

Theoretical Studies on Binding of SN6999 to the Minor Groove of DNA

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Introduction

SN6999 is a drug with **antitumor activity** (Denny, 1979). It binds to the minor groove of B-DNA, preferentially at pure AT stretches. The binding of SN6999 to the DNA dodecamer **d(GGTTAATGCGGT)-d(ACCGCATAAACC)** was recently studied by NMR spectroscopy (Chen, 1992). It was found that SN6999 is likely to bind in two orientations relative to the DNA, but no models at atomic resolution were derived. Sliding of the ligand along the DNA was excluded a priori. Here we present results of a theoretical modelling study of this complex.

Computational Methods

The torsional potentials of the rotatable backbone bonds of SN6999 were calculated using semiempirical quantum-chemical methods (MOPAC 6.0, AM1 hamiltonian). A force field for SN6999 was parameterized accordingly. Molecular dynamics (MD) simulations were run for four binding modes of the complex and for free DNA.

The AMBER suite of programs was used with the Cornell et al. (1995) force field and Ewald summation for the electrostatic forces. **Four DNA/drug complexes** were constructed with SN6999 bound in two orientations to two neighbouring sites within the 5 AT stretch. Each complex was fully solvated in a box of 3936 water molecules, 32 Na⁺ and 12 Cl⁻ (NaCl concentration approximately 0.1 M). After warming up and equilibrating for 40 ps each MD simulation was run for **500 ps** with a 1 fs time step, constant pressure (1 bar), constant temperature (300 K) and SHAKE applied to all hydrogen bond lengths.

Results

Table 1. Average distances between protons for which NOEs were observed by Chen et al. (1992). The distances are the averages over the 500 ps trajectories of each MD run. NOEs are observable if the distance between two protons is lower than 5 Å. Chen et al. have observed 26 NOEs between the DNA and SN6999. More than one distance (a, b, ...) is given for NOEs, if methyl or phenyl protons of SN6999 are involved, because these protons could not be identified unambiguously from the NMR data.

Index	SN6999	DNA	Distance / Å				
			Run 1	Run 2	Run 3	Run 4	Minimum
1a	QH1	A22:H2	18.9	14.7	4.8	3.5	3.5
1b	QH2	A22:H2	18.8	14.7	4.8	3.5	3.5
1c	QH3	A22:H2	18.9	14.6	4.8	3.5	3.5
2	QH4	AS:H2	9.2	4.7	8.7	4.8	4.7
3	QH4	A18:H2	2.4	5.4	17.1	13.2	2.4
4	QH4	A22:H2	16.9	12.4	2.3	3.5	2.3
5	QH4	C23:HI'	20.4	16.1	3.1	6.2	3.1
6	QH5	A22:H2'2	16.2	10.7	4.6	5.9	4.6
7	QH5	A22:H2	15.7	11.1	2.9	5.8	2.9
8	BQ:HI1	AS:H2	6.3	3.1	5.8	2.6	2.6
9	BQ:HI1	A21:HI'	7.1	3.1	7.1	3.8	3.1
10a	BQ:HI2	T4:HI'	12.3	7.9	5.1	9.6	5.1
10b	BQ:HI3	T4:HI'	15.5	10.5	7.8	12.2	7.6
11	BQ:HI2	AS:HI'	8.1	4.4	2.7	5.6	2.7
12	BQ:HI2	AS:H2	4.4	2.8	3.9	2.6	2.6
13	BQ:HI2	T20:HE	4.6	5.1	6.3	4.5	4.3
14	BQ:HI2	T20:HI'	2.8	6.1	8.1	4.6	2.8
15	BQ:HI2	A21:H2	8.0	3.7	2.5	5.6	2.5
16	Amide:HI5	AS:H2	3.8	4.0	3.7	3.8	3.7
17	Amide:HI5	T20:HI'	3.5	7.9	6.8	2.9	2.9
18	BP:HI6	T4:HI'	9.2	4.5	8.3	12.2	4.5
19	BP:HI6	AS:H2	7.2	4.6	2.4	4.8	2.2
20	BP:HI6	A21:H2	5.2	2.4	3.7	7.9	2.4
21a	BP:HI7	AS:H2'1	5.7	5.8	9.5	12.7	5.7
21b	BP:HI8	AS:H2'1	9.4	7.6	10.3	15.1	7.6
22	BP:HI7	T19:HI'	10.7	14.9	7.2	2.6	2.6
23	PH:22	GR:HI'	17.0	20.9	5.9	3.0	3.0
24	PH:22	A18:HI'	16.2	20.1	7.8	4.9	4.9
25	PH:22	A18:H2	13.5	18.1	4.0	2.5	2.5
26	PH:22	A22:H2	3.5	2.7	12.5	17.3	2.7

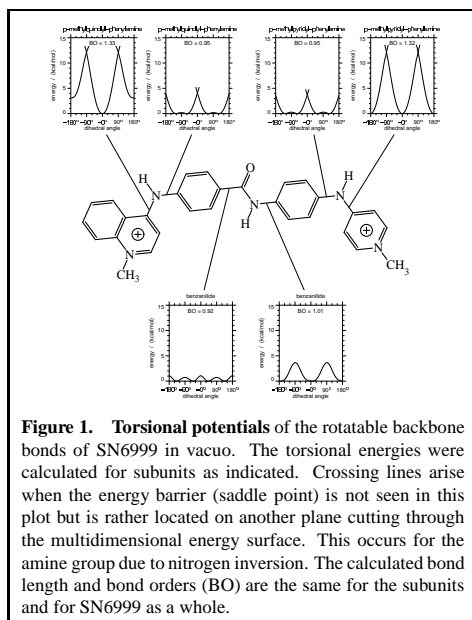


Figure 1. Torsional potentials of the rotatable backbone bonds of SN6999 in vacuo. The torsional energies were calculated for subunits as indicated. Crossing lines arise when the energy barrier (saddle point) is not seen in this plot but is rather located on another plane cutting through the multidimensional energy surface. This occurs for the amine group due to nitrogen inversion. The calculated bond length and bond orders (BO) are the same for the subunits and for SN6999 as a whole.

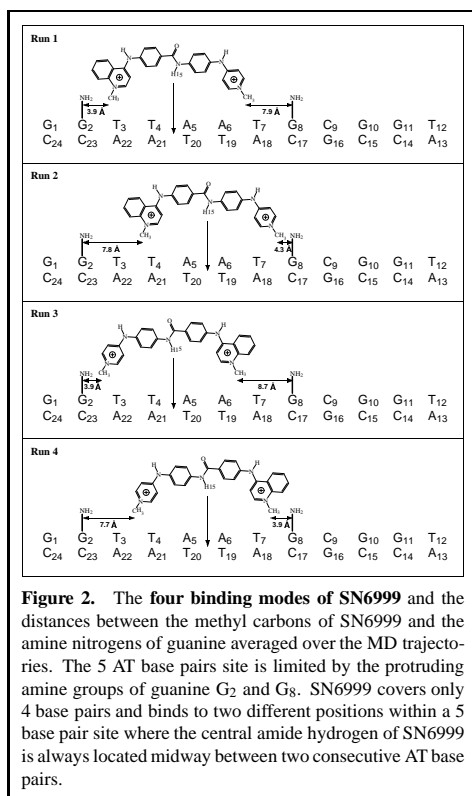


Figure 2. The four binding modes of SN6999 and the distances between the methyl carbons of SN6999 and the amine nitrogens of guanine averaged over the MD trajectories. The 5 AT base pairs site is limited by the protruding amine groups of guanine G₂ and G₈. SN6999 covers only 4 base pairs and binds to two different positions within a 5 base pair site where the central amide hydrogen of SN6999 is always located midway between two consecutive AT base pairs.

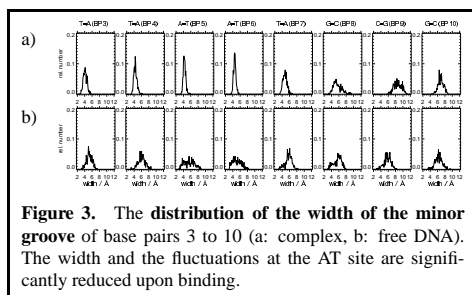


Figure 3. The distribution of the width of the minor groove of base pairs 3 to 10 (a: complex, b: free DNA). The width and the fluctuations at the AT site are significantly reduced upon binding.

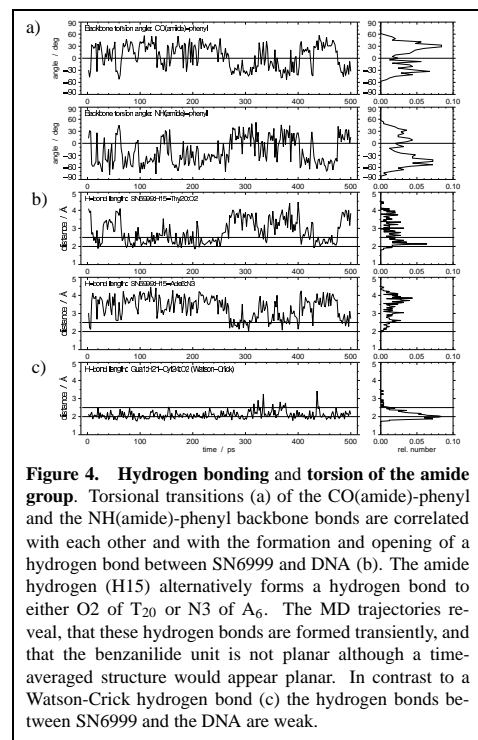


Figure 4. Hydrogen bonding and torsion of the amide group. Torsional transitions (a) of the CO(amide)-phenyl and the NH(amide)-phenyl backbone bonds are correlated with each other and with the formation and opening of a hydrogen bond between SN6999 and DNA (b). The amide hydrogen (H15) alternatively forms a hydrogen bond to either O₂ of T₂₀ or N₃ of A₆. The MD trajectories reveal, that these hydrogen bonds are formed transiently, and that the benzamide unit is not planar although a time-averaged structure would appear planar. In contrast to a Watson-Crick hydrogen bond (c) the hydrogen bonds between SN6999 and the DNA are weak.

Summary

- The central benzamide unit of SN6999 is torsionally flexible.
- The amine bonds to either phenyl or pyridine/quinoline are different in their electronical characters. Torsion about the amine bonds is restricted by sterical hindrance or by the partial double bond character respectively.
- All of the four models show stable trajectories. In general the DNA double helix is not disturbed upon binding. Only the width of the minor groove and its fluctuations are significantly reduced.
- All four binding modes are needed to account for the 26 NOEs observed by Chen et al. Our models confirm that SN6999 may bind in two different orientations relative to DNA. Moreover, the models show that the geometrical size of the binding site is 4 base-pairs only and thus there are two possible positions for binding within a stretch of 5 AT base-pairs. On a longer time-scale sliding of the ligand between the two positions may occur. We suggest a new interpretation of the NMR data based on a model with 4 binding modes in equilibrium.
- SN6999 has three hydrogen donor groups, but only the amide hydrogen faces the floor of the minor groove. The resulting hydrogen bonds to either thymine O₂ or adenine N₃ are weak as indicated by the hydrogen-acceptor distance. Hydrogen bonding seems to be less important for binding and sequence selectivity of SN6999.

References

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