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Neocarzinostatin Activation: Molecular Modeling Revisited

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Abstract: Molecular modeling studies of a neocarzinostatin-DNA complex were performed in an attempt to investigate the feasibility of several recent proposals.

Introduction Since its isolation and structure determination neocarzinostatin chromophore[1] (Fig. 1) has been the subject of a number of investigations. Studies have been directed toward the elucidation of its mechanism of cytotoxicity,[2] its total synthesis,[3] and the synthesis of functional analogs.[4] It appears that neocarzinostatin (NCS) induces cell death by cleaving cellular DNA via a diradical formed after nucleophilic addition of a thiol to C12 of NCS.[2] The radical centers then abstract hydrogen atoms from the backbone of DNA, which in the presence of oxygen leads to strand cleavage or the production of an abasic site.[2c, 2f] (Scheme 1)

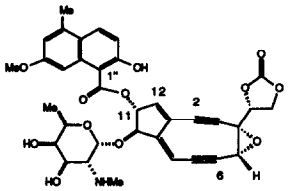
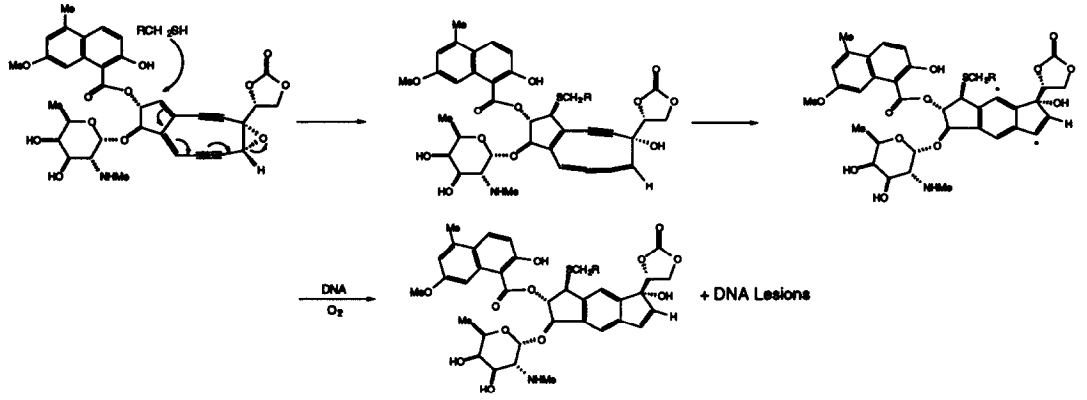


Figure 1: Neocarzinostatin Chromophore

In the course of our studies of enediyne antibiotics we developed a computer model of an NCS-DNA complex in order to rationalize the selectivity of DNA cleavage. Recently, two reports have appeared in the literature[5] that led us to reexamine the model in order to try to determine the possible relevance of postulated events. We present our results in this communication.

Scheme 1



Results and Discussion The model was developed by manually docking "activated" NCS (in which nitrogen atoms have been substituted for the carbon radicals) into an intercalation site created between A5 and G6 in the 12-mer of DNA (d-CACGAGCAGCAG),[6] which contains the known recognition site AGC.[2c, 2e, 2f] The complex was then subjected to a substructure minimization.[7] Next, a molecular dynamics simulation at 300 K was performed. The global minimum and structures within 5 kcal/mole were further minimized. The lowest energy structure was then subjected to a molecular dynamics simulation in which the complex was equilibrated for 10 ps at 300 K and then observed for 25 ps.[8]

The general features of the model are in agreement with previously developed models (Fig. 2).[9] The C6 radical makes a close contact with the C5' pro-S hydrogen of thymidine 5 with an average distance of 3.34 Å. In addition, the C4' hydrogen of the thymidine is close to C6, but the orientation appears to be such that hydrogen atom transfer may be slower. Whereas the C5' hydrogen C-H bond lies almost in the plane of the aromatized nucleus of the drug, the C4' C-H bond is displaced from this plane. The C2 radical also has a close contact, in this case to the C1' hydrogen of cytidine 7 with an average distance of 3.47 Å. These observations are consistent with the experimentally determined DNA cleavage selectivity of the natural product.

The model also suggests the existence of two interesting hydrogen bonds. These are between the hydroxyls of the fucosamine sugar of the drug and the phosphate backbone of the DNA. These postulated hydrogen bonds may help to maintain the drug in an orientation that facilitates a hydrogen atom transfer reaction with the host DNA.

One question that bears on the studies of NCS is whether intercalation precedes activation. Although it may be common for a molecule that acts via a reactive intermediate to have the binding step precede the activation step, it is not always the case. The anthraquinone antibiotics are a case in point.[10] Despite the fact that they appear to damage DNA via a quinone-methide species, it appears that the reduction of the quinone precedes intercalation of the aromatic nucleus into DNA. It has recently been shown that the initially reduced drug may pass through a long-lived intermediate prior to quinone-methide formation.[11] Studies on NCS, however, indicate that after thiol addition the cumulene intermediate has a half-life on the order of 0.5 s at 37 °C.[12] Furthermore, the diradical is rapidly trapped by solvent even at low temperature. In light of these observations it seems reasonable that in the case of NCS, DNA binding precedes activation of the drug.

With the above model and the premise that DNA binding precedes activation we set out to investigate two recent proposals concerning the mechanism of action of NCS. The first of these is from Fuchs and co-workers, who have attempted to determine if the naphthoate carbonyl oxygen may be the initial nucleophile in the activation event.[5a] In this scenario the oxygen would add to C12 with concomitant formation of the cumulene intermediate, and only subsequently would be displaced by glutathione. In order to investigate this more fully, the original starting structure for the molecular dynamics simulation was altered so that the docked drug was in the preactivation form. A molecular dynamics simulation, as described above, was then performed.

In order for the naphthoate carbonyl oxygen to add to C12, the angle between the carbonyl C=O bond and the C11-H bond must be close to 120°. This is quite far from the preferred geometry of esters of secondary alcohols in which this angle is close to 0°, i.e. these bonds tend to be parallel.[13] In fact, throughout the simulation this angle confines itself predominantly to negative values (Fig. 3) and never exceeds 10° in the positive direction. (This angular preference was also observed in the simulations with the activated drug.) More importantly, if the DNA bound ligand were to take on the required conformation, the enediyne portion would have to occupy the same space as the backbone of one of the strands of DNA. Thus, molecular modeling suggests that for the Fuchs' proposal to be operative, a significant structural change would be required of the equilibrium geometry of the complex.[14]

The second recent proposal we sought to investigate concerns the low ratio of double to single strand cleavage. Goldberg and coworkers have shown that deuterium-labeled glutathione will efficiently transfer deuterium to the C12 radical in a 1,5 hydrogen atom transfer process. The quenching of this radical site was suggested as an explanation for why single strand cleavage predominates.[5b] To investigate this possibility the DNA-activated ligand complex was once again altered. The activating agent, a "hydride" in the original case, was

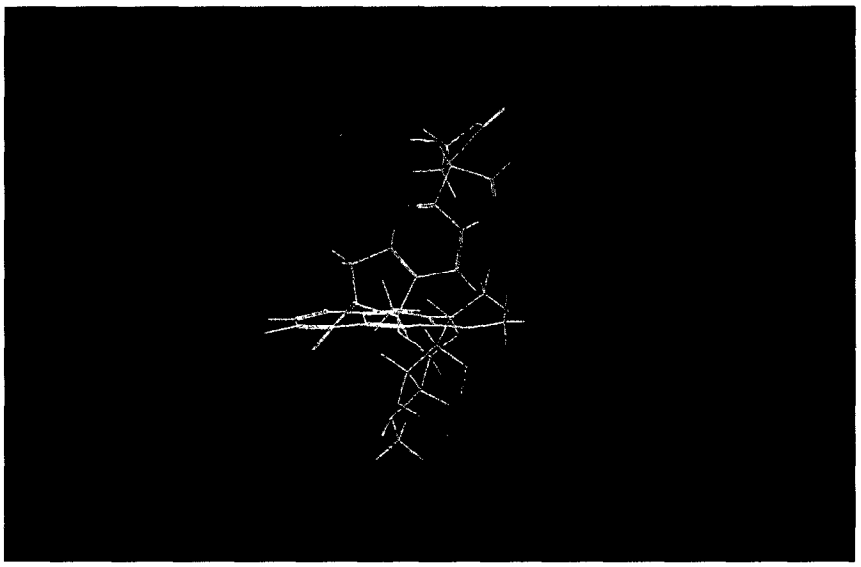


Figure 2: Close up of a representative structure of the NCS-DNA complex from the molecular dynamics simulation. The color scheme is as follows: DNA: blue, NCS: orange, C1' hydrogen of cytosine and C5' hydrogen of thymidine: green, Radical sites: red, Hydrogen bonds (see text): purple.

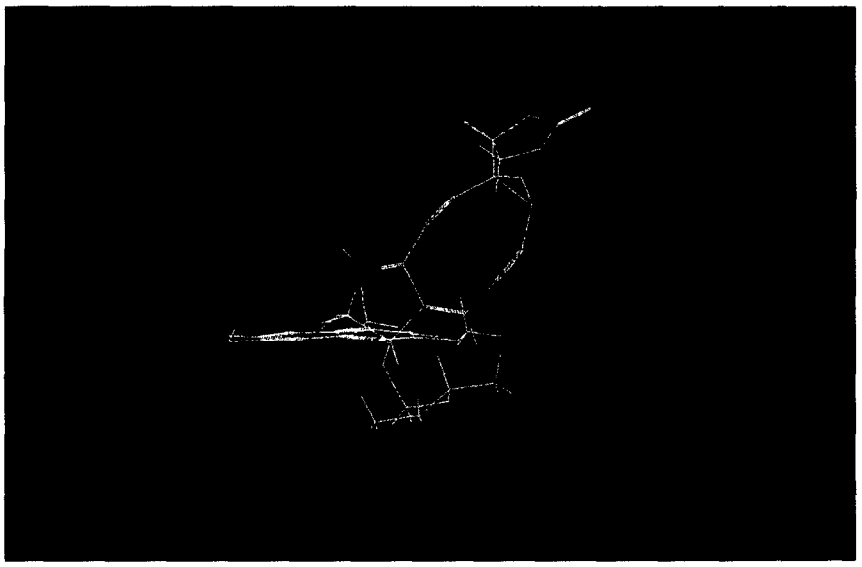


Figure 3: Detail of the naphthoate ester linkage showing the orientation of the carbonyl oxygen and C12. The color scheme is as follows: DNA: blue, NCS: orange, Carbonyl carbon and oxygen: red, C12: green.

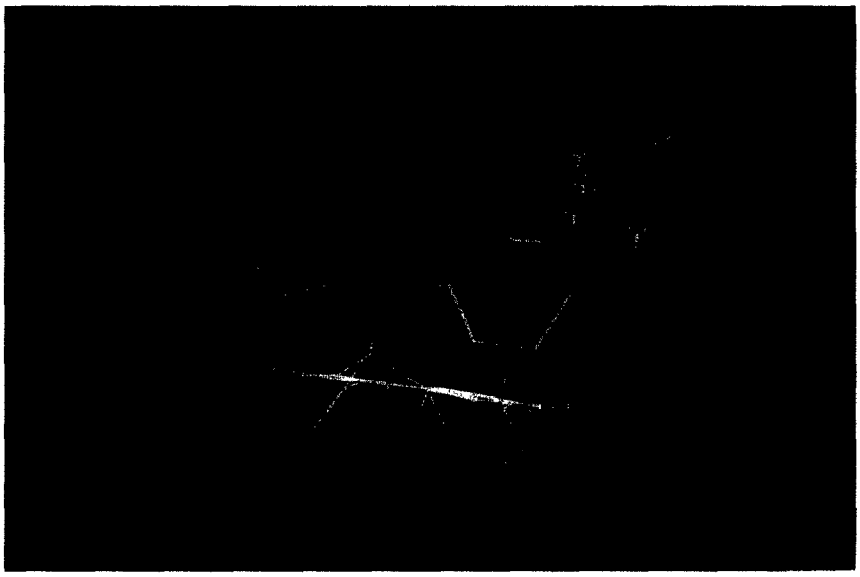


Figure 4: Structure of the thiomethane activated complex showing the van der Waals pocket in which the methyl group is situated with the C1-C12-S-C(methyl) dihedral angle of 171°. The color scheme is as follows: DNA: blue, NCS: orange, radical sites: red. The van der Waals surface is colored by atom type: oxygen: red, hydrogen: white, sulfur: yellow.

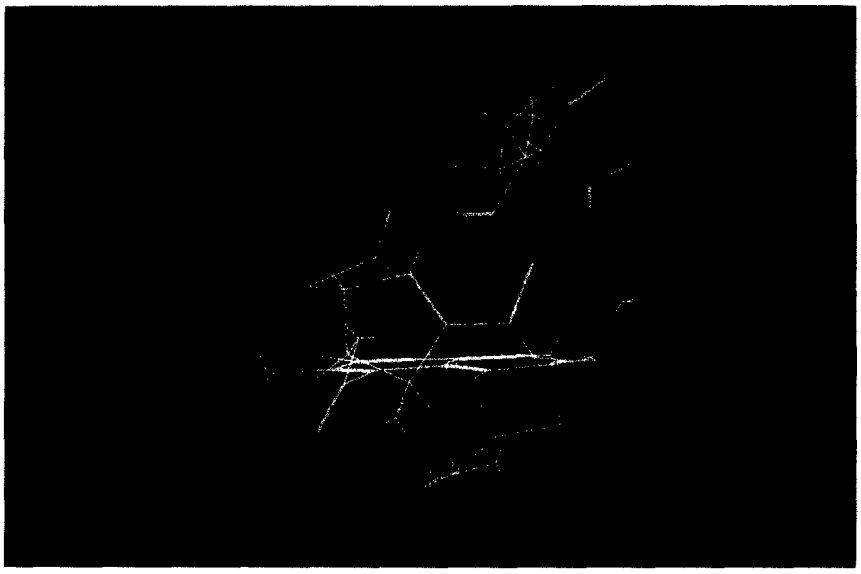


Figure 5: Structure of the thiomethane activated complex showing the van der Waals pocket in which the methyl group is situated with the C1-C12-S-C(methyl) dihedral angle of 5°. The color scheme is as follows: DNA: blue, NCS: orange, radical sites: red. The van der Waals surface is colored by atom type: oxygen: red, hydrogen: white, sulfur: yellow.

changed to a methane thiol. In developing this model we observed that there were two possible directions from which the thiol might approach C12. One of these yields the activated drug with the C1-C12-S-C(methyl) dihedral angle close to 180°; the other yields the adduct with the dihedral angle close to 0°. The complex with the dihedral at 180° was chosen for investigation first. It appears that this anti conformation would be the lower energy one based solely on intramolecular interactions that would be present in the other conformation. A molecular dynamics simulation (identical to the previous two) was performed on this complex. The results (Fig. 4) indicate that in this orientation the methyl group is too far from C2 to participate in a 1,5-hydrogen atom transfer process. Furthermore, the methyl group appears to be unable to rotate freely to the other conformation. The rigidity of this region was traced to the presence of a pocket formed by the DNA backbone and the fucosamine sugar. It therefore appears that there may be a significant energetic cost associated with the rotation that will allow for hydrogen atom transfer to occur.

An analysis of the syn conformation, however, leads to a different conclusion. Manual rotation of the dihedral angle to 5° places the methyl group into a pocket bordered by the DNA backbone and the fucosamine sugar. In this orientation it appears that the proposed 1,5-hydrogen atom transfer is possible. The C(methyl)-C2 distance is 2.9Å (Fig. 5). Thus, molecular modeling suggests that for the Goldberg hypothesis to be operative a specific orientation of the incoming nucleophile is required. Qualitatively, it would appear that both approaches are accessible. One issue that remains unresolved is the fate of the newly generated sulfur stabilized radical. As it is somewhat more solvent exposed than the C2 radical, which is buried within the minor groove, it may be quenched by water and thus be rendered harmless. However, the sulfur stabilized radical is also quite close to the C4' hydrogen of cytidine 7 (3.4 Å in this static model) and may therefore find this hydrogen to be an effective quenching agent. Interestingly, studies have shown that when double strand cleavage does occur, the lesions stem from C5' damage on one strand (as already described) and C4' damage on the second one.[15] It is conceivable that this secondary radical is responsible for abstraction of hydrogen from the second strand of DNA.

In summary, molecular modeling studies of a DNA-NCS complex suggest that it may be energetically disfavored for the drug to be activated by the naphthoate carbonyl oxygen as has been proposed by Fuchs if ligand-binding to DNA occurs prior to activation. In addition, the modeling suggests that a specific approach geometry may allow for the 1,5-hydrogen atom transfer process from C2 to the methylene of glutathione in the complex as has been proposed by Goldberg.

Acknowledgment We thank the National Institute of General Medical Sciences for support of this research.

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[6] The intercalation site was created by cleaving the DNA backbone at the appropriate site, separating the halves by 3.4 Å, and rotating by 12° so as to unwind the DNA. The resulting structure is minimized for 200 iterations to return bond lengths and angles to reasonable values.

[7] All calculations were conducted using the Macromodel program (Mohamadi, F.; Richards, N.G.J.; Guida, W.C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W.C. *J. Comp. Chem.* **1990**, *11*, 440.) using the AMBER force field (Weiner, S.J.; Kollman, P.A.; Case, D.A.; Singh, U.C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P.J. *J. Am. Chem. Soc.* **1984**, *106*, 765).

[8] The molecular dynamics simulations were conducted at 300 K using the SHAKE algorithm, and extended non-bonding cutoffs. Intermediate structures were saved at least every 0.5 ps during the observation period.

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[14] During the preparation of this manuscript a report appeared (Kappen, L.S.; Goldberg, I.H. *Science* **1993**, *261*, 1319) demonstrating NCS mediated cleavage of single stranded DNA in the absence of thiol. Preliminary spectroscopic identification of the recovered post-activated drug indicates that the structure contains a bond between C1" and C12. Although this finding supports the Fuchs proposal the conditions were neither physiologically relevant nor similar to those used for the previous double strand cleavage experiments.

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