Increased DNA Binding Specificity for Antitumor Ecteinascidin 743 through Protein−DNA Interactions?

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Ecteinascidin 743 (ET743) is a marine natural product consisting of three linked tetrahydroisoquinoline subunits and an active carbinolamine functional group (Figure 1). ET743 displays potent antiproliferative activity against a variety of tumor cells and is currently undergoing phase II clinical trials.[1] ET743 is known to bind to the minor groove of several DNA triplets containing a central guanine (e.g. AGC, CGG, TGG)[2] and to alkylate the exocyclic amino group of this purine base,[3] but the mechanism by which it exerts its anticancer activity remains to be elucidated.

We have recently studied, by molecular dynamics simulations in aqueous solution, the complexes of ET743 with two DNA target sequences and shown that drug binding brings about widening of the minor groove and bending toward the major groove.[4] The degree of bending was found to be in very good agreement with results from gel electrophoresis experiments,[5] and the major local bending element identified was an increase in roll at the base pair step involved in covalent bond formation.

Prevailing ideas about the mode of action of ET743 are that (i) by inducing bends in DNA, the drug could be serving a surrogate protein function by juxtaposing specified DNA fragments not contiguous in the primary sequence, (ii) one or more proteins could be recruited to the drug−DNA complex, and/or (iii) drug binding could disrupt the interaction of DNA-binding proteins with their target sites.[6] Indeed, it has been shown that binding of transcription factor NF-Y is impaired[7] and topoisomerase I is poisoned[8] by ET743, albeit in both cases at concentrations much greater than are necessary for achieving its cytotoxic effect.

On the other hand, at pharmacologically relevant concentrations, ET743 is also known to decrease induced activation of expression of a number of genes,[9] the best studied of which is *MDR1*,[9,10] the gene whose overexpression is responsible for multidrug resistance in cancer cells. The *MDR1* gene is regulated by NF-Y, which binds to a CCAAT box,[11] and also by Sp1, which binds to a GC box.[12] CCAAT contains a TGG site on the complementary strand, which certainly is one of the preferred binding sequences for this drug.[2,3] When looking for other possible binding sites on the 5′-flanking promoter region of *MDR1* (Figure 2),[13] we focused on the 5′-CGGGCCGGG-3′ sequence to which Sp1 binds.

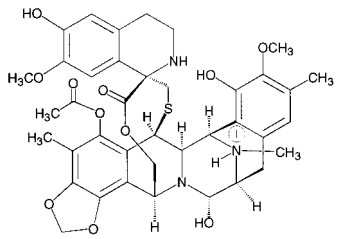

Figure 1. Chemical structure of ecteinascidin 743 (ET743).

This GC box contains two possible CGG binding sites on one strand and an alternative CGG binding site on the complementary strand. Sp1 is predicted to contain three contiguous Cys2-His2 zinc finger domains[14] near the C-terminus which are highly homologous[15] to other zinc fingers whose structures have been solved, both free and complexed to their cognate DNA sequences. Particularly noteworthy is the transcriptional regulator EGR-1 (Zif268)[16] which binds to the DNA sequence 5′-CGCCCCCGC-3′ (EGR site) and variants of which have been cocrystallized with a number of different oligonucleotides.[17] From this and related work,[18] it has been learned that each Cys2-His2 zinc finger is composed of two short β-strands followed by an α-helix[19] and makes its primary contacts with the DNA major groove in a three-base pair subsite. In doing so they cause a distinctive distortion in the DNA molecule.[20] Unexpectedly, we have found that the minor grooves of DNA when bound to the zinc fingers of EGR-1 and in the covalent complex with ET743 are virtually superimposable (Figure 3). This striking similarity raises the interesting possibility that ET743 preferentially targets the minor groove of DNA when bound to a zinc finger-containing transcription factor, very likely but probably not exclusively Sp1.[14b] In fact, molecular modeling of the Sp1−DNA−ET743 complex is relatively straightforward (Figure 3B) as the widened minor groove in the homology-based zinc finger region of the Sp1−DNA binary complex[21] and the more readily accessible 2-amino group of guanine provide a binding site for ET743 that is highly preshaped for fitting. Since no additional distortions have to be inflicted by the drug upon binding,[4] we propose that this prestructured site could provide an important additional element of selectivity so as to be preferred over any of the vast number of suitable DNA triplets present in the entire genome which are presumably found in a more or less standard B-DNA conformation. Thus, ET743 binding to DNA would depend not only on the well-defined hydrogen-bonding rules necessary for sequence recognition and adduct formation[2,3] but also on the protein-induced preorganization of a DNA stretch that becomes structurally complementary to the wedge shape of the drug molecule.[3,4]

The functional groups of ET743 and related analogues[22] not involved in DNA binding that protrude out of the minor groove and are known to be essential for activity are likely to interact with other protein residues. Their role could be either stabilizing the ternary complex, thereby preventing dissociation of Sp1, or else promoting a conformational change in a spatially con-

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(a)

5′-C**CGGGCCGGG**AGCAGTCATCTGTGGTGAGGCTG**ATTGG**CT-3′
3′-G**GCCCGGCCC**TCGTCAGTAGACACCACTCCGAC**TAACC**GA-5′

(b)

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EGR1  YACPVESCDRRFSRSDELTRHIRIHTGQKPFQCR--ICMRNFSRSDHLTTHIRTHTGEKPFACDICGRKFARSDERKRHTKIHL
      : * ::.*.:: :.:::.*  *:* ***::** *    * :.*:***.*  * ***** ***  * ::* ***. .:* * *
Sp1   HICHIQGCGKVYGKTSHLRAHLRWHTGERPFMCTWSYCGKRFTRSDELQRHKRTHTGEKKFACPECPKRFMRSDHLSKHIKTHQ
      |_____________________|    |_____________________|    |_____________________|
            Zn finger 1                Zn finger 2                Zn finger 3
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Figure 2. (a) DNA sequence of part of the 5′-flanking side of the human *MDR1* gene containing the GC and CCAAT boxes (shown in bold type). (b) Sequence alignment[15] between the zinc finger domains of human EGR-1 (Zif268) and human Sp1. Cysteine and histidine residues used to coordinate the zinc ions are shown in bold. Positions with fully conserved residues are marked with an asterisk; colons and dots denote conservation of strong and weaker groups, respectively, according to GONNET/PAM250 substitution matrices.

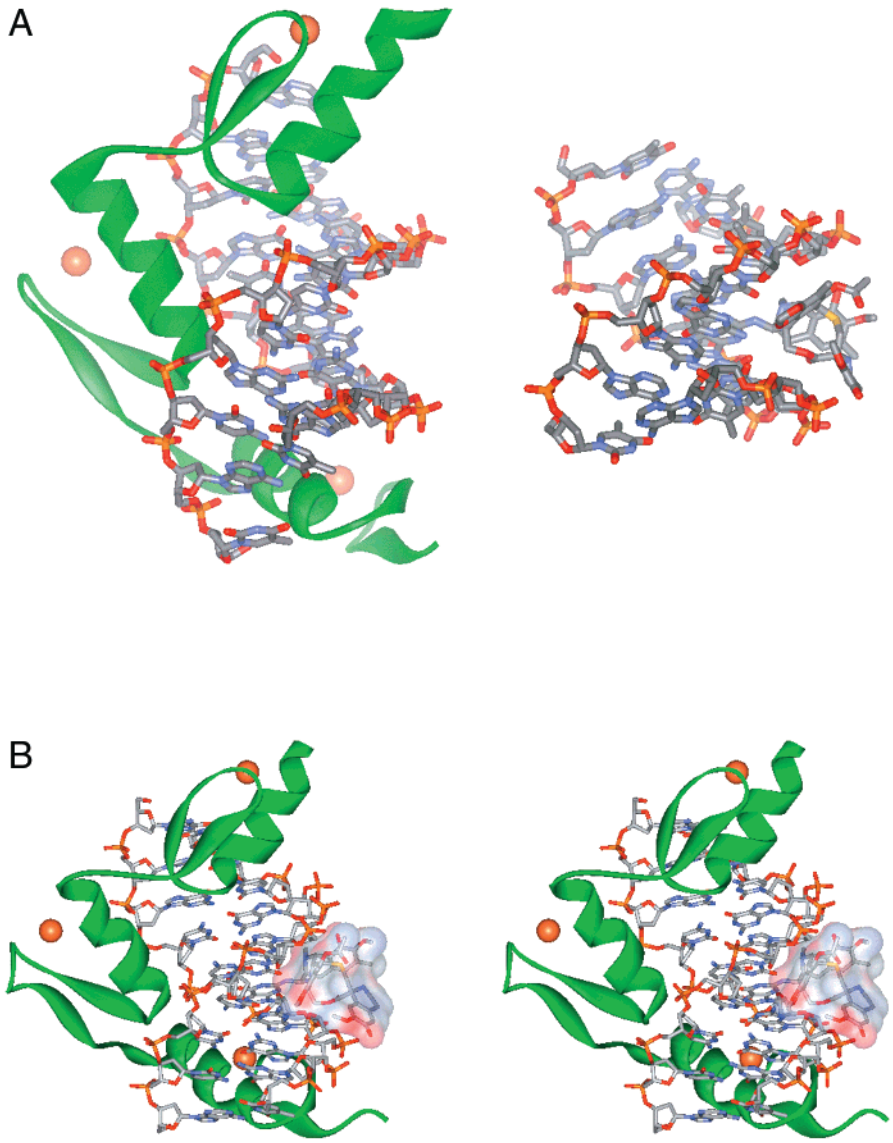

Figure 3. (A) View of the minor groove in the complexes of DNA with (left) zinc finger EGR-1 (Zif268; PDB entry 1AAY)[17] and (right) ET743 covalently bound to the central guanine of d(TAACGGTTA)$_2$ (PDB entry 1EZ5).[4] Zinc ions are displayed as orange spheres. (B) Stereoview of a preliminary model of the zinc finger domain of Sp1 (ribbon representation) in complex with its cognate GC box (5′-CGGGCC**G**GG-3′) from the *MDR1* gene promoter and ET743 (enveloped in a semitransparent surface) covalently attached to the guanine shown in bold. Coordinates of the refined ternary complex will be deposited with the Research Collaboratory for Structural Bioinformatics.

tiguous domain so as to hamper interaction with other proteins. Likely candidates could be a specific regulatory kinase[23] or another protein of the transcription machinery such as NF-Y, which binds to the promoter region (at least in the case of *MDR1*) three helical turns downstream of the GC box (Figure 2).

ET743 causes a remarkable delay in S-phase progression of the cell cycle, eventually resulting in a G_2/M block.[9] Other genes regulated by Sp1 are *c-jun*,[24] *ras*,[25] histone *H4*,[26] cytochrome c_1,[27] dihydrofolate reductase,[28] thymidine kinase,[29] and topoisomerase II,[30] all of which play an important role in cell proliferation. Induced activation of *H4*, *c-fos*, and *c-jun* has already been shown to be inhibited by ET743.[9] The present hypothesis provides new insight into the unusually high potency (low nanomolar) and unique mechanism of this class of anticancer agents[22] and opens new avenues for research.

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