Communication

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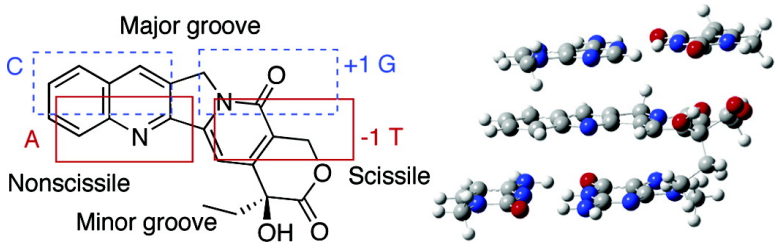
J. Am. Chem. Soc., **2005**, 127 (28), 9960-9961• DOI: 10.1021/ja042485n • Publication Date (Web): 25 June 2005



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Published on Web 06/25/2005

An Ab Initio Quantum Mechanics Calculation that Correlates with Ligand Orientation and DNA Cleavage Site Selectivity in Camptothecin−DNA−Topoisomerase I Ternary Cleavage Complexes

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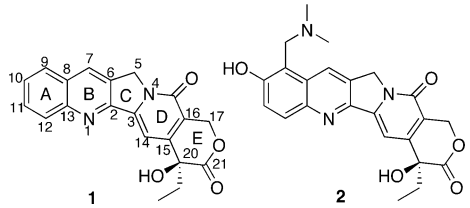
The discovery of the cytotoxic agent camptothecin (CPT, **1**) (Chart 1) from the Chinese tree *Camptotheca acuminata* not only

Chart 1. Chemical Structures of CPT (**1**) and Topotecan (**2**)



has provided a lead for the development of new anticancer drugs but also has led to the validation of topoisomerase I (top1) as a chemotherapeutic target.[1,2] Top1 is a nuclear enzyme responsible for solving the topological problems associated with DNA replication and transcription by nicking and resealing one strand of DNA in a DNA duplex.[3] These processes are achieved by a reversible transesterification reaction between Tyr723 of top1 and the phosphodiester bond of the DNA. The X-ray crystal structure of the ternary complex consisting of DNA, top1, and topotecan (TPT, **2**), a therapeutically useful analogue of CPT, shows that the pentacyclic ring system of CPT intercalates into the DNA base pair step at the cleavage site, resulting in increased physical distance between the cleaved DNA termini and, consequently, inhibition of the religation step catalyzed by top1.[4] Interestingly, none of the three previously proposed binding models of CPT at the cleavage site are consistent with the X-ray crystal structure.[5−7]

Previous experimental data have shown that CPT has a strict requirement for T at the −1 site and a strong preference of G at the +1 site of the cleaved strand.[8] This sequence preference was observed both in vitro and in vivo.[8,9] The reason for this sequence selectivity has not been explained. Since 60% of the solvent-accessible surface area of **2** is covered by base-stacking interactions and only one direct hydrogen bond is present between top1 and **2**,[4] we hypothesized that the primary $\pi-\pi$ stacking interactions between CPT and the flanking base pairs would dictate the binding orientation of CPT at the cleavage site, as well as the immediate DNA sequence selectivity. The computational evidence to support this hypothesis is the subject of the present communication.

The models used in the calculations were constructed by the following steps. (1) Topotecan and the flanking base pairs were extracted from the crystal structure (PDB 1K4T). (2) The deoxyribose rings were replaced by methyl groups, and the substituents on positions 9 and 10 of TPT were replaced by hydrogen atoms. (3) The energies of the camptothecin molecule, A−T base pair, and G−C base pair were each optimized separately at the HF/6-31G(d,p) level of theory in Gaussian03.[10] A frequency calculation was included in each case to ensure the structural minimum. (4)

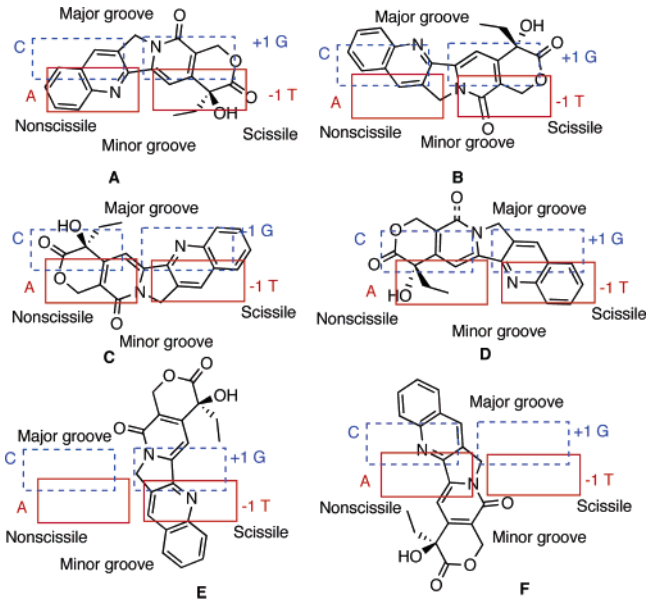


Figure 1. Two-dimensional schematic representation of different orientations of camptothecin (**1**) in the cleavage site.

Table 1. The MP2 Energies of Models **A**−**F**

models	E_{MP2} (au)[a]	ΔE_{MP2}[a,b]	$E_{MP2'}$ (au)[c]	$\Delta E_{MP2'}$[b,c]
A	−3188.935708	0.00	−3188.903671	0.00
B	−3188.867778	42.63	−3188.828201	47.36
C	−3188.899863	22.49	−3188.860667	26.99
D	−3188.921567	8.87	−3188.888630	9.44
E	−3188.915987	12.38	−3188.891051	7.92
F	−3188.926889	5.53	−3188.899196	2.81

[a] MP2 energies without BSSE. [b] ΔE_{MP2} and $\Delta E_{MP2'}$ refer to the energy difference (kcal/mol) between model **A** and other models. [c] MP2 energies with BSSE.

The three resulting individual structures were utilized to replace the original units in the complex in step 2, resulting in model **A** (Figure 1).[11] (5) CPT was then rotated to different orientations in the intercalation complex, and the ethyl group was minimized in every orientation to avoid any steric clash using the MMFF94 force field and MMFF94 charges in Sybyl,[12] providing models **B**−**F**. (6) These six models were then subjected to a single-point energy calculation at MP2/6-31G(d), where the basis set superposition error (BSSE) for the complex was calculated using the counterpoise correction method.[13] It is important to include electron correlation to accurately represent the stacking interactions.[14]

The calculated MP2 energies of the six models are listed in Table 1. In contrast to the previous molecular mechanics calculations,[7] this MP2 calculation, with or without BSSE, clearly demonstrates that the experimentally observed model **A** has the lowest energy

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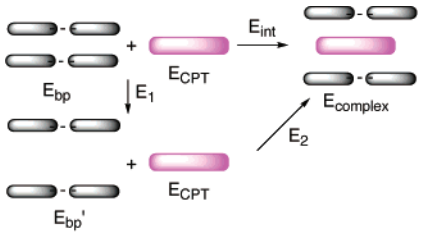

$$E_{int} = E_1 + E_2 = E_{complex} - E_{bp} - E_{CPT} - BSSE \quad Eq\ (1)$$

Figure 2. Schematic and mathematical representation of E_{int}. E_{bp} and E_{bp}' are the respective energies of the base pairs in their normal distance and their separated distance as seen in the complex.

Table 2. The Interaction Energies (kcal/mol) Calculated at the MP2/6-31g(d) Level of Theory and Observed Frequency of Different CPT Sites in the SV40 Genome

entry	−1[a]	+1[a]	E_{int}(MP2)	frequency (P)%
1	T	G	−6.69	75
2	T	A	−5.97	16
3	T	C	−5.57	7
4	T	T	−4.48	2
5	G	T	−2.76	ND[b]
6	G	G	−4.85	ND[b]
7	C	G	−5.55	ND[b]
8	A	G	−3.03	ND[b]

[a] The bases listed correspond to those next to the cleavage site on the cleaved DNA strand. [b] Not detected.

among the six models. Although the long axis of CPT is parallel to the long axis of the base pairs in all the four models **A**−**D**, the energy differences without BSSE range from 8.87 to 42.63 kcal/mol. These energy differences can be rationalized based on electrostatic complementarity between CPT and the flanking base pairs (Figure 1S).[11] Apparently, models **B** and **C** experience electrostatic repulsion between CPT and the neighboring base pairs (Figure 1S).[11] The other two models, **E** and **F**, having CPT's long axis perpendicular to that of the base pairs, surprisingly show relatively low energies. These results indicate that multiple factors, including dispersion forces, electrostatic attraction, and charge-transfer interactions, contribute to the stacking energies.[15] Solvation effects were not considered because they were assumed to be similar among all the binding processes.[16]

Having established that the primary stacking interactions could reproduce the experimental binding model, we then investigated whether the stacking interactions also govern the DNA sequence preference by mutating the immediate DNA bases and calculating the interaction energies (E_{int}) according to eq 1 (Figure 2). The interaction energies, after correction with BSSE for the complexes, with different DNA sequences are shown in Table 2. Among the eight different sequences, the interaction energy with −1 T−A and +1 G−C is the largest (−6.69 kcal/mol), which is consistent with observed experimental sequence preference.[8,9] Although the base pair separation energies for entries 1 and 3 are larger than that required for entry 4,[17] their interaction energies are larger than that in entry 4, which indicates that these interaction energy differences among different sequences are a consequence of better stacking of CPT onto certain bases instead of the differences in base pair separation energies.

The cleavage sites stabilized by CPT in the SV40 viral genome DNA were analyzed extensively by Pommier,[8] and the frequency of the CPT sites for four different sequences is presented in Table 2. All of the observed CPT sites show large interaction energies (entries 1−4). Although the possible CPT sites, as demonstrated by the relatively large interaction energy, shown in entries 6 and 7 were not detected in the SV40 genome, a high correlation (r^2 = 0.968) between the interaction energy and the known frequency of CPT sites was observed (Figure 2S),[11] suggesting that the intercalation energy is a predominant, if not exclusive, factor governing the sequence selectivity of CPT.

In conclusion, the binding orientation of CPT in the DNA−top1 cleavage site was studied by an ab initio quantum mechanics calculation, and the calculated orientation was found to match that observed experimentally. Furthermore, the DNA sequence selectivity of CPT was studied, and a good correlation was observed between the calculated interaction energy and the frequency of CPT sites in the SV40 genome. Since the calculations only involve $\pi-\pi$ stacking interactions and are capable of predicting binding orientation and binding site selectivity, it can be concluded that hydrogen bonding of the ligand to the surrounding amino acid residues of the protein, or to the base pairs, is of minor significance. The present method should be applicable to other polycyclic top1 poisons to generate similar models for further structure-based drug design.

Acknowledgment. This work was made possible by the National Institutes of Health (NIH) through support of this work with Research Grant UO1 CA89566. We thank the Rosen Center for Advanced Computing (RCAC), Purdue University for providing computing facilities.

Supporting Information Available: Three-dimensional presentation of models **A**−**F** and their Cartesian coordinates. Electrostatic potential surface maps of CPT and base pairs. Correlation figure of E_{int} versus frequency. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA042485N