti–syn* interface), the C4′-C5′

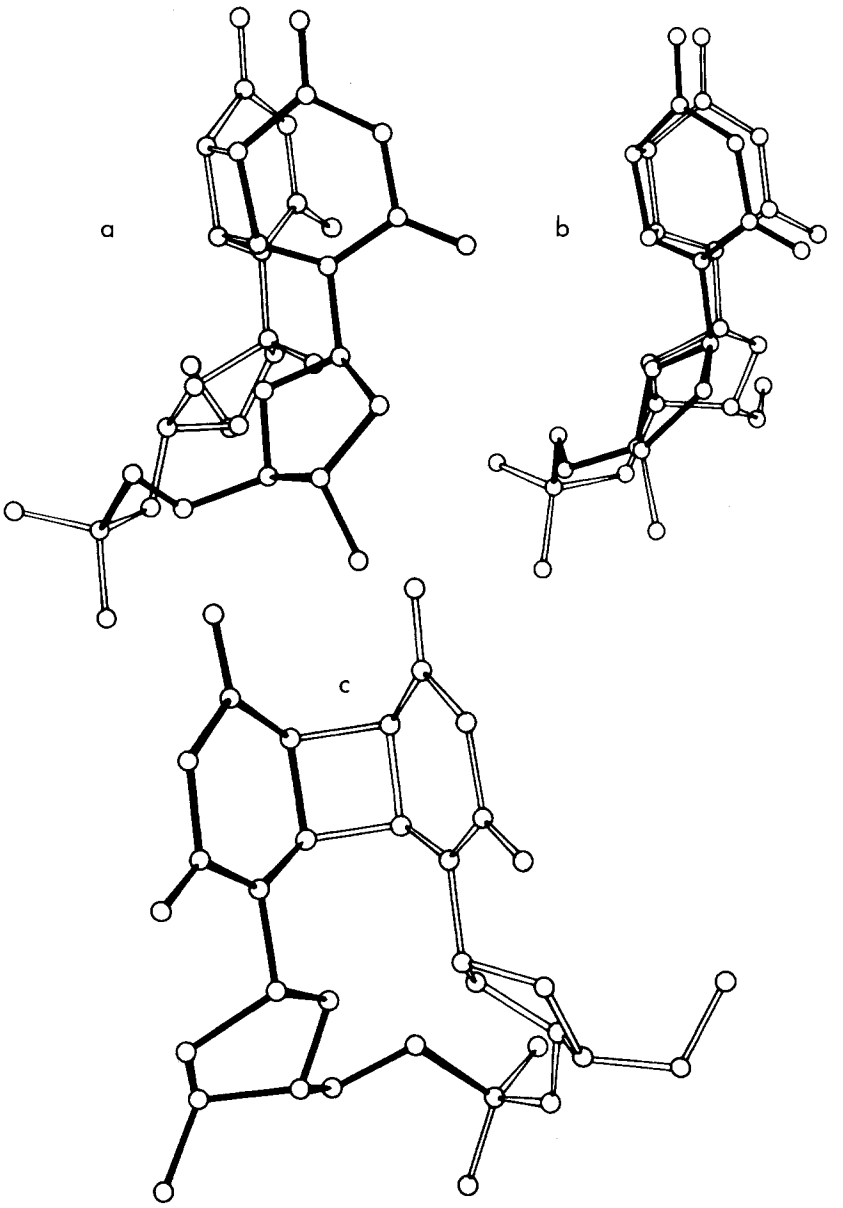

Fig. 2. Minimum-energy conformation of dUp[ ]dU with torsion angles given in Table I. (a)–(c) Three different views of the structure. The solid lines indicate structures closer to the reader.

torsion ψ is *trans*, and the sugar pucker is C4′-*exo*. However, the O3′-P and O5′-P rotations, ω' and ω, remain *gauche*$^-$, as in the A- and B-forms. While ϕ is generally close to the classical *trans* value of 180°, the low value calculated for the dimer is actually not far from 136°, reported for the most recent refinement of B-DNA fibers.[26] Table I lists torsion angles of the calculated conformation, and Fig. 1 shows the cyclobutane geometry. Figure 2 shows

ORTEP[27] drawings of this conformer. The cyclobutane ring is only slightly puckered and the bases have no significant twist. Evidently, the constraints on the cyclobutane ring arising from the nucleic acid backbone are only slightly less severe than those imposed by the -$(CH_2)_3$- in T[$(CH_2)_3$]T.[14] Both the backbone and the -$(CH_2)_3$- tend to flatten the cyclobutane ring, as compared to the case of free base dimers. It is likely that this conformation is representative of a family of conformers with very similar nucleic acid backbone conformations and slightly varying cyclobutane geometries, depending on the exact geometry of the bases. In the T[$(CH_2)_3$]T crystal, one of the two bases has C5 and C6 out of the plane defined by the other four atoms, while the other base is approximately planar.[14] Both bases were fixed planar in our calculations.

The pmr studies of Hruska et al.[17] on dTp[ ]dT in solution seem to be largely consistent with our structure, although the pmr results could not be unambiguously interpreted. The data of Hruska et al.[17] suggest changes in both glycosidic torsions compared to dTpdT, with one of them being in an extreme *anti* position. An increased preference for the *trans* domain of the C4′-C5′ torsion is also observed. The sugar is reported to prefer the C2′-*endo* pucker with a sizable C3′-*endo* contribution, but a tendency toward unusual pseudorotation parameters is noted. The cyclobutane ring is also found to be flatter than in free *cis–syn* dimers in solution, although still puckered. A comparison may also be made with the nmr studies on *cis–syn* cyclobutane dimers of abbreviated dinucleosides, in which the two bases (both thymine, or thymine and uracil) were linked via N1 to a single deoxyribose at the 5′- and 1′-carbons.[28] The base carbonyls in these molecules were found to be *syn* to O1′. On the other

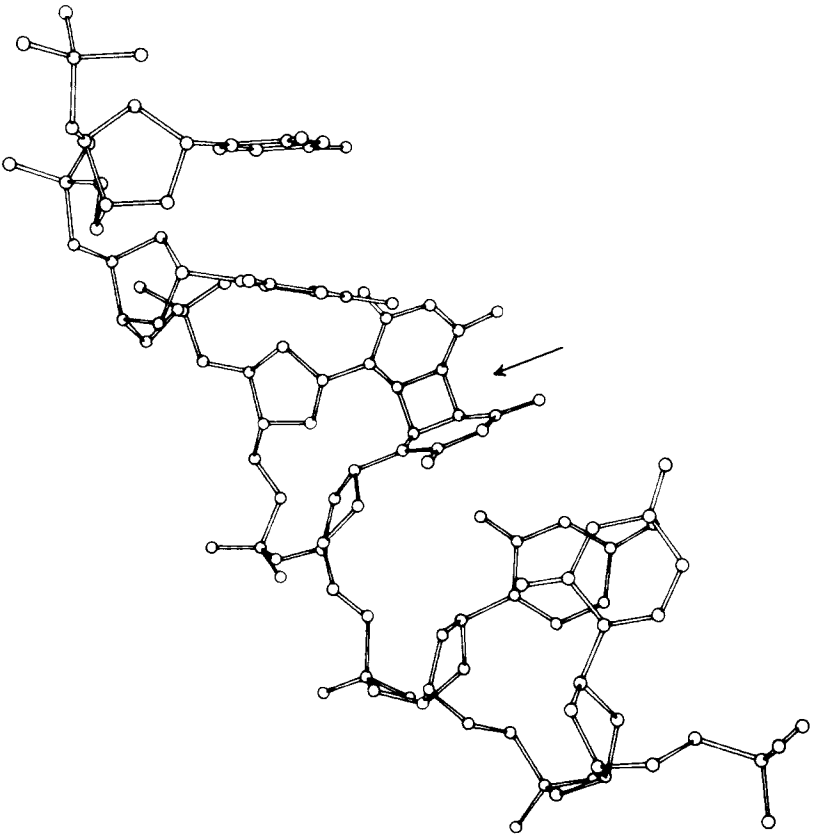

Fig. 3. Hexameric single-stranded segment of DNA incorporating dUp[ ]dU minimum-energy conformation where indicated by arrow. Other residues are in B-DNA conformation (Ref. 30).

hand, nmr results on the *cis–syn* dimer of dTp[ ]dU have been interpreted to be consistent with *anti* glycosidic torsions.[29] Our results, which place χ' at the extrema of the high-*anti* range, may bridge these conflicting findings.

In order to evaluate how this covalently linked dimer might alter the normal B-form, we incorporated the computed minimum-energy conformation into a model of single-stranded B-DNA. This model assumes that the adjacent sites are in the B-form. Figure 3 shows an ORTEP[27] drawing of the calculated dUp[ ]dU conformer as part of a single-stranded hexameric segment whose other residues are in the B-DNA conformation.[30] The dimer causes a severe bend in the helix, with adjacent base pairs ruptured, according to solution studies (7). Recent experimental results[18,19] reveal that pyrimidine[ ]pyrimidine dimers block DNA synthesis in replicating systems, causing a termination site at the locus of the lesion. A conformational distortion similar to the one presented here might be responsible for this malfunction.

CONCLUSION

Minimized potential energy calculations have been made for the *cis–syn* cyclobutane dimer of dUp[ ]dU. The unique conformational features of the dimer are as follows: the 3′-linked base has the glycosidic torsion in the high-*anti* region, the sugar pucker is C4′-*exo*, and the C4′-C5′ rotation is *trans*. The O3′-P and O5′-P torsions both remain in the *gauche*$^-$ domain of B-DNA. This conformer produces a severe bend in the helix when incorporated in B-DNA, which could cause the termination site that these dimers produce in replicating DNA.[18,19]

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