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Molecular modeling of the interaction of anthracenyl-amino acid topoisomerase inhibitors with the DNA sequence d(CGTACG)

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Anthracenyl-amino acid and dipeptide conjugates represent new classes of topoisomerase (topo) inhibitors. To investigate the structural basis for their different selectivity against topo I and II and varying potency, the binding of six compounds to d(CGTACG) was studied by molecular modeling. Modeling data were in good agreement with physical data showing that five compounds intercalated DNA with the anthraquinone chromophore orientated in parallel to the long dimension of the d(CpG) base pairs and the amino acid placed in the minor groove. Differences in binding modes emerged which correlated to different biological properties. The amino acid chain of the topo I inhibitor (NU/ICRF 600, gly–phe) extended significantly out from the helical axis horizontal. The amino acid side chains of two topo II inhibitors (NU/ICRF 510, arginine and NU/ICRF 512, methionine) were inserted into the minor groove, whereas the C-terminal groups (hydrazide) of two potent topo II inhibitors (NU/ICRF 500 and 506, serine) were placed into the minor groove while the amino acid side chains pointed away from the minor groove. These data provide structural information which may prove valuable in rational design of second generation analogs.

Key words: anthracenyl-amino acid conjugates, d(CGTACG), DNA binding, molecular modeling, topoisomerase I and II inhibitors.

Introduction

Anthracenyl-amino acid conjugates (for structures and biochemical properties, see Figure 1) represent a new class of compound which exhibit a range of specificities as inhibitors of DNA topoisomerase (topo) I and II.[1] Conventional inhibitors of topo I and II stabilize a ternary complex between the drug, DNA and the enzyme referred to as the cleavable complex.[2,3] Recently, new compounds have been described which do not stabilize the cleavable complex but act as catalytic inhibitors of enzyme activity.[4] Various mechanisms have been proposed to explain catalytic enzyme inhibition ranging from non-specific DNA binding[5] to a selective interaction with a specific conformation of the topo protein formed during its complex catalytic cycle.[6]

In the absence of crystallographic studies, structure–activity relationships coupled to computer modeling have been utilized to identify drug molecular features necessary for stabilization of the ternary complex.[7,8] A common pharmacophore has been proposed for topo II inhibition consisting of a polycyclic ring system for DNA binding by intercalation and a further aromatic ring substitution with a sterically unhindered hydroxy or amino function required to extend out of the plane of DNA and interact with a hydrophobic pocket of the enzyme. In this present investigation, molecular modeling has been employed to study the interaction of anthracenyl-amino acid conjugates as well as a dipeptide derivative with the DNA sequence d(CGTACG) in order to explore binding modes for this class of compound. These data may provide insights into mechanisms of topo inhibition and prove informative in the design of second generation compounds.

Materials and methods

Experimental compounds

Anthracenyl-amino acid conjugates and the dipeptide were synthesized through the reaction of α-amino acid esters with [2H,3H]-9,10-dihydroxyanthracene-1,4-dione as described in detail (Cummings and Mincher, UK patent GB 9205859.3; International Application Number PCT/GB93/00546, published 30 September 1993).

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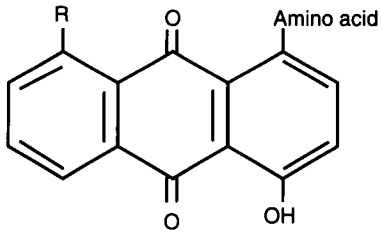

Code name	Amino acid	R	C-terminus	Mechanism of topo Inhibition[b]	DNA binding[a] UV thermal melt (ΔT_m) 1	2	Topo I unwinding assay	IC_{50}^{c} A2780 (μM)
NU/ICRF 500	serine	H	$-CONHNH_2$	catalytic inhibitor topo II	1.2 ± 1.7	1.3 ± 0.4	+	5.4
NU/ICRF 505	tyrosine	H	$-COOC_2H_5$	stabilizes topo I cleavable complexes	0	0	–	7.4
NU/ICRF 506	serine	OH	$-CONHNH_2$	catalytic inhibitor topo I and II	4–9	ND	++	3.4
NU/ICRF 510	arginine	H	$-COOCH_3$	catalytic inhibitor topo II	4.2 ± 1.1	2.1 ± 1.2	+++	11.2
NU/ICRF 512	methionine	H	$-COOCH_3$	weak catalytic inhibitor of topo II	25.9 ± 0.5	9.5 ± 2.2	–	NA
NU/ICRF 600	gly–phe	H	-COOH	stabilizes topo I cleavable complexes, catalytic inhibitor topo I and II	0	0	–	NA

Figure 1. Molecular structures and biological properties of anthracenyl-peptides. [a,b,c]Bulk of data taken from Meikle *et al.*[1] In the DNA binding studies, sequence **1** is d(CGTACG) and sequence **2** is d(CGCGAATTCGCG). The topo I DNA unwinding assay used relaxed pBR322 plasmid DNA as a substrate and unwinding was detected as the spontaneous reformation of supercoiled plasmid DNA in the presence of excess topo I. The degree of unwinding was scored on the basis of effective concentration: –, no unwinding; +, unwinding at 50 μg/ml; ++, unwinding at 5 μg/ml and +++, unwinding at 1 μg/ml. IC_{50} values refer to the level of *in vitro* cytotoxicity against the A2780 human ovarian cancer cell line. ND, not determined; NA, not active.

Molecular modeling

Minimum energy conformations of anthracenyl-amino acids. Molecular modeling was performed using the Quanta and Charmm programs (Molecular Simulations, Burlington, MA). The compounds were built using Chemnote, imported into Quanta and energy minimized using the Steepest Descents program (50 iterations) followed by minimization using the Adopted Basis Newton–Raphson (ABNR) algorithm (800 iterations). A conformational search of the anthraquinone–NH and the NH–C torsions was then performed using the conformational search program in Quanta. For each structure the torsion angles of two selected bonds were altered stepwise by 30° to 360° and energy minimized at these fixed positions using the ABNR procedure following each step. This process produced a two-dimensional contour plot which was analyzed to yield the minimum energy conformations associated with the selected torsion angles of the structures. For each molecule a further conformational search of the minimum energy conformation derived above was carried out on the next two bonds. This process again produced a contour plot of torsion angle versus potential energy. In these studies, the structures calculated to have the minimum energies from the two above conformational searches were used in modeling DNA binding.

Docking of anthracenyl-amino acids with the DNA sequence d(CGTACG). The DNA sequence d(CGTACG) was built in Quanta. Each of the six anthraquinones (see Figure 1) was inserted in parallel and perpendicular modes between the four end bases (CG.CG). All the DNA atoms except those in the CG.CG were constrained (i.e. fixed rigidly in space) and the complex energy minimized (Steepest Descents 50 iterations, ABNR 200 iterations). Next all of the DNA atoms were constrained and the anthraquinone was energy minimized whilst still positioned within the hexamer (Steepest Descents

50 iterations, ABNR 200 iterations). Finally the whole complex was energy minimized (no constraints applied, ABNR 25 iterations). All six anthraquinones are unsymmetrically substituted, therefore the insertion of the tricycle within the DNA was performed with the side chain pointing into either the major or minor groove. The resulting complexes were analyzed in Quanta to: (1) view the position of the drug molecule with respect to the DNA, (2) determine intramolecular hydrogen bonds and (3) measure the potential energy of the complex.

Results

The nucleotide sequence chosen in the present work d(CGTACG) exhibits the standard B-form right-handed DNA double helical structure[9] and has been used extensively in molecular modeling as well as crystallographic studies of several related anthraquinoid/anthracycline drugs.[10–12] d(CGTACG) has also been employed, together with the dodecamer d(CGCGAATTCGCG), to measure binding of anthracenyl-amino acids to DNA by UV-thermal melt analysis (see Figure 1).[1]

The present studies represent the first report of molecular modeling being applied to study DNA binding of this new class of potential anticancer drugs. By molecular modeling, five of the six compounds (see Table 1) were determined to form a stable interaction with d(CGTACG) where the planar anthraquinone ring system was orientated in parallel to the long dimension of the d(CpG) base pairs at either end of the sequence with the amino acid was inserted into the minor groove (see Figures 2–4 for examples). No DNA binding was tolerated with the two serine compounds (NU/ICRF 500 and 506)

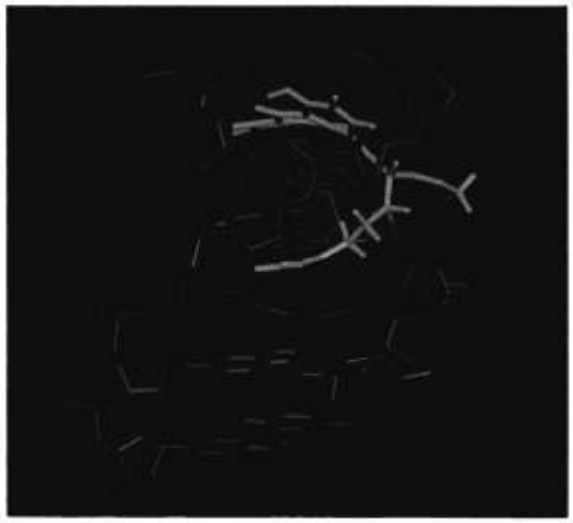

Figure 3. Molecular modeling of the interaction of NU/ICRF 510 with d(CGTACG). The complex is viewed essentially down the 2-fold axis of the minor groove with the drug highlighted in yellow, the DNA sequence in red and hydrogen bonds depicted as broken lines.

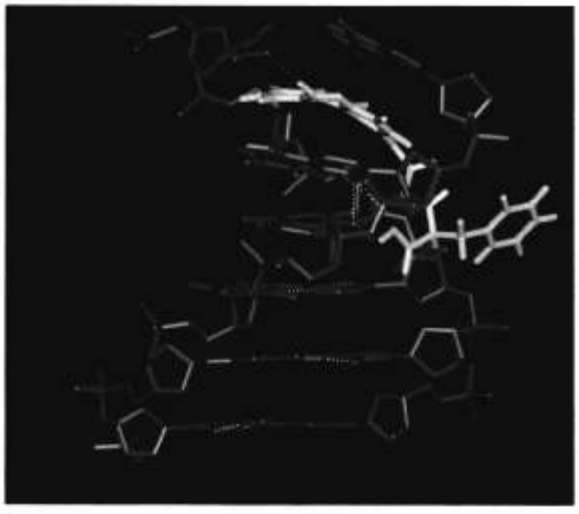

Figure 2. Molecular modeling of the interaction of NU/ICRF 600 with d(CGTACG). The complex is viewed essentially from the 2-fold axis horizontal with the drug highlighted in yellow, the DNA sequence in red and hydrogen bonds depicted as broken lines.

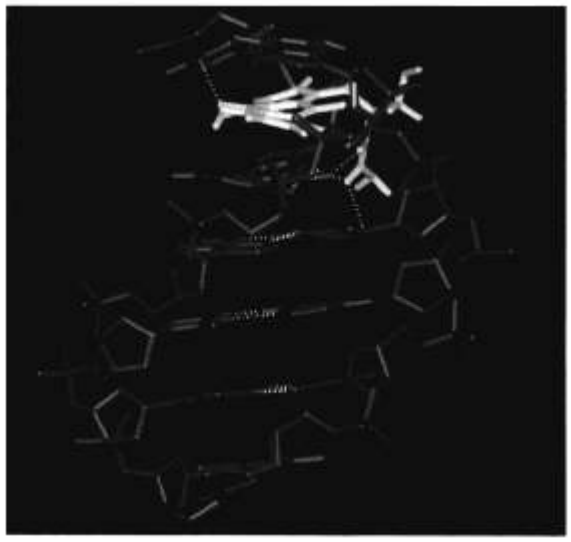

Figure 4. Molecular modeling of the interaction of NU/ICRF 500 with d(CGTACG). The complex is viewed essentially down the 2-fold axis of the minor groove with the drug highlighted in yellow, the DNA sequence in red and hydrogen bonds depicted as broken lines.

Table 1. Molecular modeling of the interaction of anthraquinone-amino acid topoisomerase inhibitors with the DNA sequence d(CGTACG)

Compound	Interaction with DNA after modeling[a]	Potential energy (kcal/mol)	No. of hydrogen bonds	Groove placement of amino acids
NU/ICRF 500	binding	−65	4	minor
NU/ICRF 505	non-binding			
NU/ICRF 506	binding	14	1	minor
NU/ICRF 510	binding	63	2	minor
NU/ICRF 512	binding	−229	1	minor
NU/ICRF 600	binding	−224	3	minor

[a]The anthraquinone ring system was orientated in parallel to the long dimension of the d(CpG) base pairs.

when the amino acid was inserted into the major groove prior to energy minimization. With the other four compounds (NU/ICRF 505, 510, 512 and 600), these were pushed out of the DNA complex after minimization. A parallel intercalative binding mode has also been shown previously to be the most stable DNA association observed with a series of closely related mono- and disubstituted *N*-alkyl anthraquinones.[13] Such a binding mode is different from the anthracyclines where the larger ring systems stack perpendicular to the base pairs but is similar in that the anthracycline sugar side chains also lie in the minor groove.[10] The potential energy associated with the minimum energy conformations for the binding of the five compounds to d(CGTACG) and number of hydrogen bonds formed between the drug and DNA sequence are also contained in Table 1.

Discussion

Molecular modeling studies have predicted that four (NU/ICRF 500, 506, 510 and 512) out of the five anthracenyl-amino acid conjugates and the single dipeptide (NU/ICRF 600) should bind to DNA by intercalation. These data are in good—but not perfect—agreement with results from physical studies (see Figure 1).[1] Based on UV-thermal melt analyses, NU/ICRF 500, 506, 510 and 512 bind to DNA to varying degrees but NU/ICRF 505 does not bind, in accordance with the molecular modeling data (see Table 1). Where there is disagreement is with the dipeptide (gly–phe) NU/ICRF 600. According to computer modeling, this compound should also be capable of binding to DNA since formation of the complex is energetically favorable and is stabilized by three hydrogen bonds. However, the molecular modeling programs employed primarily evaluate the stability of a drug–DNA complex once the interaction has occurred after computer placement of the drug between the planes of the base pairs and does not take into account the fact that the compound may not be able to insert between the base pairs in the first place. Both NU/ICRF 505 and 600 contain bulky aromatic amino acid residues (tyrosine and phenylalanine, respectively) and this may explain why these two compounds were unable to intercalate with DNA in the physical binding studies.[1] The poor agreement between topo inhibitory properties and *in vitro* cytotoxicity (see Figure 1) has now been partly explained by a lack of drug uptake into cancer cells for both NU/ICRF 510 and NU/ICRF 600.[14,15]

NU/ICRF 505 (and probably NU/ICRF 600) share two properties in common with the classic topo I poison camptothecin (CPT); they stabilize topo I cleavable complexes and they do not bind to DNA.[1,3] However, CPT has been shown to interact with a site on the binary macromolecular intermediate formed between DNA and topo I during the catalytic cycle of the enzyme.[16] The enzyme catalyzed cleavage of the phosphate backbone that takes place during this process may permit access of NU/ICRF 505 and 600 to bind to DNA, and thus stabilize the cleavable complex.

A major emphasis of the present study was to establish whether molecular modeling could provide a structural explanation for the different selectivity and potency of anthracenyl-amino acids as topo inhibitors. A significant difference was observed between the orientation of the amino acid residue of NU/ICRF 600 (Figure 2) and NU/ICRF 500, 510 (Figures 3 and 4), 506 and 512. In the case of the dipeptide NU/ICRF 600 the aromatic phenylalanine side chain extended away from the helix axis horizontal, whereas with the other four compounds the amino acids residues inserted into the minor groove and this clearly demarcated a biological difference between stabilization of topo I cleavable complexes and catalytic inhibition of topo II (see Figure 1). A large number of existing topo II

inhibitors are also known to intercalate DNA and contain substituents which bind to the minor groove.[5] The strong implication concerning NU/ICRF 600 is that the phenyl ring associates with a hydrophobic site on the topo I protein facilitating stabilization of the cleavable complex, in keeping with analogous models proposed mainly for topo II inhibitors which stabilize the cleavable complex.[7,8,17–19] It is feasible that a similar binding mode to NU/ICRF 600 (Figure 2) occurs with NU/ICRF 505 after it interacts with the binary topo I–DNA macromolecular complex. The presence of the *para*-hydroxyl group on the tyrosine may further stabilize drug protein binding and account for the 4-fold greater activity of NU/ICRF 505 compared to NU/ICRF 600 against topo I.[1]

An interesting structural variation in DNA binding modes was observed between the four topo II catalytic inhibitors. In the case of NU/ICRF 510 (arginine) and 512 (methionine) the amino acid side chains inserted into the minor groove (e.g. see Figure 3 with NU/ICRF 510) whereas the *O*-methyl ester modified C-terminus tended to project away from the minor groove. In contrast, with NU/ICRF 500 (serine) and NU/ICRF 506 (serine) the modified C-terminal hydrazide group inserted into the minor groove (e.g. see Figure 4 with NU/ICRF 500), whereas the side chain tended to project away from the minor groove. Thus, with NU/ICRF 500 and 506 a hydroxyl function is potentially available to interact with a topoisomerase while with NU/ICRF 510 and 512 this functionality is absent. Both NU/ICRF 500 and 506 are more potent catalytic inhibitors than NU/ICRF 510 and 512, with 506 also possessing topo I inhibitory activity. Tighter protein binding of NU/ICRF 500 and 506 might explain their increased biological potency. A close structural analog of NU/ICRF 500 which contains the serine residue but has the amino acid C-terminus modified as an ethyl ester (analogous to NU/ICRF 510 and 512), proved to be far inferior to the parent drug as a topo II inhibitor.[1] Whether this is due to re-orientation of the amino acid in the minor groove is presently under investigation.

Similar conclusions to above, i.e. that an available hydroxy function is necessary to interact with topo II for enzyme inhibition, can be drawn from structure–activity relationship studies carried out with established topo II inhibitors. Removal of the 9-hydroxy external group from ellipticine produces inactive analogs[17] and alterations to the 9-hydroxy position on the anthracycline ring system significantly reduces activity.[20] More recently, it has been demonstrated with a series of 7H-benzo[*e*]pyridol[4,3-*b*]indoles that for topo II inhibition an 'outside binding mode' is required extending from the minor groove of DNA mediated via the hydroxyl group of the A-ring of the chromophore.[19]

The modeling data appear to have provided a structural basis for the different selectivity of anthracenyl-amino acids for topo I and II. However, whilst NU/ICRF 600 was an effective topo I inhibitor and this is proposed to be due to an outside binding mechanism mediated via the phenyl ring, studies with intoplicine analogs suggest that topo I inhibition is favored by a deep intercalative binding mode.[19] Topo II inhibition is normally favored via external hydrogen bonding mechanisms, in keeping with numerous previous studies and the data generated in the present studies on four topo II catalytic inhibitors[7,8,17–20] In conclusion, the molecular modeling data presented have furnished insights into the structural basis for topo inhibition by a series of novel anthracenyl-amino acid conjugates. These data may prove informative in future rational design of second generation analogs.

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