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Modelling basic features of specificity in DNA-aureolic acid-derived antibiotic interactions

K.-X. Chen, N. Gresh, X. Hui, B. Pullman and K. Zakrzewska

Institut de Biologie Physico-Chimique, Laboratoire de Biochimie Théorique associé au CNRS, 13, rue Pierre et Marie Curie, 75005 Paris, France

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The nonintercalative groove binding of a simplified model of olivomycin, to sequences $d(CGCGCGC)_2$, $d(TATATAT)_2$, and $d(CICICIC)_2$ is investigated. A significant preference is displayed for the minor groove of the d(CG) sequence. This is due predominantly to the formation of H-bonds between the hydroxyl groups on the aglycone of the drug and the 2-amino group of the central guanine of the oligonucleotide.

Antitumor antibiotic; Groove binding; Minor groove GC specificity

1. INTRODUCTION

Olivomycin, mithramycin and chromomycin A_3 are antitumor glycoside antibiotics derived from aureolic acid which exert their action by binding to DNA. They possess very similar structural features consisting of an aglycone moiety and five attached hexopyranoses. The structure of one of them, olivomycin, is illustrated in fig. 1. Although footprinting patterns obtained with the three drugs are not identical they do indicate a definite specificity of all of them for GC-rich sequences of DNA [1, 2], a result confirmed via other physicochemical techniques (see e.g. [3,4]).

Another important feature of the interaction is association of the specificity essentially with the aglycone moiety: thus, GC specificity is maintained upon successive elimination of the sugars down to the derivative consisting of the aglycone and sugar D only [3]. However, the sugars do affect the strength of binding which they increase significantly, in particular by decreasing the rate of dissociation of the DNA-drug complex [3].

Correspondence address: B. Pullman, Institut de Biologie Physico-Chimique, Fondation Edmond de Rothschild, 13, rue Pierre et Marie Curie, 75005 Paris, France

While there exists a general consensus on the above features of specificity, no understanding is available on the nature of the factors responsible. In fact, strikingly divergent opinions are held even as regards the fundamental characteristics of the interaction of these antibiotics with their nucleic acid receptor. Thus, while some authors [5,6] originally proposed an intercalative mode of association, they have more recently considered a groove binding mode [7,8], the latter being also advocated by others [4,9,10]. In view of the data presented particularly in [10] groove binding indeed appears to be the most probable mode of interaction. Differences in opinion persist, however, among the protagonists of the groove binding mechanism as to whether the interaction involves the major [7,8] or minor [4,10] groove of DNA. Moreover, quite different architectures of the minor groove interaction were considered in the latter two studies, involving e.g. the interaction of the side-chain keto oxygen of the aglycone [4] or of its O_{11} hydroxyl [10] with the NH_2 group of guanine.

Significant divergence in opinions also prevails as concerns the role of Mg^{2+} in the association. While it is frequently considered that the presence of Mg^{2+} constitutes an absolute requirement for

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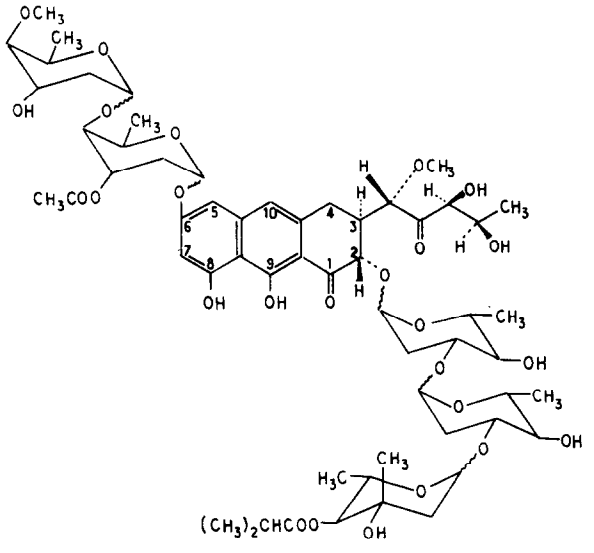

Fig.1. Olivomycin.

binding to occur [11,12], with, however, differing views on the ion's exact role and positioning [3,4,10], a recent publication [13] indicates that at pH 4.5 binding of the drug, which is present then in neutral monomeric form, does not require divalent cations (although the strength of binding is greatly enhanced in their presence). At pH > 7.0 and low DNA/drug ratios (<20), metal cations are necessary, but at high DNA/drug ratios an appreciable proportion of the drug is bound even in the absence of the metal. These authors consider that the insertion of Mg^{2+} into the drug-DNA complex is accompanied by deprotonation of the drug. Following [10] the divalent cation is implied in interaction of the dimeric form of the drug in an anionic form with the DNA receptor.

Here and as a first step towards elucidating the

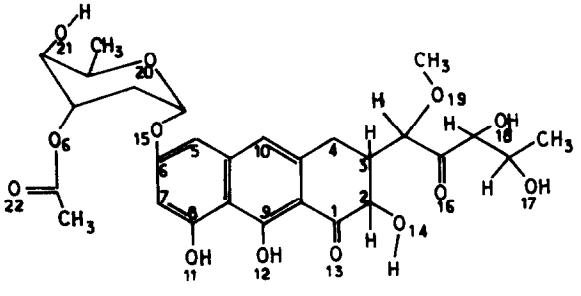

Fig.2. The reduced model of olivomycin: ASD.

essential factors governing the groove and base sequence preferences of this type of antibiotic, we have investigated the binding to three heptanucleotides, $d(CGCGCGC)_2$, $d(TATATAT)_2$ and $d(CICICIC)_2$, of a shortened model of olivomycin composed of its aglycone and its D sugar (fig.2). This shortened form of the natural antibiotic (symbol ASD) was considered in its neutral monomeric form and the interaction was investigated in the absence of any divalent cation. Although we are well aware of the fact that such a simplified model is far from corresponding to the usual conditions and mode of interaction of the natural aureolic acid-derived antibiotics with DNA, it seems nevertheless plausible, on the basis of the literature reviewed above, that this investigation could nevertheless demonstrate the basic features responsible for the specificity of this type of drug for GC sequences of DNA and yield information on their groove preference.

2. METHODOLOGY

The procedure employed here is the Jumna method [14], used in similar investigations (e.g. [15,16]) described recently. The procedure will therefore not be repeated here.

3. RESULTS AND DISCUSSION

The base numbering adopted for the investigated double-stranded heptamers is shown in fig. 3 for the representative C-G sequence. The results of computations for the three investigated sequences are listed in tables 1 and 2. Table 1 reports the values of the ligand-oligonucleotide interaction energies (ΔE_{int}), the conformational energy variations of ligand (ΔE_{lig}), and oligonucleotide (ΔE_{DNA}) referred to their most stable respective conformation energies being taken as energy zeros, the resulting energy balances (ΔE) and the differences (δ) of energy balances with respect to the best value of δE being taken as energy zero. Table 2 lists the hydrogen bond interactions that are involved in stabilization of the complexes.

Two binding configurations were explored and energy-minimized for the interaction of ASD with the grooves of the $d(CGCGCGC)_2$ and $d(TATATAT)_2$ through its hydrophilic (fig.2, lower) or hydrophobic (fig.2, upper), side. The

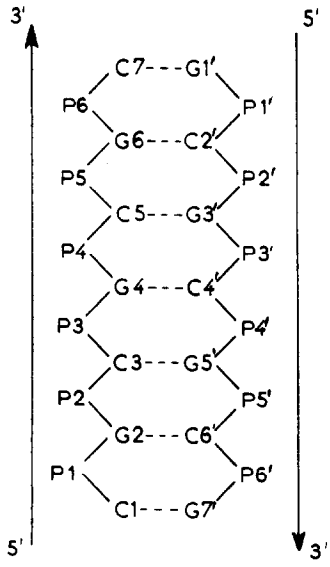

Fig.3. Base and phosphate numbering in the heptanucleotides.

corresponding results are presented in columns a and b of tables 1 and 2, respectively.

Examination of tables 1 and 2 shows that:

(i) The most stable association occurs in the minor groove of the GC oligonucleotide and involves the hydrophilic side of the chromophore (columns a). Structurally, this preferential stabilization is due essentially to hydrogen bond interactions of the hydroxyl oxygens of O_{11} and O_{12} of the chromophore with the 2-amino group of guanine G4 and of the hydrogen of OH_{12} with O1′ of S5′. It may nevertheless be noted that the complex formed through the interaction of the hydrophobic side of the drug with the same oligonucleotide (columns b) is only 1.5 kcal/mol less stable than the previous one. Its stabilizing interactions involve hydrogen bonds of the hydroxyl groups of the side chain of the aglycone with the amino group of G3′ and with O_1′ of S4′ and also of the hydroxyl hydrogen of O_{21} of the chromophore with O1′ of S6′.

(ii) The similar interactions with the minor groove of the AT oligonucleotide are significantly weaker (by 7.2 and 6.3 kcal/mol) than those corresponding with the GC oligonucleotide. Although they also involve a series of hydrogen bonds between the drug and the nucleic acid receptors, these bonds occur essentially between the proton donor hydroxyl groups of the aglycone side chain and different acceptor oxygen atoms on the oligo-

Table 1

Values of the binding energies of ASD to $d(CGCGCGC)_2$, $d(TATATAT)_2$ and $d(CICICIC)_2$

	$d(CGCGCGC)_2$		$d(TATATAT)_2$		$d(CICICIC)_2$
	(a)	(b)	(a)	(b)	(b)
(1) Minor groove binding					
ΔE_{inter}	−63.2	−56.9	−60.2	−52.2	−55.5
E_{el}	−25.4	−19.9	−21.3	−17.0	−19.5
E_{rep}	33.5	35.5	31.1	25.9	31.1
E_{disp}	−71.0	−72.1	−69.6	−60.8	−66.8
ΔE_{lig}	3.6	1.1	6.3	1.5	3.1
ΔE_{DNA}	7.4	5.0	8.9	4.8	6.5
δE	−52.2	−50.7	−45.1	−45.9	−45.9
δ	0.0	1.5	7.2	6.3	6.3
(2) Major groove binding					
ΔE_{inter}	−52.6	−54.0	−43.4	−47.8	−52.0
E_{el}	−20.4	−19.4	−16.0	−15.4	−20.9
E_{rep}	24.6	25.6	20.7	25.3	21.7
E_{disp}	−56.4	−59.8	−47.8	−57.5	−52.4
ΔE_{lig}	0.5	4.4	0.0	1.7	4.5
ΔE_{DNA}	8.6	8.5	5.4	5.2	6.9
δE	−43.4	−41.1	−38.0	−40.8	−40.7
δ	8.8	11.1	14.0	11.4	11.6

Energies in kcal/mol; see text for definitions

Table 2

List of hydrogen-bond distances (in Å) between ASD and the oligonucleotides d(CGCGCGC)$_2$, d(TATATAT)$_2$ and d(CICICIC)$_2$

d(CGCGCGC)$_2$		d(TATATAT)$_2$		d(CICICIC)$_2$
(a)	(b)	(a)	(b)	(b)
(1) Minor groove complexes				
O_{12}-H_2N(G4)2.57	O_{18}-H_2N(G3′)1.95	$O_{17}H$-O_2(P′1)2.57	$O_{17}H$-O′$_3$(P4′)2.00	$O_{17}H$-N_3(I3′)2.03
O_{12}-H_2N(G4)2.24	O_{17}-N_3(G3′)2.04	$O_{18}H$-O_2(P′1)1.82	$O_{17}H$-O_2(P4′)2.58	$O_{18}H$-O′$_1$(S4′)1.98
HO_{12}-O′$_1$(S5′)2.54	$O_{18}H$-O′$_1$(S4′)1.98	HO_{12}-O_2(T′4)2.00	$O_{18}H$-O_2(P4′)2.21	$O_{21}H$-O′$_1$(S6′)2.26
	$O_{21}H$-O′$_1$(S6′)2.61	HO_{14}-O′$_1$(S′4)2.16	$O_{21}H$-O′$_1$(S6′)2.22	
(2) Major groove complexes				
				O_{19}-H_2N(C3)2.15
O_{12}-H_2N(C2′)2.37	O_{19}-H_2N(C3)2.06	O_{11}-H_2N(A3′)2.46	$O_{17}H$-O′$_5$(P3)2.16	$O_{21}H$-O_1(P2)1.94
O_{13}-H_2N(C5)2.04	HO_{11}-O_1(P2)2.07	O_{11}-H_2N(A4)2.23	$O_{17}H$-O_1(P3)2.57	$O_{17}H$-O_1(P4)2.54
HO_{12}-O_6(G4′)2.06	$O_{17}H$-O′$_5$(P4)2.60	O_{12}-H_2N(A4)2.14	$O_{18}H$-O_1(P3)1.87	$O_{18}H$-O_1(P4)1.79
HO_{21}-O_1(P1′)1.97	$O_{17}H$-O_1(P4)2.55	HO_{12}-N_7(A4)2.50	O_{15}-H_2N(A3′)2.39	O_{22}-H_2N(C5)2.32
HO_{14}-O_1(P4)2.66	$O_{18}H$-O_1(P4)1.78	HO_{14}-O_1(P3)1.83		
		HO_{21}-O_1(P1′)2.19		

nucleotide. They are obviously unable to produce as strong an association as the bonds formed with the GC oligonucleotide by the interactions involving the NH_2 group of guanine. The interactions with the IC oligomer are essentially similar to those found with the AT oligomer.

(iii) Energetically, the preference for the minor groove of GC sequences over AT sequences is due essentially to the greater values of the electrostatic and dispersion components of the interaction energy and the lower DNA deformation energies in the former than in the latter association.

(iv) Complexes with the major groove of the oligonucleotides, irrespective of the hydrophilic or hydrophobic side of the drug being involved, are in all cases significantly weaker than those formed in the minor groove. Binding to the AT oligonucleotide is particularly disfavored in this case. This decrease in affinity is due essentially to reduction of the values of the electrostatic and dispersion components of the drug-DNA interaction energy.

In conclusion, it seems that the ASD model of the aureolic acid-derived antibiotics correctly reflects the major aspects of specificity in the interaction of these drugs with DNA. It confirms the specificity of this drug system for GC sequences, and moreover, supports its preference for the minor groove of these sequences. The major structural features which appear to be responsible for this specificity are the hydrogen bonding interactions between the hydroxyl groups O_{11} and O_{12} of the chromophore and the 2-amino group of guanine G4.

As is well known, groove binding antibiotics generally show a marked specificity for the minor groove of AT sequences [17,18]. The particular behavior of the aureolic acid-derived antibiotics is thus worth stressing. It may be observed that in the few cases in which binding of drugs is observed to the minor groove of GC sequences, they always seem to involve a hydrogen bonding interaction between an oxygen atom of the drug and the 2-amino group of guanine on the nucleic acid receptor [19].

Work is being engaged presently in view of enlarging the model to full scale for this type of antibiotic so as to determine the role in its interaction with DNA of the structural factors omitted here (e.g. the remaining sugars, Mg^{2+}, ionization and possible dimerization of the drugs, etc).

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