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A 1:2 crystalline complex of ApA:proflavine: a model for binding to single-stranded reigons in RNA

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ABSTRACT

The structure of a 1:2 complex of adenylyl-(3',5')-adenosine phosphate and proflavine hemisulfate has been determined using the methods of x-ray crystallography. Since the ApA does not form a mini double helix, it may serve as a model for the interaction of planar molecules with single stranded nucleic acids. The dinucleotide adopts an extended conformation with the adenines in adjacent molecules forming base pairs. A most unusual feature of the molecule is that it does not obey the "rigid nucleotide" concept although none of the torsion angles occur in energetically unfavourable regions. This is most probably due to the strong interactions between the proflavine and the oligonucleotide.

INTRODUCTION

A large number of drugs, carcinogens and mutagenic agents act biologically by binding to nucleic acids. Interactions with double-stranded nucleic acids, especially DNA have been the subject of many investigations[1,2], and a number of structural studies on both the natural polymer as well as model systems have attempted to elucidate details of the molecular mechanisms of binding and the structures of the complexes formed[3-5]. It is already apparent from some of these studies that the conformational changes induced in double-stranded nucleic acids by even the simple mutagen proflavine are complex, and our knowledge of them is far from complete. A recent examination of the model dinucleoside phosphate intercalated complexes[6] has shown that the torsion angle changes induced in them are within the accepted ranges of the rigid nucleotide hypothesis[7], though only just so in some instances. This widely-accepted theory in essence states that the conformational flexibility of both nucleic acids and their individual nucleotide constituents, falls within certain well-defined angular ranges, and therefore that some conformations are distinctly not preferred.

We have now extended our previous structural studies on a double-heli-

cal model complex of proflavine, to one of this drug with a single-stranded dinucleoside phosphate. Single-stranded nucleic acids have long been thought to be of considerable functional importance - examples are looped-out regions of DNA (of importance in mutagenesis), and the non-base-paired regions in tRNAs (which are responsible for much of their functionality). This study reports crystallographic studies on a complex of adenylyl-(3', 5')-adenosine phosphate (ApA), which cannot form an anti-parallel stranded miniature double helix, although in principle a poly A-type double helical fragment could be formed[8].

METHODS

Slow evaporation of a solution of proflavine hemisulfate and ApA by vapour diffusion methods yielded deep-red rectangular crystals. Ultraviolet spectroscopic tests indicated that the crystals contained both proflavine and ApA. A crystal sealed in a glass capillary was used for all subsequent X-ray crystallographic measurement.

Crystals were orthorhomic, of space group $\underline{P2_12_12}$ and had unit cell dimensions $\underline{a}$ = 32.157 Å, $\underline{b}$ = 21.450 Å and $\underline{c}$ = 10.175 Å. A total of 5695 unique data up to 2θ (Cu$\underline{K}\alpha$) = 120° (which corresponds to a resolution of 0.9 Å), were collected on a SYNTEX $P\bar{1}$ diffractometer.

The structure was solved by direct methods (using a procedure which first obtained the phases of $\underline{hk}0$ reflections only, then extended these phases to all reflections) and independently by Patterson superposition techniques. A blocked full matrix least squares technique was employed for the structure refinement. The current discrepancy factor $\underline{R}$ is 0.15 based on 3431 observed reflections.

RESULTS AND DISCUSSION

The structure analysis shows that the asymmetric unit contains a 1:2 complex of ApA:proflavine. The molecular structure of ApA is shown in Fig. 1.

The molecule is protonated at N1 at the 5' end and is thus a zwitterion. It adopts an extended conformation, with adenines in adjacent molecules base-pairing together to form infinite chains of ApA units. This feature of opened-out bases is not unlike in general appearance that of adenylyl-(3',5')-uridine when complexed to 9-aminoacridine[9]. The base pairs, involving N6 and N7, have been previously observed in the crystal structures of ApApA[10] and UpA[11], and is of the type proposed for the structure of acid

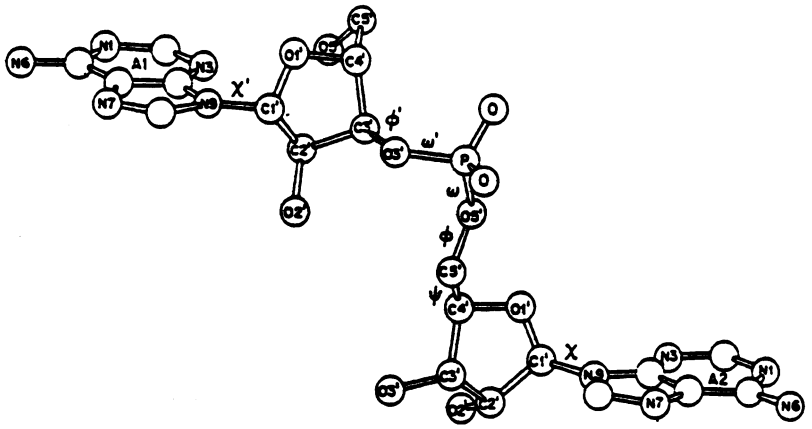

Figure 1. A single molecule of ApA. The conformational nomenclature for the torsion angles is shown.

poly(rA)[8]. Each base pair has stacked (3.4 Å apart) on either side of a proflavine cation, with their long axes both aligned with the base pair. Furthermore, each independent proflavine is stacked with the other such that the order of planar groups looking along the stacking direction is base-pair, proflavine, proflavine, base-pair, proflavine, etc. (Figure 2). This situation is reminiscent of that in both proflavine CpG[6] and in a platinum-AMP intercalation complex[12]. The rest of the lattice is filled up with water molecules and counter ions which are involved in extensive hydrogen bonding and ionic interactions with the proflavines and the dinucleotide.

The conformational features of the dinucleoside phosphate are unusual as shown in Table 1.

In the other oligonucleotide molecules studied to date the nucleoside portions have been shown to obey (at least approximately) the "rigid" nucleotide concept[7]. In rigid nucleotides, the glycosidic angles are anti(χ = 0-90°); the torsion angles around C5'-O5' and C3'-O3' are trans ($\phi \sim \phi' \sim 180°$); the torsion angle around the C4'-C5' bond is gauche (ψ = 60°) and the ribose sugars are either C3' or C2' endo. Only ω and ω' show large conformational flexibility. Almost all the exceptions to this principle are found in this ApA molecule. The two halves of the ApA molecule have very different conformations. The glycosidic linkage of the 5' adenosine is <u>syn</u> ($\chi \sim$ -

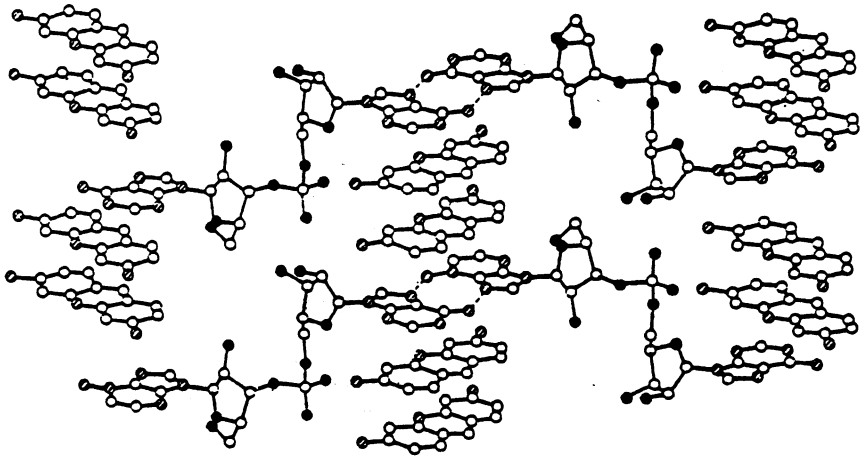

Figure 2. The arrangement of ApA and proflavine moieties in the crystal structure.

Table 1

Torsion Angles for the Nucleotide Unit in Various Structures

	χ'	φ'	ω'	ω	φ	ψ	χ	Reference
Prof-ApA	-119	272	290	293	175	168	71	This study
Prof-CpG	17	201	290	289	231	52	85	(5)
RNA-11	13	213	281	300	175	50	13	(21)
UpA 1	12	206	81	82	203	55	37	(11)
UpA 2	19	224	164	271	192	54	44	
ApA^+	8	223	283	298	161	53	28	(10)
A^+pA^+	28	209	77	93	188	56	26	
ApU-9 amino-acridine	76	222	100	86	202	63	72	(9)

The values which deviate significantly from those in RNA-11 are underscored.

119°). Usually, the syn conformation is found only in molecules in which a chemical modification actually excludes an anti-conformation, for example in 8-bromoinosine[13]. (χ has the values -85° and -76° for the two independent molecules in that structure.) A syn conformation has also been observed for the formycin nucleoside antibiotics - formycin B hydrochloride has χ = -140°[14] and oxoformycin B has χ = -164°[15]. The other unusual features can be rationalized more easily. The conformation about the C4'-C5' bond is trans,

a feature which has been observed in some nucleotide structures such as deoxyguanosine 5'-phosphate with $\chi = 175°$[16] but in no other oligonucleotides. It has also been implicated in forming kinks in DNA when wound round histone proteins in the nucleosome[17]. Moreover, it appears to be an important feature of the loop regions in tRNA[18].

The high anti χ conformation of the 3' adenosine as well as the mixed sugar puckering has precedent in other dinucleotides with drugs. In the intercalated molecules the C2' endo conformation is found at the 3' rather than the 5' end of the dinucleotide. In the non intercalated ApU-9 aminoacridine[9] the sequence of puckering is the same in this structure. The high value of the ϕ' angle can be correlated with the C2' endo sugar pucker, as observed in pdTpdT[19] and tRNA[20]. Interestingly, the torsion angles ϕ, ω and ω' have values normally found in helical ribonucleic acids[21,22]. It has always been thought that variation in the latter two angles in particular is responsible for the flexibility in nucleic acids[11].

The crystal structure of transfer RNA has now been refined independently by four groups[20,23-27]. The conformations of some of the loop regions are rather ill-defined, and indeed the four analyses do not always agree on interpretation. However, it is likely that at least some of the conformational features observed here in ApA are present in tRNA loop regions. Even the rare syn glycosidic conformation may be present in, for example, residue 76 (the end of the amino-acid acceptor stem)[23].

Although the overall conformation that we find for ApA has not been described previously for either a nucleic acid constituent or a polymer, none of the variable angles occur in energetically unfavourable regions. Since we are not observing in this analysis the structure of a 'naked' nucleic acid fragment, but one in a drug-interactive situation (albeit a frozen one), it is tempting to suggest that the binding of the drug is largely responsible for these unexpected conformational features. Furthermore, since the functionality of nucleic acids is often expressed in single-stranded regions, we can expect that protein or drug binding can induce rather more variable and profound conformational changes than have been hitherto suspected, with almost if not all the torsion angles in a nucleotide unit capable of considerable flexibility.

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