

The Determination of Adduction 'Hot Spots' via Mass Spectrometry and Molecular Modeling

Terrence Black^{*}; Mary Jo Ondrechen; Paul Vouros
Barnett Institute and Dept. of Chemistry, Northeastern University, Boston, MA
02115

Molecular modeling allows for *in silico* analysis of chemical systems. Commercially available modeling programs such as InsightII and Amber can be utilized to simulate the adduction of polyaromatic hydrocarbons to oligonucleotide model systems. The energies of these systems can be calculated using forcefields. The energies from multiple positional isomers of the adduct can be compared to determine the most energetically favored point of adduction. These proposed modification sites can then be compared with adduction points determined by other methods, such as liquid chromatography - mass spectrometry (LC-MS) to establish correlations between the calculated and experimentally obtained results.

Molecular modeling was used to create an *in silico* oligonucleotide analogous to a system previously investigated in our laboratory. In the prior study, a double stranded sequence (5'-TAGTCA₆A₇GGGCA-3') was modified by reaction with benzo[c]phenanthrene diol epoxide (BcPDE) and investigated by LC-MS. This analysis revealed chemoselective binding at both A₆ and A₇, with three times more prevalent adduction at A₇. In order to investigate molecular modeling as a complementary tool to LC-MS, InsightII was used to simulate the adduction of BcPDE. A Consistent Valence ForceField (CVFF) was applied to this system. Energy minimizations were performed with the program Discover, by the methods of steepest descents and conjugate gradient. Counterions and a solvent shell were added to the system before minimizing the energy once again.

Adductions have been simulated at A₆, A₇, and G₉ with the InsightII program. An initial minimization was performed to obtain reasonable starting values for bond angles and bond lengths in the adduct. Sodium was added as a counterion to offset the negatively charged phosphate in the backbone of the oligonucleotide. A 10 Å solvent shell of water was added in order to make the system comparable to an *in vivo* environment. At this point conjugate gradient minimizations were performed until a derivative of less than 0.03 was reached. Upon completion of the minimizations both the coulombic and total energies of the system could be determined.

In order to determine the most probable point of adduction, one must consider many factors. The first is the propensity for the adduct to select either dA or dG for adduction. Also one must consider that if there are multiple dA and dG sites available, that the flanking sequence to that particular base must have an impact on the selection of the point of adduction. To take these flanking bases into

consideration one must look to the non-bonding energies (such as coulombic energy) to determine if a particular point of adduction is energetically favored.

Point of Adduction	Total Energy (kcal)	Coulombic Energy (kcal)
No Adduct	-25620.510769	-31716.54143
A7	-24068.623025	-30172.90366
A6	-24117.491766	-30167.54058
G9	-24081.395199	-29968.75499

Table 1. Energies calculated after all energy minimizations.

It was determined that the calculated coulombic energies (Table 1.) agreed with the results of the previously described LC-MS study. Adduction at the found chemoselective hot spot (A₇) proved to have the lowest coulombic energy, in contrast to adduction at G₉, which had the highest coulombic energy. It would stand to reason that G₉ would produce the highest coulombic energy as this is consistent with LC-MS data which showed no adduction at that site. Molecular modeling also shed light on the variations in structural conformation of B[c]PDE when adducted to different bases (Figure 1.)

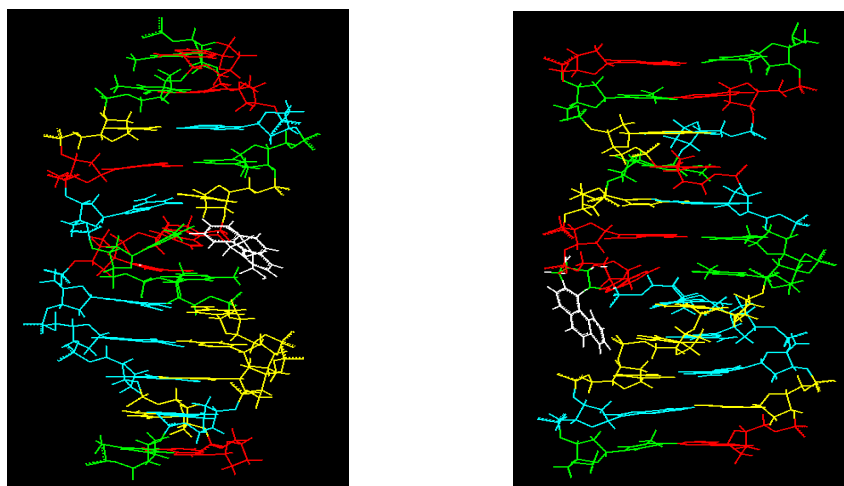


Figure 1. Minimized structure of the adducted oligonucleotide system for both A₆ (left) and A₇ (right). Adduct shown in white, dA in red, dT in green, dG in blue, dC in yellow. The water solvent shell and sodium counterions were hidden to clarify the adduct location

The models shown above identify the structural differences that are present between adduction of the same carcinogen, at the same base pair with different flanking bases. As shown in Figure 1, B[c]PDE is sandwiched between bases when adducted to A₆, whereas the adduct resides outside of the double helix when adducted to A₇. This information demonstrates how molecular modeling may serve as a useful complementary tool to an LC-MS study.