Pluramycins. Old Drugs Having Modern Friends in Structural Biology

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It has been 40 years since the first pluramycin antibiotics were isolated by Umezawa[1] and found to have antimicrobial and anticancer activity. For 37 years, they remained as chemical evolutionary oddities, wearing clothes superficially borrowed from diverse groups of natural products. During this period, isolated pockets of information provided tantalizing snippets for how the pluramycins might interact with DNA and produce their potent biological effects. However, until the application of technology derived from structural chemistry and molecular biology, it was difficult to knit together the assortment of seemingly unmatched patterns that we now know represent an elaborate costume tailored for a very special role. Here we examine the structural components that constitute the costume, reassemble the separate units, and cast the fully costumed structure of the pluramycins in a role in the larger scheme of things. Finally, we ask how the character wearing the costume may have been conceived. In this broader context, we examine the possible role of a DNA-reactive drug in sidelining a transcriptional control system and ask how the molecule was evolutionally derived.

In this Account, we first describe the characterization of the structure of the pluramycin−DNA adduct and propose a novel mechanism for the DNA sequence selectivity of the pluramycins. On the basis of this insight, we then use the pluramycin to probe an important aspect of asymmetry in transcriptional control and demonstrate the formation of a quite unique ternary complex between pluramycin and the TBP−TATA box specific binding complex. This example provides an additional way in which DNA-reactive agents can achieve enhanced sequence selectivity, in this case, by virtue of a protein-induced unwinding of DNA. A summary of drug targeting of transcriptional control has recently appeared.[2]

Pluramycin Structures

The pluramycins are a group of highly structurally evolved DNA-reactive agents that represent a range of 4*H*-anthra[1,2-*b*]pyran-4,7,12-trione structures with attached carbohydrate and epoxide moieties on the corners of their planar chromophores (Figure 1).[3] This structurally evolved complexity is predictive of a complex molecular mechanism giving rise to sequence selective interactions with DNA. Reminiscent of nogalamycin, the pluramycins intercalate through the DNA molecule and interact in both grooves of the DNA helix, and those compounds displaying epoxides have the ability to alkylate N7 of guanine in the major groove.[4−6] These compounds display a novel mode of DNA recognition in which carbohydrate interactions in both grooves of the DNA are transmitted through the helix via an intercalating chromophore to sequence selectively align the epoxide in the major groove for nucleophilic attack by N7 of guanine.[7]

The pluramycins display a range of structures, from the aglycon pluramycins, or pluramycinones, to the heavily glycosylated compounds such as hedamycin and altromycin B (Figure 1). Sapurimycin is typical of the simplest analogs of the pluramycin family, which lack any carbohydrate substitution. The more complicated agents that have multiple sugar moieties are broken down into two subgroups, the "classical pluramycins" and the "altromycins", on the basis of variation in structure and, as will be discussed later, reactivity and sequence selectivity.

Covalent Modification of DNA by Pluramycins

Multiple studies have been conducted that show that the pluramycins interact with DNA. Two of the earlier most insightful studies showed that hedamycin can make at least two types of interactions with DNA. Both interactions are proposed to be intercalative in nature, but in only one is there a covalent bond formed with the DNA.[8,9] Subsequent work has shown that hedamycin forms a stable complex with DNA, causing associated alkali labile strand breaks that occur at deoxyguanosine.[10] However, the exact mechanism of reaction at guanine remained unclear until investigations involving altromycin B were initiated. By isolating and characterizing a thermally-released depuri-

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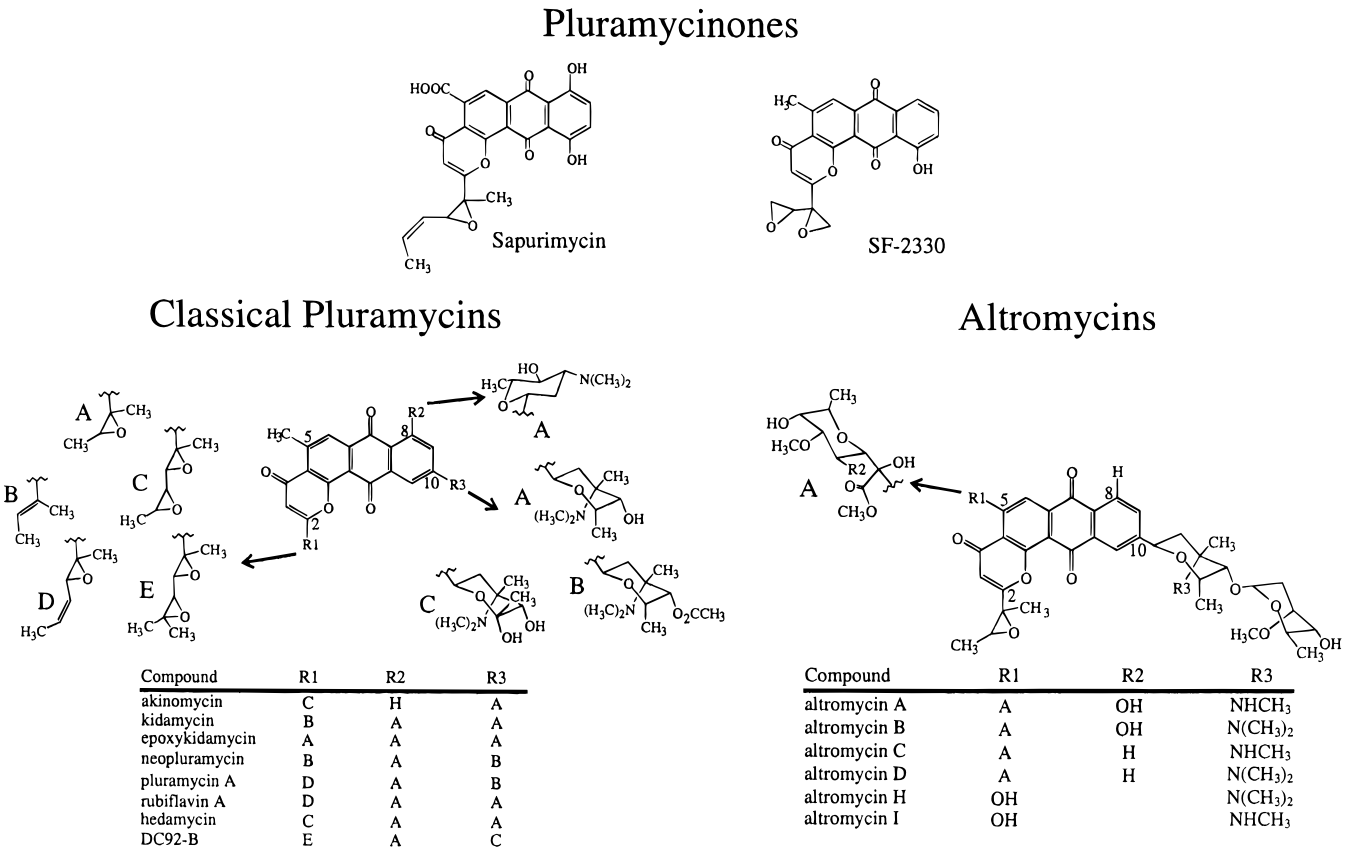

Compound	R1	R2	R3
akinomycin	C	H	A
kidamycin	B	A	A
epoxykidamycin	A	A	A
neopluramycin	B	A	B
pluramycin A	D	A	B
rubiflavin A	D	A	A
hedamycin	C	A	A
DC92-B	E	A	C

Compound	R1	R2	R3
altromycin A	A	OH	$NHCH_3$
altromycin B	A	OH	$N(CH_3)_2$
altromycin C	A	H	$NHCH_3$
altromycin D	A	H	$N(CH_3)_2$
altromycin H	OH		$N(CH_3)_2$
altromycin I	OH		$NHCH_3$

Figure 1. Structures of representative pluramycin antibiotics. This family of antibiotics is divided by structure into several subgroups. Shown are the pluramycinones, the classical pluramycins, and the altromycins.

nation product of the altromycin B−DNA reaction, the probable mechanism of covalent modification was determined.[4] Using high-field NMR in combination with proton−nitrogen correlations of the depurinated product, it was shown that covalent attachment occurs through C16 of altromycin B to N7 of guanine, presumably through electrophilic addition of the drug epoxide to N7 of guanine. On this basis, it was proposed that the drug epoxide undergoes electrophilic addition to N7 of guanine, creating a resultant lesion that is cationic in nature and that readily depurinates under elevated temperatures, and produces the experimentally observable single-strand breaks through a mechanism similar to the Maxam and Gilbert sequencing reaction for guanine (Figure 2). Studies of pluramycin-modified oligomers further confirm this mechanism of covalent modification.[5,6]

DNA Reactivity

Using a 189-base-pair restriction enzyme fragment, the alkylation sequence selectivities and relative reactivities of multiple epoxide-bearing pluramycins (altromycins B, H, and I, pluramycin A, rubiflavin A, and hedamycin) were determined by a strand breakage assay.[7] In general, the classical pluramycin antibiotics showed at least a 5-fold higher reactivity in the alkylation of DNA than altromycin B, H, and I, indicating the importance that substitutions at the C2, C5, C8, and C10 positions play in DNA reactivity. Of the classical pluramycins, hedamycin was the most reactive, followed by pluramycin A and then rubiflavin A. On the basis of these results, it would appear that compounds having a double epoxide (hedamycin) have greater reactivity than those having an olefinic epoxide (pluramycin A and rubiflavin A). In addition, acetylation of the C10 sugar (pluramycin A) increases reactivity over the nonacetylated analog rubiflavin A. This, however, is to be expected, because neopluramycin, an analog that has a C2 vinyl substituent but is otherwise identical to pluramycin A, binds to DNA with greater affinity than kidamycin, an analog that also has a C2 vinyl group but is otherwise identical to rubiflavin A. The relative reactivities of pluramycin A and rubiflavin A therefore probably reflect differences in binding affinity to DNA.[11] In the case of the altromycins, altromycin B demonstrated greater reactivity than altromycin H and I, suggesting the importance of the C5 altrose substituent. Altromycin H, which has a dimethylamino group on the C10 disaccharide, was roughly 5 times more reactive than altromycin I, which instead has a monomethylamino group (see Figure 1).

Sequence Selectivity of Pluramycin Compounds

Several studies have shown that the pluramycin antibiotics show significant DNA sequence selectivity of covalent modification at the lowest drug concentrations tested.[7,10,12] Compounds can be broken down into three groups of sequence selectivity, each demonstrating an overall different 2-base-pair sequence selectivity that correlated with the substitution pattern at the C5, C8, and C10 positions of the tetracyclic chromophore. The first group, the classical pluramy-

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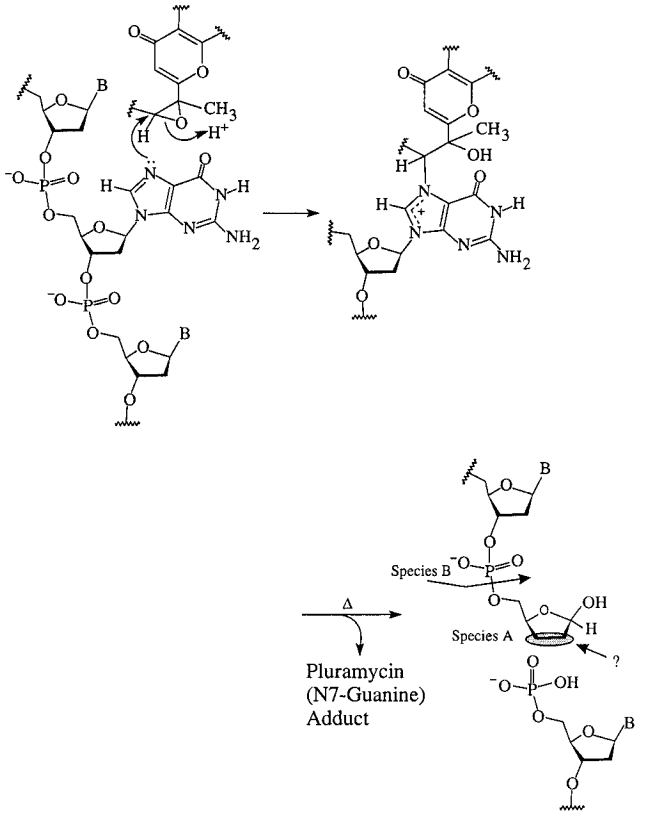

Figure 2. Mechanism of covalent modification of DNA by the pluramycin-type compounds. The N7 of guanine performs nucleophilic attack on the epoxide, forming a cationic lesion on the DNA. Subsequent thermal depurination results in DNA strand breakage.[4]

cins, represented by hedamycin, rubiflavin A, and pluramycin A, showed 5′CG* > 5′TG* ≫ 5′AG* = 5′GG* sequence selectivity (the asterisk designates the covalently modified guanine). Of the pluramycins, only the sequence selectivities of DC-92B and hedamycin have been studied *in vivo*.[13] Using primer extension methods to identify damaged sites on the DNA from treated human cells, both of these compounds demonstrated similar selectivity previously reported in *in vitro* studies. Significantly, nucleosomal linker regions showed increased reactivity relative to core regions.[13]

The altromycins have been divided into two groups of sequence selectivity, those agents with a C5 altrose substituent (altromycin B) and those without (altromycin H and I).[7] Altromycin B preferred 5′AG* (high) and 5′TG* (medium) sequences but had low reactivity with both 5′CG* and 5′GG* sequences (5′AG* ≥ 5′TG* ≫ 5′CG* = 5′GG*). Interestingly, altromycin analogs that lack the C5 neutral altrose moiety (altromycin H and I), in addition to diminished overall reactivity, also lack the ability to discriminate between 5′AG* and 5′TG* sequences (5′AG* = 5′TG* ≫ 5′CG* = 5′GG*). In only the classical pluramycins is there a possible preference for a base pair to the 3′ side. One study showed that for the classical pluramycins there exists some preference for thymine,[12] while our own study revealed a slight preference for guanine at this position.[7] This is not surprising, because both thymine and guanine position electronegative functionalities in the major groove adjacent to the reactive guanine, which would be expected to enhance both the nucleophilicity and accessibility of N7.

Sequence selectivity can arise from noncovalent binding interactions that stabilize the bound complex in certain sequences (thermodynamic affinity), or it can be influenced by binding interactions that act to facilitate the alkylation step (kinetic acceleration). If precovalent binding plays a significant role in achieving alkylation specificity, one would expect to see binding selectivity in noncovalent analogs that parallels observed alkylation selectivity. To study this, the alkylation selectivity of pluramycin A was compared to the binding selectivity of neopluramycin, a noncovalent but otherwise structurally equivalent analog.[7] While pluramycin A alkylation selectivity can be determined using the strand breakage assay, DNase I footprinting was used to assay neopluramycin DNA binding selectivity. Neopluramycin failed to show defined DNase I footprinting patterns in the same DNA sequence in which pluramycin A showed the expected 5′(Py)G* alkylation selectivity. It is therefore proposed that the pluramycins achieve sequence selectivity of alkylation largely through the covalent reaction step, as opposed to the precovalent binding step. This mechanism to attain sequence specificity is analogous to that of (+)-CC-1065, where the determinant of sequence selectivity is achieved at the *covalent bonding reaction* and is controlled by *kinetic* rather than thermodynamic factors.[14] In contrast, the sequence selectivity of (−)-CC-1065 is achieved at the noncovalent binding step.[14c]

Substitution of inosine for guanine changes the structure and reactivity of DNA and allows a study of how these factors affect pluramycin reactivity and sequence selectivity.[7] Absence of the exocyclic 2-amino group decreases the number of hydrogen bonds made to cytosine, removes electrostatic and steric bulk from the minor groove, and decreases the nucleophilicity of N7 of guanine. In strand breakage studies in which guanine reactivity was compared to the reactivity of inosine in otherwise identical oligomers, inosine was much less reactive and sequence selective. The small amount of sequence selectivity that remained differed from that of guanine set in like sequences. On the basis of this, there would appear to be only a small "window" of reactivity for which the DNA environment can effectively perpetuate sequence selective alkylation by the pluramycins. By decreasing the reactivity at the reaction site, the ability of cognate sequences to selectively accelerate alkylation is also reduced, and therefore the experimentally observed selectivity reverts to approximating that of precovalent bonding.

Structural Interactions Made with DNA

Gel electrophoresis data are predictive of the three-dimensional interactions pluramycin makes with DNA. Experiments using supercoiled DNA demonstrated

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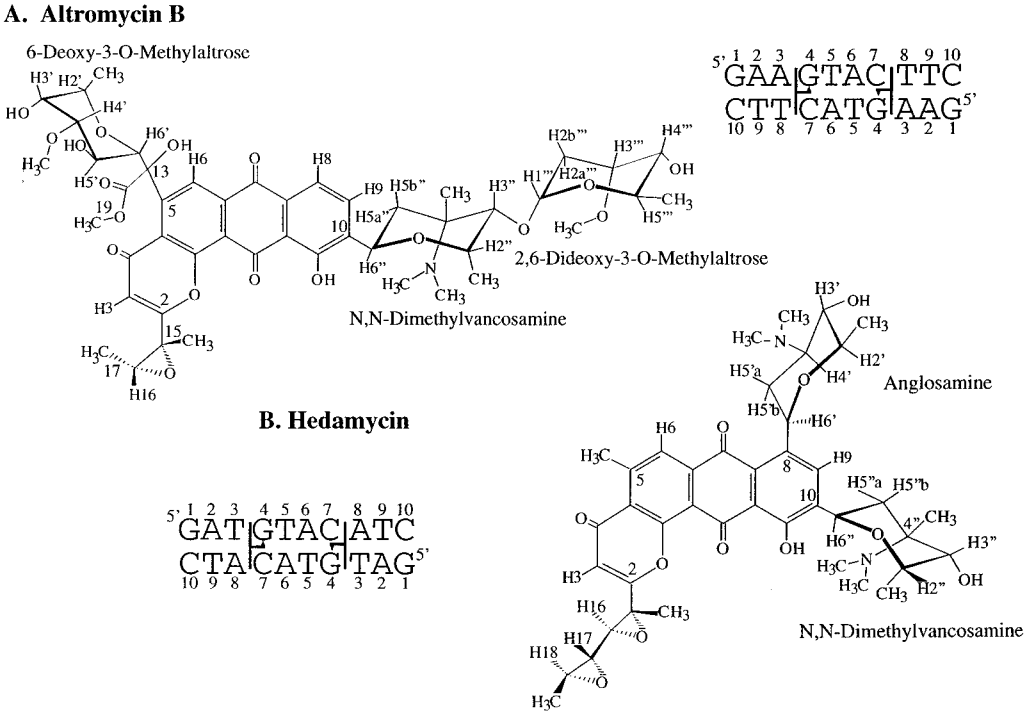

Figure 3. DNA sequence and numbering schemes used in two-dimensional NMR studies performed on the bis(altromycin B)−[d(GAAG*TACTTC)]$_2$ diadduct (A) and the bis(hedamycin)−[d(GATG*TACATC)]$_2$ diadduct (B).

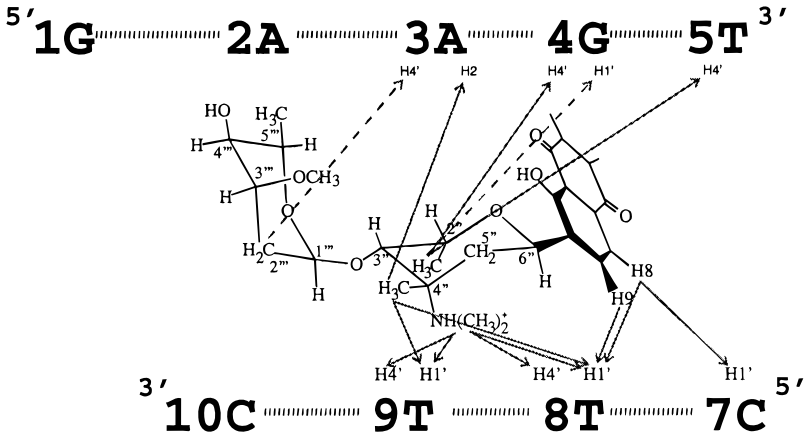

Figure 4. Summary of key NOESY connectivities between altromycin B's C10 disaccharide and the minor groove. Medium and strong connectivities are shown in solid arrows, and weak connectivities are shown in hatched arrows.

that noncovalent compounds intercalate the DNA, and studies with oligomeric DNA reveal that alkylating pluramycins display sequence selectivity predominantly for a single base pair to the 5′ side of the covalently modified guanine in strand breakage assays.[4,7] On the basis of these results, it is easy to propose that the pluramycins as a class covalently modify N7 of guanine, intercalate to the 5′ side of this modified guanine, and place multiple saccharide moieties into both grooves of the DNA helix. To gain a greater understanding of the complexity of these interactions, two of the most highly substituted and biologically potent compounds, hedamycin and altromycin B, were adducted to oligomers containing their preferred alkylation sequences and studied by high-field NMR (Figure 3).

NMR studies of the 2:1 bis(altromycin B)−[d(GAAG*TACTTC$_2$ self-complementary diadduct (the asterisk indicates the site of covalent modification, and the intercalation site is underlined) revealed that the tetracyclic chromophore intercalates to the 5′ side of the modified guanine in an orientation perpendicular to the base pairs and places the C10-linked aminodisaccharide into the minor groove and the C5 altrose moiety into the major groove.[5] The *N*,*N*-dimethylvancosamine moiety, by positioning itself near 8T on the nonmodified strand, forms a hydrogen bond between the protonated dimethylamino and the pyrimidine's O2 carbonyl, while the terminal sugar, or C3″ neutral altrose moiety, shows only a few weak NOEs to the 3A residue on the modified DNA strand (Figure 4). Because proton resonances associated with the 2A and 3A residues are broadened in comparison with the rest of the duplex, it is proposed that the C3″ altrose is conformationally flexible, making no distinct contacts with this region of the DNA and possibly functioning only to fill space in the minor groove. In the major groove the hydrophobic face of the C5 neutral altrose sugar makes van der Waals contact with the 7C and 8T residues on the nonmodified strand, while the more hydrophilic side of this sugar faces out of the major groove to interact with solvent.

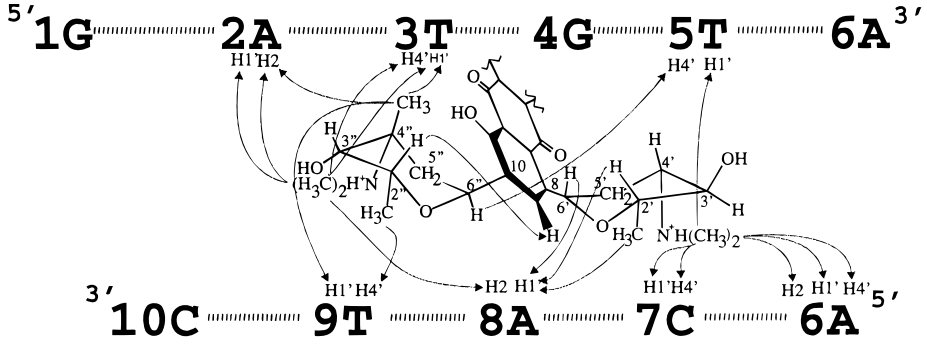

Figure 5. Summary of key NOESY connectivities between hedamycin's C8 and C10 sugar moieties and the DNA minor groove.

In the 2:1 bis(hedamycin)−[d(GATG*TACATC)]$_2$ self-complementary diadduct, the conjugated chromophore intercalated in a similar perpendicular orientation and also stacked to the 5′ side of the modified guanine.[6] Both the C8 anglosamine and the C10 *N*,*N*-dimethylvancosamine amino sugars of hedamycin interact in the minor groove (Figure 5). The C8 anglosamine is oriented to the 3′ side of the modified guanine in close proximity to the cytosine base-paired to the modified guanine. Oriented to the 5′ side of the modified guanine is the C10-linked *N*,*N*-dimethylvancosamine, which makes NOE contacts to 3T located on the covalently modified strand. Interestingly, on the basis of ^{13}C and ^{1}H chemical shifts, it appears that it is C18 of the terminal epoxide that forms the covalent bond to N7 of guanine. The internal epoxide, or the epoxide equivalent to that present in altromycin B, appears to be chemically unchanged in the adduct. Although compounds that have a C2 olefinic epoxide could conceivably also alkylate N7 of guanine similarly at the C18 position, NMR studies of sapurimycin adducted to oligomeric DNA reveal that they do not. The olefinic epoxide of sapurimycin instead forms the covalent bond between guanine and C16 of the epoxide in a fashion similar to that of the altromycins.[15]

Rationale for Sequence Selectivity

Molecular modeling of the bis(altromycin B)−5′-(GAAG*TACTTC)$_2$ and bis(hedamycin)−5′(GATG*TACATC)$_2$ duplex diadducts, based upon two-dimensional ^{1}H-NMR results, provides important insights into why the altromycins prefer 5′AG* and the classical pluramycins prefer 5′(T/C)G*.[7] These ^{1}H-NMR studies show that, upon intercalation of the planar drug chromophore into the DNA, the C5, C8, and C10 glycoside binding interactions cooperatively function to steer their reactive epoxide into proximity of the nucleophilic N7 of guanine in the major groove, thereby accelerating the reaction with DNA in a sequence selective fashion.

Key interactions in sequence recognition between pluramycins and the DNA minor groove are through the C8 and C10 amino sugars. NMR studies involving altromycin B and hedamycin covalently bound to their respective *highly* reactive sequences reveal a preference for the C8 and C10 amino sugars to interact directly with pyrimidines in the minor groove, presumably through hydrogen bonding in the protonated state with the negatively charged O2 carbonyl. In the case of altromycin B adducted to its preferred sequence, 5′AG*, the C3″ neutral altrose moiety interacts in the minor groove near the modified strand with residues 2A and 3A, forcing the C10 amino sugar into proximity of 8T on the *noncovalently* modified strand (Figure 6).[5] This is in contrast to the structure of hedamycin adducted to its highly reactive 5′TG* sequence, in which the C8 anglosamine positions near 7C on the *noncovalently* modified strand, thereby driving the C10 *N*,*N*-dimethylvancosamine into proximity of 3T on the *covalently* modified strand (Figure 7).[6] Also, altromycin B has the added interaction of the C5 altrose in the DNA major groove between the hydrophobic face of this sugar and the hydrophobic pocket formed in the major groove by the two pyrimidines (7C and 8T) adjoining the intercalation site on the nonmodified strand.

The reactivity of a site is determined by the ability of a sequence to favorably form these interactions and steer the reactive epoxide into proximity of N7 of guanine in the major groove to facilitate alkylation. In the case of the altromycins, high reactivity for 5′AG* sequences arises from the association of the *N*,*N*-dimethylvancosamine with this noncovalently modified strand near the thymine base-paired to the 5′ adenine, thereby creating a minor groove pocket into which the terminal neutral 6-deoxy-*O*-3-methylaltrose can fit and favorably align the C5 substituent for interaction against the floor of the major groove. In the less reactive case, the 5′TG* bonding site, the amino sugar would be limited either to hydrogen bonding to the thymine carbonyl located on the covalently modified strand, which would sterically crowd both the terminal neutral 6-deoxy-3-*O*-methylaltrose in the minor groove and the C5-linked 2,6-dideoxy-3-*O*-methylaltrose against the floor of the major groove, or to forming a less optimal hydrogen bond with N3 of the base-paired adenine on the floor of the minor groove. In the case of the C5 altrose-deficient altromycins, in addition to lower reactivity, these agents demonstrate diminished selectivity for 5′AG* over 5′TG*. The loss of steric interactions in the major groove potentially allows for greater freedom to rotate the intercalating chromophore toward the covalently modified strand (see Figure 7) in the minor groove to permit hydrogen bonding by the C10 amino sugar to 5′ thymine O2 on the covalently modified strand. The C5 altrose-deficient compounds therefore have less steric conflicts in reactions with 5′TG* but as a consequence have an overall lower reactivity with DNA.[7] The low reactivity of altromycins with 5′CG* and 5′GG* is probably due to steric and electrostatic hindrance of the guanine exocyclic *N*2-amino substituent, which would inhibit proper interaction of the C10 disaccharide with both strands in the minor groove.

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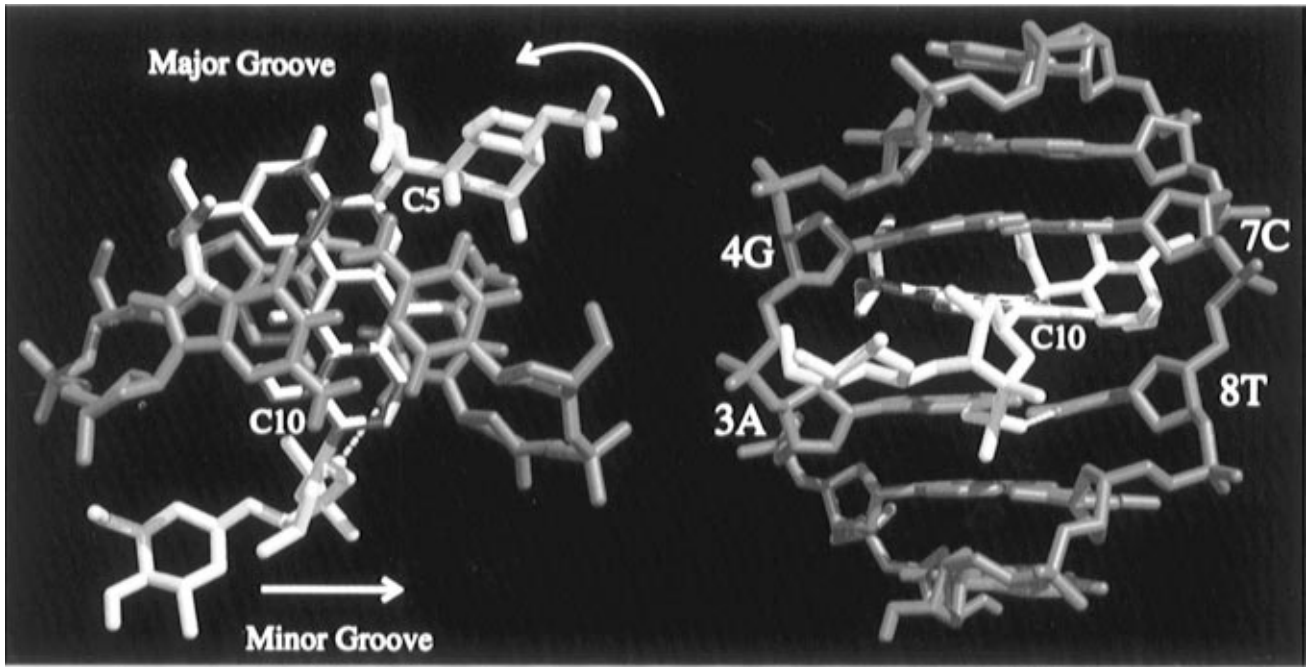


Figure 6. Molecular model of the bis(altromycin B)−[dGAAG*TACTTC)]$_2$ diadduct. Shown is the interaction made by altromycin B with its most-preferred sequence, 5′AG*. On the right is the interaction of the altromycin B amino disaccharide (yellow) in the minor groove, and on the left is the proposed steering reaction of the altromycin chromophores (gray) by the C5 and C10 glycosides (yellow) to perpetuate alkylation to N7 of guanine (cyan).

In the case of the classical pluramycin subfamily, the highly reactive 5′TG* and 5′CG* sequences allow for interaction of both the C8 anglosamine and the C10 *N*,*N*-dimethylvancosamine with pyrimidines in the minor groove. The C8 amino sugar can interact on the nonmodified strand with the cytosine base-paired to the modified guanine, while the C10 amino sugar can favorably interact with the 5′ pyrimidine. In the low reactivity case, 5′AG*, the C8 amino sugar that interacts with the base-paired cytosine limits the C10 amino sugar to interact with the 5′ adenine. Interactions of the C10 amino sugar with the noncovalent strand, specifically the pyrimidine, would sterically crowd the C8 amino saccharide. Therefore, these interactions lead to a 5′(Py)G* selectivity, which positions a pyrimidine to the 5′ side of the modified guanine on the drug-modified strand for interaction with the C10 amino sugar and a pyrimidine on the noncovalent strand base-paired to the modified guanine for interaction with the C8 amino sugar.

Importance of "Epoxide Reach"

While binding interactions that steer the epoxide into proximity of N7 of guanine affect covalent reactivity and sequence specificity, it is also apparent that the overall level of alkylation reactivity differs markedly between the pluramycins. The classical pluramycins hedamycin and rubiflavin have a 5-fold greater reactivity than pluramycin A and any of the altromycins. We propose that variable reach and/or flexibility of the alkylating substituent at C2 on the anthrapyran system can have a significant effect on the reactivities of the pluramycin compounds. The alkylating epoxide at the C2 position is quite variable, ranging from the diepoxide of hedamycin to the olefinic epoxide of rubiflavin and the unconjugated epoxide of the altromycins (Figure 1). ^{1}H-NMR studies show that, while reaction takes place at the C16 position of altromycin B and sapurimycin, in the case of hedamycin, covalent reaction takes place at the C18 position of the terminal epoxide. Since there is no reason to expect the alkylating epoxides of the altromycins or hedamycin to have different inherent reactivities, we propose that the higher reactivity of hedamycin is due to the longer reach of the epoxide, which reduces the need for guanine to distort from its optimally base-paired position in order for alkylation to occur. Evidence to support this minimized distortion proposal is available from molecular modeling of duplex adduct structures of the hedamycin and altromycin B adducts. While in the case of the altromycin B−DNA adduct, the covalently modified guanine is tilted into the major groove, for the hedamycin−DNA adduct, the longer reach allows for a planar orientation of the equivalent alkylated guanine.

Evolution of the Pluramycins

It is interesting that the pluramycins display distinct glycosidic substitution patterns that divide them into two subfamilies. Although the pluramycins have certain structural commonalities, each subgroup has components that are unique and have not been observed in combination. Specifically, a compound that bears an amino saccharide at the C8 position and also has a C5 or C3″ altrose substitution has not been reported. On the basis of models of the altromycin B and hedamycin−DNA adducts, steric limitations imposed by the DNA helix would decrease the effectiveness of these "mixed" analogs to covalently modify DNA. For example, a hypothetical compound having the C10 amino disaccharide and a C8 amino sugar would not easily accommodate both of these substituents in the minor groove. Similarly, an analog possessing a major groove binding C5 substituent and a minor groove binding C8 sugar substitution would

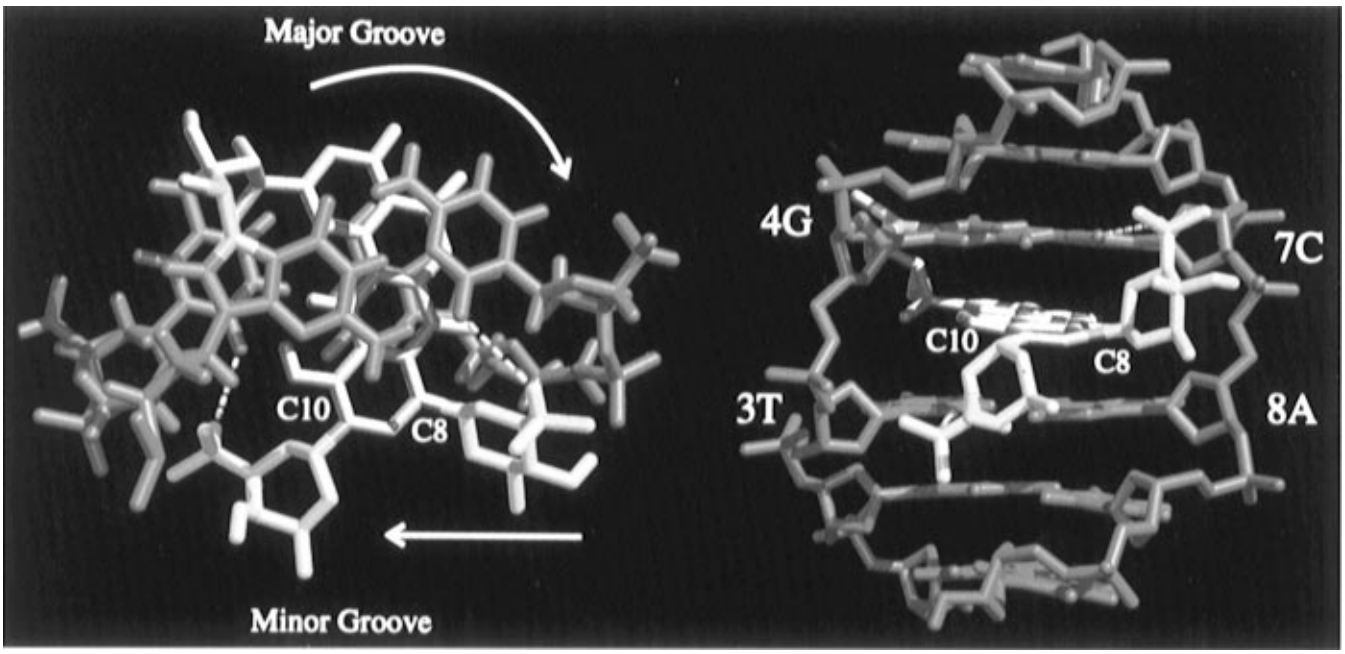


Figure 7. Molecular model of the bis(hedamycin)−[d(GATG*TACATC)]$_2$ diadduct. Shown is the interaction made by hedamycin with its highly reactive 5′TG* sequence. Shown on the right is the interaction of the C8 anglosamine and the C10 *N*,*N*-dimethylvancosamine (yellow) in the minor groove. Shown on the left is the steering reaction of the drug chromophore (gray) by the C8 and C10 substituents (yellow) for alkylation of N7 of guanine (cyan).

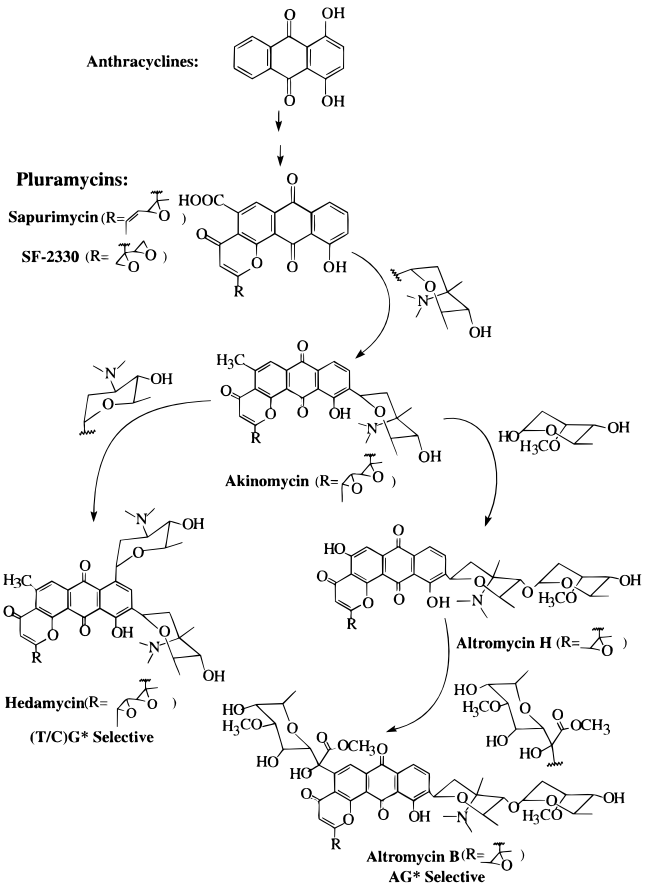


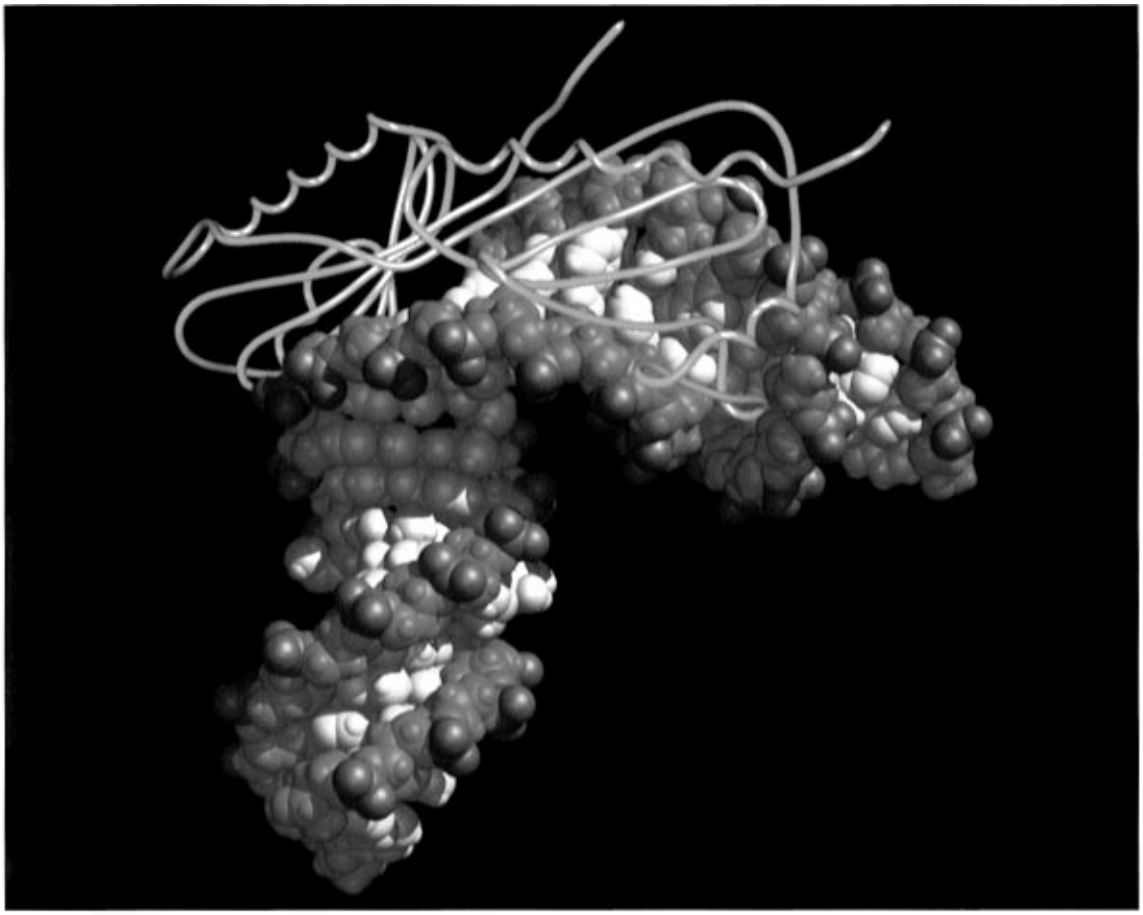
Figure 8. A proposed evolutionary pathway beginning with the anthracyclines and ending with the most potent compounds from each pluramycin subfamily.

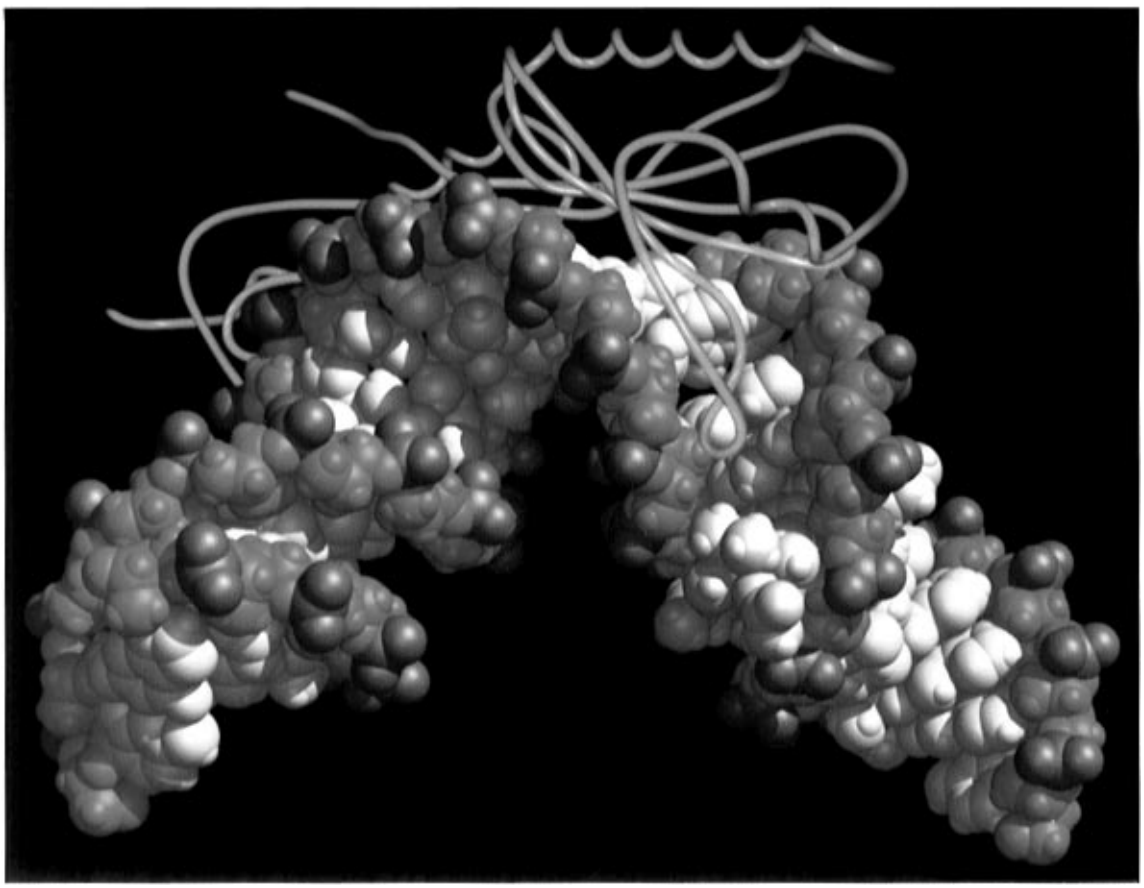
not easily span the DNA double helix from groove to groove. On the basis of these observations, the evolutionary scheme shown in Figure 8 is proposed for the biosynthesis of the pluramycin class of antibiotics.

In light of the structural incompatibilities between subfamilies, the earliest pluramycin antibiotic probably evolved from the anthraquinones, creating a compound very similar to sapurimycin. It is then likely that the antibiotic-producing organism added an *N*,*N*-dimethylvancosamine, common to both families, to the C10 position to yield a compound similar to akinomycin, which would probably display similar reactivity toward sequences preferred by the classical pluramycins and the altromycins. Divergence occurred at this point, creating two independent subfamilies of compounds. The altromycins would be created by adding neutral altroses to the C3″ position and the C5 position, and the classical pluramycins would be created by attaching an anglosamine sugar moiety to the C8 position. Consequently, the most structurally advanced and biologically potent analogs of each subfamily, altromycin B and hedamycin, have independently evolved from a common ancestor to form the most DNA-reactive compounds of the pluramycin family, each preferentially recognizing mutually exclusive DNA sequence targets.

Pluramycins as Probes of Protein-Induced Unwinding of DNA

Because of their dual roles as intercalators and alkylators, the pluramycins make unique chemical probes of dynamic aspects of DNA structure and protein-induced unwinding of DNA. As a requirement for pluramycin reaction with DNA, the helix must be unwound and the minor groove must be accessible to the sugar moieties of pluramycin. Pluramycin has a critical advantage over other, better known unwinding probes such as MPE·Fe(II) or Cu−phenanthroline as a molecular probe for protein-induced unwinding of DNA, because it forms a stable covalent attachment at the intercalation site. The other agents “hit and run”, leaving strand breaks at the intercalation site. In addition to identifying unwound or loosely base-





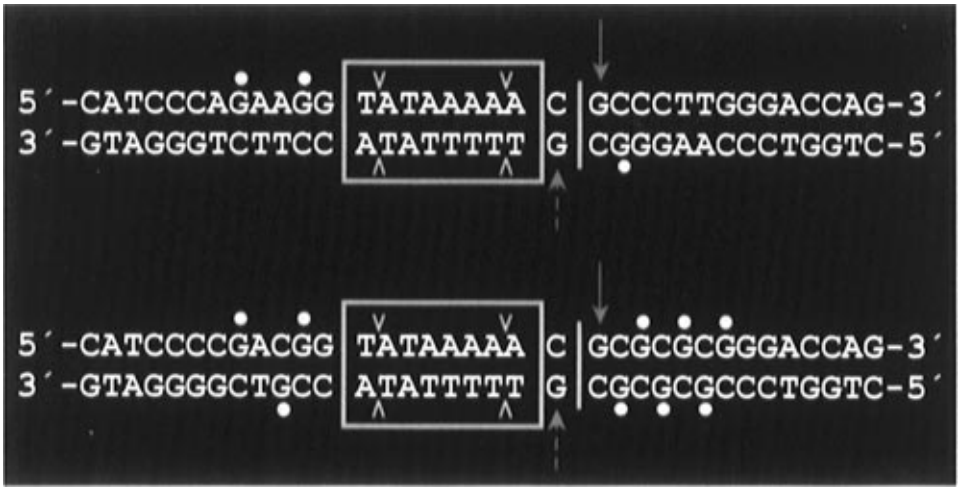


Figure 9. Molecular models (previous page, top and bottom) of the proposed ternary complex among TBP (orange), the TATA box (cyan and purple), and pluramycin A (yellow, with the site of covalent linkage to the DNA in blue). The ternary complex contains the following duplex DNA sequence:

where the arrows show phenylalanine insertions, the vertical line is the pluramycin intercalation site, and G* shows the alkylated guanine. The DNA sequences used in the gel study are shown on this page. Solid dots indicate the site of pluramycin inhibition, the broken arrow represents an unchanged reaction site, and the solid arrow represents the enhanced cleavage site. Also shown are sites of TBP−phenylalanine insertion (carets) into the DNA helix and the site of intercalation (vertical line).

paired sites on the DNA helix, the effects of drug interaction on DNA structure and dynamics of the complex can also be determined. Despite the stated advantages of pluramycins as probes of DNA−protein interactions, it is important to be cautious in the interpretation of results because of the additional distortions produced as a consequence of drug interactions.

In studies of protein−DNA interactions, the pluramycins are able to probe changes in DNA helix conformation caused by protein binding. A major component found in the TFIID transcriptional complex is TATA-binding protein or TBP.[16] Upon complexation of TBP with the transcriptional machinery, the template strand is committed to transcription. Because the crystal structures of the DNA bound to the C-terminal domain of TBP show that the protein folds into a roughly symmetric structure,[17] the mechanism by which the seemingly symmetric TBP−DNA complex initiates an asymmetric event, i.e., transcription in a single direction, remains unresolved.

By probing the TBP−DNA complex with pluramycin, the dynamic effects propagated from the TATA box asymmetrically along the DNA can be monitored. Enhanced pluramycin-induced cleavage downstream of the TATA box suggests that TBP induces transient unwinding of the DNA duplex on a single side of the TATA box, which forms a kinetically favored pluramycin reaction site (see Figure 9).[18] In addition to enhanced DNA modification, interaction of pluramycin to the 3′ side of the TATA box region significantly stabilizes the DNA−protein complex, causing a significant increase in the complex half-life.[18] On the basis of this data, it is proposed that TBP induces transient unwinding of the DNA downstream of the TATA box, creating a pluramycin-enhanced reactivity site that would otherwise act as the initiation site for unwinding of DNA involved in transcriptional activation. By trapping this specific binding mode of the TBP−TATA box complex, pluramycin may prevent access to this kinetically available intercalation site by a normal component of the transcriptional complex and thereby produce unproductive transcriptional regions. This would lead to down-regulation of specific gene transcription, and perhaps even general transcription, because of the resulting depletion of transcriptional factors.

Conclusions

Through this comprehensive study of the pluramycins, we have gained insight into the interaction of the pluramycins with DNA for which prior mechanistic and structural information could only be speculated upon. On the basis of an understanding of the precise interactions that lead to sequence specific recognition of DNA, we have demonstrated how this group of antitumor agents can achieve higher selectivity in the presence of DNA binding proteins that transmit dynamic effects to adjacent sites. The pluramycins have proven to be a novel group of compounds that combine characteristics of other classes of DNA-reactive agents. Like the anthracycline nogalamycin, the pluramycins intercalate through the DNA molecule *via* a threading mechanism that positions saccharide structural groups into the minor groove as well as the major groove. In addition, these agents can alkylate N7 of guanine *via* an epoxide mechanism reminiscent of aflatoxin B in a sequence selective fashion. Last, our studies on the ternary complex formed among pluramycin, TBP, and the TATA box have provided important insight into how intercalat-

(16) Roeder, R. G. *Trends Biochem. Sci.* **1991**, *16*, 402−408 and references therein.
(17) (a) Kim, Y.; Geigler, J.; Hahn, S.; Sigler, P. *Nature* **1993**, *365*, 512−520. (b) Kin, J.; Nikolov, D.; Burley, S. *Nature* **1993**, *365*, 520−527.
(18) Sun, D.; Hurley, L. H. *Chem. Biol.* **1995**, *2*, 457−469.

ing agents in general may achieve increased specificity and, in this particular case, how potent biological effects may result from stabilization of transcriptional complexes.

The emperor has been reclothed, and now we see him in all his isolated splendor, but whether we have cast him in his true role in the greater scheme of life and death yet remains to be determined.

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