A theoretical evaluation of the effect of netropsin binding on the reactivity of DNA towards alkylating agents

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ABSTRACT

The effect of netropsin binding on the electrostatic potential of DNA reactive sites is presented. Calculations are performed for atoms N7 and O6 of guanine, N3 and N7 of adenine of model, 25 base pair long, DNA-netropsin complexes. An important weakening of the potential is found spreading along all the oligonucleotide chain studied. The results are discussed in connection with the inhibitory effect of a related ligand, distamycin A, on DNA methylation.

INTRODUCTION

Interactions between alkylating agents, many of which are potent carcinogens or/and mutagens, and DNA are intensively studied (for reviews see e.g. 1, 2) and the elucidation of the factors which influence these interactions is important for a better understanding of their nature. A particularly interesting problem, which attracted significant attention recently (see e.g. 3) concerns the possible modulating effect on such interactions of the binding to DNA of different types of groove specific ligands. Such a binding will necessarily result in a modification of the electrostatic properties of DNA active sites. Now, it has been shown in our laboratory (4,5) that these electrostatic properties, in particular the molecular electrostatic potential, are able to account satisfactorily for the evolution of the reactivity of a large number of different types of electrophiles towards the principal reactive sites of DNA. We may thus expect that the evaluation of the effect on this property of the binding of a ligand will contribute to the understanding of the associated modification in the reactivity of DNA.

In this paper we wish to present the results of model calculations demonstrating the influence of an important representative of the minor groove binding ligands, netropsin, on the electrostatic potentials of particularly important DNA reactive sites: N7 and O6 of guanine and N3 and N7 of adenine.
METHOD

The B-DNA models used in our calculations were built from 50 5'-nucleotides, producing 25 base pair oligomers, the geometry of these helices being that of Arnott et al. (6). Two model sequences were studied, the first containing 25 AT base pairs and the second 5 central AT base pairs surrounded by 10 GC base pairs on each side. A model counterion screening of the nucleic acid segments was performed by placing a magnesium cation at the bridge position (1.99 Å from each anionic oxygen) of every second phosphate and out of step in the two phosphodiester chains (7). In this way 25 magnesium cations were positioned, resulting in a total charge on the nucleic acid of +2.

The geometry of netropsin-DNA complex was obtained by the energy minimization technique described in (8). The minimization was done independently for the two sequences studied. In both DNA segments the optimally bound netropsin was located close to the center of the segment, deep in the minor groove, following the results obtained in (8).

The molecular electrostatic potentials presented in figs. 1 and 2 represent average values over all the accessible surface of the atoms studied. This technique is described in detail in (9), here we recall only its main features. In order to find the accessible points of an atom within a macromolecule we cover its van der Waals sphere with a grid of uniformly distributed points, generated by the Korobov technique (10). The attacking species, a sphere of a chosen radius, in this case 1.8 Å corresponding to CH$_3^+$ (11), is placed in contact with each one of the grid points and it is checked whether the attacking sphere intersects any of the van der Waals spheres of the other atoms composing the macromolecule. If no such intersections exist the associated grid point is classed as accessible. For each of these accessible grid points a molecular electrostatic potential is calculated and finally an average value for the atom is obtained.

The electrostatic potential is calculated with the use of optimized monopoles, obtained by the Hückel-Del Re procedure, reparametrized (9) to reproduce the electrostatic potentials of the nucleic acid subunits, calculated with the use of Overlap Multipole Expansions (5) derived from ab initio wave functions. Charge redistribution between the subunits was accounted for by calculating the reparametrized Hückel-Del Re monopoles for macromolecular fragments containing centrally the subunits whose charges are desired (9). A net charge of -1 was maintained for a nucleotide to a precision of 0.0004e.

RESULTS

In fig. 1 we present the electrostatic potentials calculated for the B-
FIGURE 1. The effect of netropsin binding on the electrostatic potential of (dA)$_{25}$,(dT)$_{25}$ screened by Mg$^{2+}$. a) Effect on Adn(N7) and b) effect on Adn(N3). The full curve corresponds to the nucleic acid without netropsin and the dotted curve to the DNA-netropsin complex. The dark bar in b) represents the position of netropsin.

DNA model with the AT sequence. The lower full curves correspond to the counterion screened nucleic acid alone, the upper dotted curves to its complex with netropsin, located in the minor groove between the 11-th and 15-th base pairs. Fig. 1a shows the potential calculated for the N7 atoms of the adenines, located in the major groove of DNA, and fig. 1b shows the potential of the N3 atoms of this base located in its minor groove. The middle part of fig. 1b shows, by the dark bar, the region where netropsin is actually bound making the N3 atoms inaccessible. Both figures show a common feature, namely an important decrease of the electrostatic potential upon netropsin binding. For Adn(N7) the potential in the middle part of the sequence even becomes positive, but the fact which is particularly interesting is that the effect is very long range, remaining roughly 0.8 volts even 10 base pairs away from the netropsin binding region.

We now consider our second model DNA fragment consisting of 5 AT base pairs, to which netropsin can bind, surrounded by 10 GC base pairs on each side. The results obtained for the atoms N7 and O6 of guanine are shown in fig. 2a and fig. 2b respectively. In the middle of the fig. 2a, the N7 atoms of the five central adenines are also included and are clearly distinguishable due to their weaker potentials compared to those of the guanines. Both parts of fig. 2 show again that netropsin binding weakens the potentials of the reactive sites of neighbouring guanines, the reduction reaching roughly 2 volts for the closest bases and almost completely destroying their negative potentials at N7 and O6. As in the case of the model of fig. 1 the effect of netropsin is long range, still causing potential reductions of about 0.7 volts ten base pairs away.
The results obtained in this study show that netropsin binding to DNA induces an important modification of its electrostatic properties. This effect is long range and although the binding occurs selectively in the minor groove it affects sites in both the major and minor grooves, reducing their associated electrostatic potentials. This phenomenon can be expected to modify the reactivity of DNA bases both in the vicinity of the ones involved in the ligand binding and also at a significant distance from them.

Direct experimental results for the effect of netropsin binding on the consequent reactivity of DNA are not available. However, it seem interesting to indicate here recent results (3) obtained on the inhibition of the methylation of DNA by N-methyl-N-nitrosourea (MNU) due to the binding of the closely related drug distamycin A. The formation of N7-methylguanine, O6-methylguanine and N3-methyladenine was equally affected. Distamycin A, like netropsin, shows a preference for the minor groove of A-T rich sequences. Although the detailed mode of its binding to DNA may be somewhat different from that of netropsin (12), it may be reasonably presumed that the overall electrostatic effect that these drugs produce will be similar. The fact that computations carried out for the effect of netropsin account for the experimental results obtained from the effect of distamycin A supports this viewpoint. Altogether it appears thus that the modification of the molecular electrostatic potential of DNA upon ligand binding could be one of the major factors governing the modulation of subsequent reactions.
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REFERENCES