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The structure of drug-deoxydinucleoside phosphate complex; generalized conformational behavior of intercalation complexes with RNA and DNA fragments

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ABSTRACT

A 2:2 complex of proflavine and deoxycytidylyl-3',5'-guanosine has been crystallized and its structure determined by x-ray crystallography.

The two dinucleoside phosphate strands form self complementary duplexes with Watson Crick hydrogen bonds. One proflavin is asymmetrically intercalated between the base pairs and the other is stacked above them. The conformations of the nucleotides are unusual in that one strand has C3',C2'endo mixed sugar puckering and the other has C3',C3'endo deoxyribose sugars. These results show that the conformation of the 3'sugar is of secondary importance to the intercalated geometry.

INTRODUCTION

It is now well established that a wide range of drugs, carcinogens, and mutagens can interact with double-stranded nucleic acids by intercalative mechanisms (1,2). The detailed stereochemistries of these processes are not yet known; suggestions have been made on the basis of both theoretical model-building (3,4), and with the crystal structures of dinucleoside phosphate duplex complexes as starting points (5,6). A number of ribodinucleoside phosphate complex structures have now been reported - ethidium (Et) with i^5CpG (5-iodocytidylyl-(3'-5')guanosine) (7) and i^5UpA (5-iodouridylyl-(3'-5')adenosine) (8), acridine orange (AO) with i^5CpG (9) and with CpG itself (10), and proflavine (PF) with CpG (11,12); only one complex deoxyribo-structure has been determined - 2-hydroxyethanethiolato-2,2',2"-terpyridine-platinum (II) (TPH) with deoxyCpG (13). The conformational characteristics of intercalated ribodinucleoside phosphates have been analyzed (14), where it has been shown that the major changes required to produce intercalation are in the torsional angles of the C5'-O5' (δ) and glycosidic (χ) bonds at the 3' end. It was also demonstrated by computer model-building, that the structural differences observed between the proflavine-CpG structure on the one hand, and the ethidium complexes on the other, are not significant. In

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particular, patterns of sugar pucker, be they all C3'-endo (in the proflavine complex), or alternating C3'-endo-(3'-5')-C2'-endo (in all others), are relatively unimportant and are not a prerequisite for, or a fundamental property of, intercalation and not responsible for site exclusion.

Doubt has been cast on these assertions (13,15); it has been stated that the all C3'-endo pucker associated with proflavine intercalation is an exception to the general alternating-pucker rule, arising from the peculiar nature of the proflavine cation and its interaction with CpG in that there are intermolecular hydrogen bonds between the drug and phosphate oxygen atoms. However, the behavior of proflavine with both DNA and RNA (1,2), reveals no abnormal features compared to other drugs, and shows similar site exclusion properties.

In this study we report conformational details from the crystal structure of a 2:2 deoxyCpG-proflavine (dCpG·PF) complex. This reveals patterns of intercalative behavior analogous with, but distinct from, the ribo analogue. Moreover, the sugar pucker observed here clearly show that proflavine is not a "special case," at least for the reasons cited above. Most significantly, these results demonstrate conclusively that sugar pucker is a soft parameter in the context of intercalation into RNA or DNA dimeric subunits.

METHODS

Orange platelike crystals were grown from solutions of deoxyCpG (dCpG) (Collaborative Research, Inc.) and proflavine hemisulphate (Sigma). One of dimensions 0.3 x 0.3 x 0.05 mm was mounted in mother liquor and used for data collection. Cell dimensions were: $\underline{a}$ = 32.991 Å, $\underline{b}$ = 21.995 Å and $\underline{c}$ = 13.509 Å in space group $P2_12_12$ with two dCpG anions, two proflavine cations, and numerous solvent molecules in the asymmetric unit. These cell parameters are almost identical with those of the crystal structure of the 2:2 dCpG·TPH complex. However, these two structures are not isostructural since dCpG·TPH has space group $P2_12_12_1$ which is different from that of the present structure. Data were collected on a Syntex P$\bar{1}$ automated diffractometer using CuKα radiation. 8571 independent reflections were measured (corresponding to a maximum resolution of 0.83 Å); 2654 had intensities greater than $2\sigma(I)$, and were used in structure solution and refinement. The structure was solved by the difference resolution Patterson technique, which located the phosphorus atoms. Superposition methods revealed the bonded O3'

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atoms; the rest of the structure was solved by successive E, F and difference Fourier syntheses. Considerable difficulty arose in the early stages of the analysis due to the extreme weakness of odd c-axis reflections, which initially suggested the space group to be $P2_12_12_1$. Patterson syntheses were however only fully interpretable on the basis of $P2_12_12$. Examination of the solved structure showed that many atoms were approximately related in pairs x_1, y_1, z_1 and x_2, y_2, $1/2 + z_1$. In contrast, the dCpG·TPH complex crystal has space group $P2_12_12_1$ with a pseudo 2-fold axis parallel to a which corresponds to b in the present system.

The structure was refined by full-matrix least-squares techniques to a crystallographic residual of 0.15. The average estimated standard deviations of the bond lengths are 0.04 Å, and of the angles 2°. Table 1 gives the atomic coordinates and the thermal parameters for the dCpG, proflavine and water molecules.

RESULTS AND DISCUSSION

Figure 1 shows a view of the complex of dCpG and proflavine (PF) which is a highly hydrated structure. The two dinucleoside phosphate strands form self complementary duplexes with Watson Crick hydrogen bonds with one proflavine molecule asymmetrically intercalated between the base pairs and the other stacked above them. Because of the almost identical cell parameters, it is not surprising that this structure has some features, such as its hydrogen bonding patterns and base orientations in the cell, that are similar to the structure of dCpG·TPH. However, because of the different intercalator molecules and space groups, these two structures are distinctly different. Indeed, the present structure has an unusual and quite unexpected characteristic that has not been reported before for intercalated dinucleoside phosphate in that the two strands are not symmetric with respect to one another. In one both deoxy ribose sugars are in the C3'-endo conformation (strand 1); in the other the 5' deoxyribose has C3'-endo pucker and the 3' deoxyribose has C2'-endo (strand 2). In the first strand, the phase angles of pseudorotation (16) for the deoxyriboses are 27° and 35°, in the second they are 25° and 172°. As in other related structures, the glycosidic torsion angles for the cytosine rings are anti and for the guanine are high anti although the guanosine with the C2'endo pucker has a value for χ considerably higher than for the one with the C3'endo pucker. Comparison of this structure with other intercalated complexes reveals a distinct conformational pattern (Table 2): 1) the torsion angles in the

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Table 1

Atomic Coordinates and Thermal Parameters for Molecular Complex of dCPG.PF

ATOM	X	Y	Z	B	ATOM	X	Y	Z	B
		PF(1)					PF(2)		
C1PF1	1.0424(10)	0.181(1)	0.261(3)	6.2(1.0)	C1PF2	1.0302(10)	0.154(1)	0.767(3)	5.4(0.9)
C2PF1	1.0700(10)	0.226(1)	0.260(3)	6.2(1.1)	C2PF2	1.0637(10)	0.188(1)	0.772(3)	5.6(1.0)
C3PF1	1.0553(8)	0.286(1)	0.260(4)	8.3(1.3)	C3PF2	1.0569(8)	0.252(1)	0.771(4)	6.6(1.2)
C4PF1	1.0138(8)	0.301(1)	0.262(4)	7.8(1.3)	C4PF2	1.0180(8)	0.279(1)	0.771(4)	9.3(1.5)
C5PF1	0.8737(8)	0.250(1)	0.260(3)	7.2(1.2)	C5PF2	0.8734(8)	0.257(1)	0.749(3)	6.8(1.2)
C6PF1	0.8440(9)	0.206(1)	0.254(4)	9.8(1.5)	C6PF2	0.8392(9)	0.219(1)	0.749(4)	7.4(1.3)
C7PF1	0.8557(10)	0.145(2)	0.271(3)	6.7(1.1)	C7PF2	0.8392(10)	0.154(2)	0.753(3)	7.4(1.2)
C8PF1	0.8738(11)	0.126(2)	0.263(4)	9.6(1.6)	C8PF2	0.8764(11)	0.131(2)	0.761(4)	5.1(1.0)
C9PF1	0.9661(9)	0.152(2)	0.269(3)	6.1(1.0)	C9PF2	0.9546(9)	0.139(2)	0.764(3)	4.6(0.9)
N10PF1	0.9451(7)	0.248(1)	0.268(3)	6.5(0.9)	N10PF2	0.9463(7)	0.265(1)	0.761(3)	6.6(0.9)
C11PF1	0.9858(9)	0.255(2)	0.270(5)	10.7(1.6)	C11PF2	0.9848(9)	0.240(2)	0.765(5)	4.8(0.9)
C12PF1	0.9262(9)	0.169(2)	0.263(4)	7.9(1.3)	C12PF2	0.9152(9)	0.160(2)	0.762(4)	6.1(1.1)
C13PF1	0.9989(10)	0.191(2)	0.268(4)	8.5(1.3)	C13PF2	0.9888(10)	0.176(2)	0.764(4)	4.0(0.8)
C14PF1	0.9126(9)	0.229(1)	0.267(3)	5.2(1.0)	C14PF2	0.9110(9)	0.232(1)	0.762(3)	5.9(1.1)
N15PF1	1.0839(10)	0.332(1)	0.262(4)	12.6(1.5)	N15PF2	1.0909(10)	0.289(1)	0.773(4)	12.2(1.5)
N16PF1	0.8042(8)	0.221(2)	0.252(4)	12.4(1.5)	N16PF2	0.8015(8)	0.246(2)	0.748(4)	10.6(1.3)
		dCPG(1)					dCPG(2)		
N1C1	0.7999(7)	0.181(1)	-0.015(2)	2.9(0.5)	N1C2	1.0978(7)	0.209(1)	0.532(2)	4.6(0.7)
C2C1	0.8428(8)	0.168(1)	-0.002(2)	2.7(0.6)	C2C2	1.0570(8)	0.193(1)	0.525(2)	6.5(1.1)
O2C1	0.8508(5)	0.115(1)	0.017(2)	2.7(0.4)	O2C2	1.0516(5)	0.136(1)	0.519(2)	5.5(0.6)
N3C1	0.8643(6)	0.215(1)	0.009(2)	2.1(0.5)	N3C2	1.0261(6)	0.229(1)	0.520(2)	2.9(0.5)
C4C1	0.8529(12)	0.271(2)	-0.003(4)	6.4(1.0)	C4C2	1.0315(12)	0.287(2)	0.530(4)	4.9(0.9)
N4C1	0.8782(8)	0.324(1)	0.007(2)	5.3(0.7)	N4C2	0.9999(8)	0.326(1)	0.516(2)	6.4(0.8)
C5C1	0.8082(11)	0.280(2)	-0.014(3)	4.5(0.8)	C5C2	1.0715(11)	0.317(2)	0.523(3)	5.6(0.9)
C6C1	0.7850(11)	0.235(2)	-0.022(3)	5.6(1.0)	C6C2	1.1022(11)	0.272(2)	0.535(3)	6.0(1.0)
C1'C1	0.7738(10)	0.125(1)	-0.026(3)	3.8(0.8)	C1'C2	1.1274(10)	0.163(1)	0.541(3)	4.8(0.9)
O1'C1	0.7370(7)	0.143(1)	-0.069(2)	4.8(0.6)	O1'C2	1.1657(7)	0.189(1)	0.573(2)	4.8(0.6)
C2'C1	0.7620(11)	0.102(2)	0.076(3)	4.1(0.8)	C2'C2	1.1378(11)	0.125(2)	0.405(3)	4.3(0.9)
C3'C1	0.7211(9)	0.130(1)	0.099(2)	2.9(0.7)	C3'C2	1.1729(9)	0.175(1)	0.413(2)	4.4(0.9)
C4'C1	0.7000(10)	0.130(2)	-0.006(3)	4.8(0.8)	C4'C2	1.1989(10)	0.181(2)	0.507(3)	5.6(0.9)
C5'C1	0.6643(11)	0.179(2)	-0.030(3)	5.1(0.9)	C5'C2	1.2283(11)	0.235(2)	0.518(3)	4.6(0.8)
O5'C1	0.6804(6)	0.239(1)	-0.014(2)	3.9(0.5)	O5'C2	1.2092(6)	0.289(1)	0.504(2)	8.4(0.8)
O3'C1	0.6945(7)	0.096(1)	0.162(2)	5.2(1.4)	O3'C2	1.2034(7)	0.148(1)	0.338(2)	4.3(1.3)
P1	0.6921(4)	0.111(1)	0.278(1)	5.5(0.7)	P2	1.1979(4)	0.155(1)	0.222(1)	4.9(0.6)
O1A	0.6923(8)	0.175(1)	0.301(1)	5.3(1.4)	O2A	1.1940(8)	0.219(1)	0.196(1)	5.3(1.4)
O1B	0.6541(7)	0.072(1)	0.317(2)	7.3(1.7)	O2B	1.2292(7)	0.128(1)	0.176(2)	7.9(1.9)
O5'G1	0.7346(10)	0.088(1)	0.315(2)	7.2(1.7)	O5'G2	1.1559(10)	0.124(1)	0.191(2)	4.6(1.3)
C1'G1	0.8142(10)	0.047(2)	0.513(3)	4.2(0.8)	C1'G2	1.0950(10)	0.098(2)	-0.006(3)	4.7(0.8)
O1'G1	0.8044(8)	0.035(1)	0.413(2)	5.9(0.6)	O1'G2	1.0918(8)	0.064(1)	0.088(2)	4.6(0.6)
C2'G1	0.7819(16)	0.039(2)	0.579(4)	9.6(1.5)	C2'G2	1.1370(16)	0.112(2)	-0.034(4)	1.1(0.5)
C3'G1	0.7483(11)	0.034(2)	0.504(3)	4.3(0.8)	C3'G2	1.1542(11)	0.045(2)	0.005(3)	2.5(0.6)
O3'G1	0.7180(13)	-0.009(2)	0.552(3)	15.2(1.4)	O3'G2	1.1439(13)	0.000(2)	-0.062(3)	3.1(0.5)
C4'G1	0.7677(14)	0.002(2)	0.414(3)	7.1(1.1)	C4'G2	1.1288(14)	0.037(2)	0.108(3)	2.4(0.6)
C5'G1	0.7417(11)	0.022(2)	0.316(3)	4.8(0.9)	C5'G2	1.1535(11)	0.060(2)	0.192(3)	5.6(1.0)
N1G1	0.9451(7)	0.180(1)	0.512(2)	2.7(0.6)	N1G2	0.9526(7)	0.189(1)	0.020(2)	2.4(0.5)
C2G1	0.9402(10)	0.119(2)	0.516(3)	3.9(0.8)	C2G2	0.9648(10)	0.129(2)	0.011(3)	2.0(0.6)
N2G1	0.9736(9)	0.082(1)	0.507(3)	6.8(0.8)	N2G2	0.9343(9)	0.090(1)	0.008(3)	4.5(0.6)
N3G1	0.9053(8)	0.090(1)	0.511(2)	4.5(0.6)	N3G2	1.0032(8)	0.116(1)	0.018(2)	2.7(0.5)
C4G1	0.8728(10)	0.131(1)	0.515(3)	3.5(0.7)	C4G2	1.0278(10)	0.161(1)	0.017(3)	1.6(0.5)
C5G1	0.8715(12)	0.193(2)	0.512(3)	5.2(0.9)	C5G2	1.0197(12)	0.221(2)	0.020(3)	5.9(1.0)
C6G1	0.9096(10)	0.221(2)	0.511(3)	4.1(0.8)	C6G2	0.9781(10)	0.243(2)	0.017(3)	4.2(0.8)
O6G1	0.9217(7)	0.277(1)	0.510(2)	5.5(0.6)	O6G2	0.9645(7)	0.295(1)	0.025(2)	3.9(0.5)
N7G1	0.8339(10)	0.218(1)	0.506(3)	6.8(0.8)	N7G2	1.0559(10)	0.254(1)	0.021(3)	4.0(0.6)
C8G1	0.8127(10)	0.165(2)	0.506(3)	4.4(0.8)	C8G2	1.0854(10)	0.214(2)	0.018(3)	2.6(0.6)
N9G1	0.8311(8)	0.116(1)	0.507(2)	4.6(0.7)	N9G2	1.0687(8)	0.157(1)	0.014(2)	2.6(0.5)
				WATER MOLECULES					
*OW1	0.0000(0)	0.000(0)	-0.080(3)	8.7(1.3)	OW14	0.9297(0)	-0.029(0)	0.627(3)	18.1(1.8)
OW2	0.2677(11)	0.057(2)	0.033(3)	13.2(1.7)	OW15	0.1898(11)	0.448(2)	0.242(3)	18.4(1.7)
OW3	0.3208(10)	0.157(2)	0.491(3)	13.2(1.2)	OW16	1.0821(11)	0.423(2)	0.742(3)	16.7(1.6)
OW4	0.3025(11)	0.122(2)	0.294(3)	10.2(1.0)	OW17	0.2958(11)	0.133(2)	-0.309(3)	25.4(2.6)
OW5	0.1391(13)	0.458(2)	0.497(4)	16.3(1.5)	OW18	0.6360(13)	-0.045(2)	0.228(4)	12.6(1.2)
OW6	0.5649(13)	-0.111(2)	0.251(3)	14.2(1.4)	OW19	0.3990(13)	0.097(2)	0.503(3)	19.3(1.8)
OW7	0.5689(12)	0.119(2)	0.017(4)	15.5(1.4)	OW20	0.2008(12)	0.107(2)	0.758(4)	13.4(1.3)
OW8	0.1436(11)	0.034(2)	0.748(3)	12.8(1.2)	OW21	0.6145(11)	0.047(2)	-0.099(3)	23.4(2.3)
OW9	0.0694(13)	0.025(2)	0.402(3)	13.2(1.3)	*OW22	0.0151(13)	0.009(2)	-0.296(3)	17.1(3.6)
OW10	0.0387(11)	0.006(2)	0.223(3)	12.3(1.1)	OW23	1.2594(11)	0.508(2)	0.144(3)	13.5(1.3)
OW11	0.2178(11)	0.321(2)	0.295(3)	12.8(1.2)	OW24	0.3463(11)	0.071(2)	-0.227(3)	32.0(3.5)
OW12	0.1755(8)	0.217(1)	0.813(2)	7.2(0.8)	OW25	1.0713(5)	0.605(1)	0.268(2)	21.3(2.2)
OW13	0.3506(10)	0.052(2)	0.019(3)	11.2(1.0)	OW26	0.9752(10)	0.424(2)	-0.015(3)	28.0(2.7)

* These atoms have occupancy factors of 0.5.

backbone $\alpha,\beta,\gamma,\delta,\varepsilon$ (for definitions see Table 2 and reference 17) are similar in all the structures as illustrated in Figure 2; 2) the X values at the 3' end of each strand are at least 60° higher than those at the 5' end and 3) the sugar at the 5' end is always C3'endo whereas the one at the 3'

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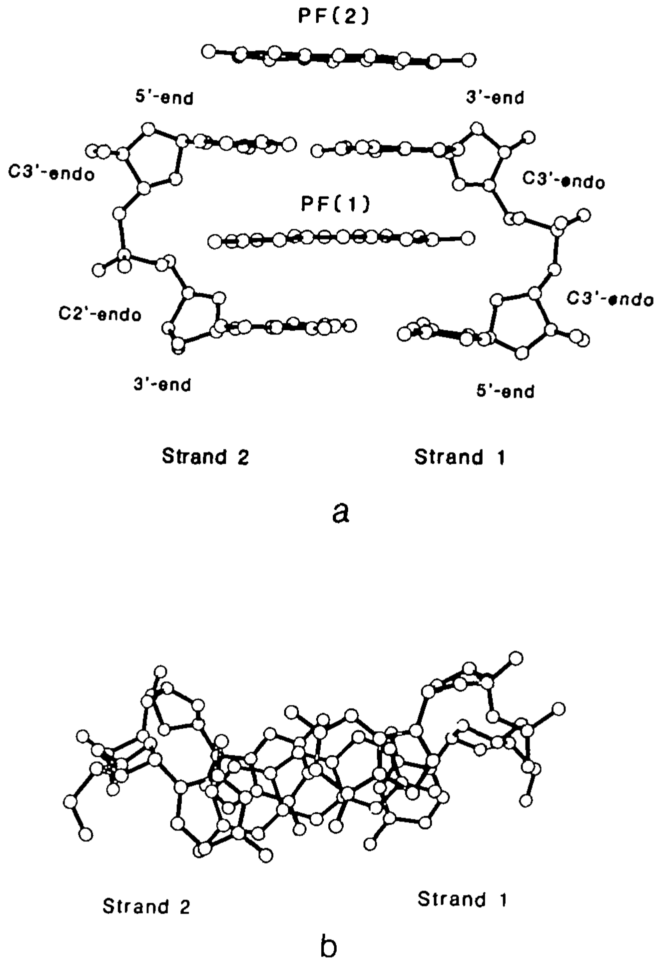

Figure 1. Two views of the 2:2 complex of dCpG and proflavine (dCpG·PF)
a) a view parallel to the base pairs.
b) a view perpendicular to the base pairs (non-intercalated proflavine is not shown here).

end is either C3'endo or C2'endo. The $\chi(3')$ values correlate with the sugar pucker at the 3' end in that values of 80–90° and 100–110° match C3'endo and C2'endo pucker, respectively. These results show that the greatest flexibility occurs at the 3' end of the dinucleoside phosphate molecules and

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Table 2 Conformational Angles for Dinucleoside Phosphate and Drug Complexes

	α	β	γ	δ	ε	χ(5')	χ(3')	Sugar Pucker 5'-end	Sugar Pucker 3'-end	Ref.
dCpG·PF1	210	290	290	219	46	16	80	C3'-endo	C3'-endo	this study
2	203	300	287	218	73	10	113	C3'-endo	C2'-endo	
dCpG·TPH1	201	287	282	226	57	32	114	C3'-endo	C2'-endo	13
2	194	292	308	217	84	34	117	C3'-endo	C2'-endo	
CpG·PF	204	292	287	234	53	18	87	C3'-endo	C3'-endo	11,14
CpG·AO1	211	301	288	237	50	9	105	C3'-endo	C2'-endo	10
2	225	298	297	226	40	8	115	C3'-endo	C2'-endo	
i5CpG·Et1	226	281	286	210	72	3	101	C3'-endo	C2'-endo	8
2	225	291	291	224	55	24	109	C3'-endo	C2'-endo	
i5UpA·Et1	207	286	291	236	52	26	99	C3'-endo	C2'-endo	7
2	218	302	276	230	70	14	100	C3'-endo	C2'-endo	
Average	211(11)	293(7)	290(8)	225(9)	59(14)	18(10)	104(12)			
Average for dinucleoside duplexes	217(5)	289(4)	290(6)	174(7)	57(7)					18
A-RNA	213	281	300	175	49	14	14	83	83	19
A'-DNA	205	290	299	176	51	13	13	83	83	20
A-DNA	175	315	270	211	47	27	27	83	83	19
B-DNA	159	261	321	209	31	85	85	157	157	19

The conformational angles are defined by Seeman et al. (17):
α = C4'-C3'-O3'-P, β = C3'-O3'-P-O5', γ = O3'-P-O5'-C5', δ = P-O5'-C5'-C4', ε = O5'-C5'-C4'-C3',
ζ = C5'-C4'-C3'-O3', and χ is the torsional angle about glycosidic bond.

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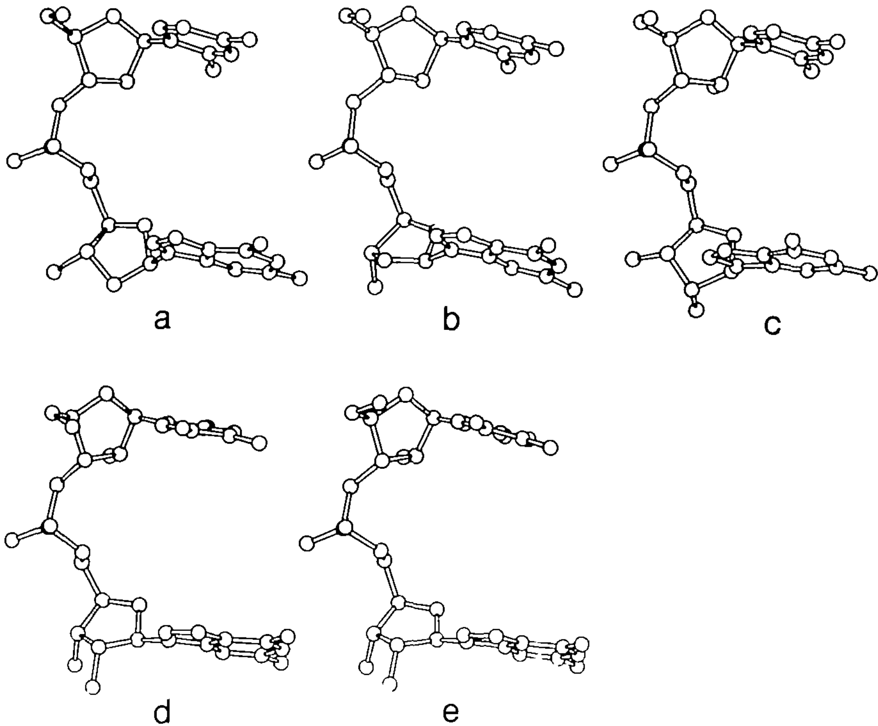

Figure 2. The structures of some of the dinucleoside phosphates involved in complexes. The backbones from C3' at the 5' end to C4' at the 3' end are shown in the same view for each of the structures.

a) strand 1 of dCpG·PF
b) strand 2 of dCpG·PF
c) CpG (in the CpG·PF complex)
d) strand 1 of i^5CpG·Et
e) strand 2 of i^5CpG·Et.

there appears to be no simple relationships among the conformations of the 3' nucleosides and the nature of the intercalating drug. Comparison of these structures with DNA and RNA is difficult because they are dimers and not polymers. It is not even possible to describe easily the conformational transition which would allow a dimer structure with a B-DNA conformation and base pairs 3.4 Å apart to assume the intercalation geometry described here with the base pairs 6.8 Å apart. However, to transform a deoxydinucleoside phosphate with a A'-DNA conformation or a ribodinucleoside phosphate with an

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A-RNA conformation, to an intercalated structure it is only necessary to increase δ by 50° and $\chi(3')$ by at least 60°. These observations parallel those obtained utilizing computer modelling which also showed that the alteration in the χ and δ angles were the only essential differences between the intercalated ribodinucleoside structures and A-RNA (14). The variations in the other backbone angles between different intercalated dinucleoside phosphates including the puckering of the 3' ribose would appear to be due to subtle differences in stacking patterns and hydrogen bonding.

Since all the dinucleoside phosphate-drug complexes have the same phosphate backbone conformations and the flexibility of the 3' nucleoside has been proven difficult to correlate with the drug structures, other geometric parameters such as the base turn and tilt angles between two base pairs and the bend and twist angles between two bases may be useful in discussing the structural differences effected by drug molecules and counterions . In the structure of dCpG·PF complex, the base turn angle is 17°, and both the twist angles of the base pairs and the tilt between them are essentially 0°. Table 3 presents the definitions of these geometric parameters and summarizes their values for the structure dCpG·PF along with those found in related complex structures.

It has already been shown that in a dinucleoside phosphate the base turn angle is more a function of the local base pairing geometry than of the backbone conformation (14) and can be correlated with the shape of the intercalated drug molecule. The bulky phenyl group of ethidium, for example, forces the base turn angle to be small while complexes with planar molecules such as 9-amino acridine, acridine orange and proflavine have angles ranging from 8° (CpG·AO) to 32° (CpG·PF). Although in a polymer the base turn angle is a measure of unwinding, this is not necessarily true in dinucleoside phosphate drug complexes. Since the glycosidic angles $\chi(3')$ and $\chi(5')$ in the dimer are asymmetric, if these structures were to be found in a polynucleotide, the sites adjacent to the intercalated one would have conformational changes as well. It is thus necessary to ascertain the conformations of all the areas in the strands affected by the intercalated drug and calculate a base turn angle over all affected residues which will then be related to unwinding of the polymer.

The twist and bend angles of the bases in each base pair may be functions of the counterion rather than of the drug structures so that dCpG·PF has a zero value for the twist whereas CpG·PF which has disordered $SO_4^{=}$ groups coordinated to both cytosine groups in the major groove has very

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Table 3

Some Geometric Features of Drug Dinucleoside Phosphate Complexes

	Base Turn[1]	Bend[2] base pairs	Twist[2] base pairs	Twist[2] stacked bases	Tilt[3]	Distance between phosphorus atoms on the two strands
dCpG·PF	17°	-1.7°	0.3°	-3.1°	-0.7°	16.7 Å
		8.3	-0.8	0.3		
CpG·PF	32	-5.5	-13.4	-8.3	-6.2	15.9
		5.5	-13.4	-8.3		
i^5CpG·Et	4	-6.8	0.8	13.9	10.4	17.1
		-0.3	2.6	8.1		
i^5UpA·Et	5	-6.8	1.5	5.9	7.5	16.6
		3.3	1.4	4.9		

[1] If we project two C1'-C1' vectors of the two adjacent base pairs to their average least squares plane, the base turn angle is the angle between these two projected vectors.

[2] Bend and twist angles of a base pair are the two components of the dihedral angle between two base planes. We choose N3···N1 hydrogen bond as our reference axis. The twist angle is the component along this axis and the bend angle is the one along the axis perpendicular to the reference axis. Both axes are parallel to the base plane. In the stacked bases, the reference axis is defined by C4···N1 in purine base or C6···N3 in pyrimidine base for reference axis.

[3] Tilt angle is a meaure of the inclination of one base pair to another. It is obtained by defining a reference axis and calculating the component of the dihedral angle between two base pairs along this axis. The reference is so chosen that if we view through the minor groove side of the duplex, the N3···N1 hydrogen bond on upper base pairs is used as the reference axis. The resulting + sign of the angle means opening-up in the minor groove side and - sign means in the major groove side.

large twist values. An analogous situation occurs in the structure of ApU (17) in which a sodium ion coordinates to the uracil groups in the minor groove causing the twist angles of those base pairs to have opposite values to those found in other oligonucleotide structures (18).

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On the other hand, the twist value of the stacked bases which is a measure of the inclination of one base to another in each single strand, and the tilt angle which is a measure of the inclination of the two stacked base pairs in a duplex may be related to the shape and location of the drug molecules as seen in Figure 2. In the ethidium complexes the twist and tilt of the stacked bases are positive, indicating a twist of the bases toward the major groove in which there are no bulky substituents. In the dCpG·PF complex these values are zero which is a reflection of the planarity of the intercalating drug. The highly negative value of these parameters for CpG·PF appears to be influenced by the presence of the $SO_4^=$ group.

The stacking patterns of the proflavines and base pairs in this structure are quite unlike that found in the CpG·PF structure (11,12) as shown in Figure 3. In that duplex the intercalated proflavine is symmetrically placed between the base pairs and hydrogen bonded to the phosphate oxygen atoms in both strands, whereas in this deoxy structure the proflavine is asymmetrically positioned with respect to both the base pairs and the phosphate oxygens to which it forms no hydrogen bonds. In dCpG·PF the proflavine overlaps both bases of strand 2 and only the guanine of strand 1 in a pattern which bears striking resemblance to that exhibited by the overlap of acridine orange in the CpG·AO complex (10). If the proflavine were translated by .5 Å toward the backbone of strand 1 one exocyclic amino group would form a hydrogen bond with one phosphate oxygen. However, the stacking pattern would then change to one that is possibly less favorable. To hydrogen bond the proflavine to strand 2 requires it to be rotated and translated and results in much less overlap between the dye and the base pairs. In any circumstance it is not possible to hydrogen bond both amino groups of the proflavine simultaneously to the phosphate groups of both strands. This is partially due to the fact that the phosphorus-phosphorus distance is 16.7 Å compared with 15.9 Å in CpG·PF and 17.5 Å in uncomplexed ribodinucleosides.

The non-intercalated proflavine stacked above the base pair of one duplex is also stacked below the base pair of a translationally related duplex so that there is a continuous alternation of base pairs and proflavines in the crystal. The stacked proflavine is orientated in roughly the same direction as the intercalated one and in this respect the structure more resembles that of ApA·PF (21) than that of CpG·PF.

The main conclusions about intercalated ribo- and deoxy-dinucleoside phosphate duplexes that can be drawn from this crystal structure analysis are 1) the χ values of the 3' ends are higher than those at the 5' ends 2)

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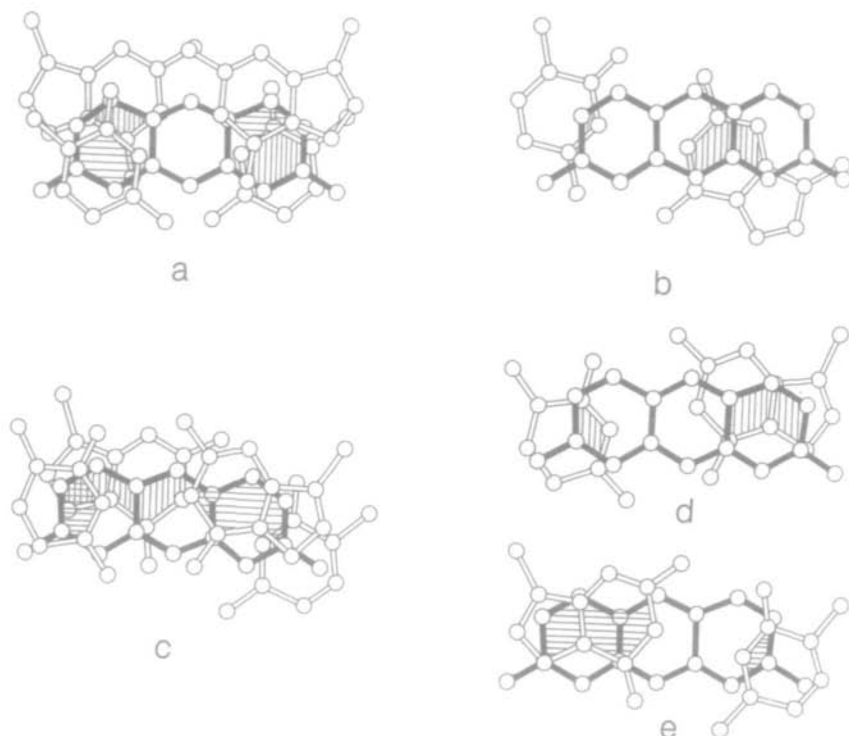

Figure 3. A comparison of the stacking patterns of proflavine with dCpG and CpG

a) Intercalated PF with two base pairs in CpG·PF
b) Non-intercalated PF with one base pair in CpG·PF
c) Intercalated PF with two base pairs in dCpG·PF (strand 2 bases are on left)
d) Non-intercalated PF with one base pair in dCpG·PF
e) Non-intercalated PF with the other base pair in dCpG·PF

the conformations of the backbone angles $\alpha, \beta, \gamma, \delta$ and ε assume very similar values in all the structures and 3) the puckering of the ribose (or deoxy) sugar at the 3' end is of secondary importance and is certainly not a necessary feature of intercalated structures. It must be emphasized here that the puckering of the sugar at the 5' end is important because as has been shown by Jack et al. (22), the spacing between this ribose and its next neighbor is affected by a change from C3'endo to C2'endo puckering. Hence

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the puckering of the 3' end of these dimers is of importance in modelling of larger oligonucleotides although it is still not necessary to have mixed sugar puckering for site exclusion as shown by computer model building (6) of intercalated tetranucleotides. This analysis also shows an interesting difference between the potential for dye binding in RNA and DNA structures. In the CpG structure (11,12) proflavine molecules were found bound to the outside of the duplexes via the O2' hydroxyl of the ribose sugar. This type of outside binding could not and does not exist in the dCpG structure. However outside binding of proflavine molecules to the phosphate oxygens (as was also observed in the CpG·PF complex) is not precluded in deoxynucleotides although it is not exhibited in this structure. We also see here that the presence of a symmetrical intercalating molecule does not ensure symmetrical conformations of the duplex itself.

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