CRYSTAL STRUCTURE OF d(A–T)$_2$ AND SEQUENCE SPECIFIC DNA-PROTEIN RECOGNITION*

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ABSTRACT

We have obtained the crystal structure of the deoxy tetranucleotide d(A–T)$_2$. The structure shows (dA–dT)·(dA–dT) dinucleotide duplex fragments. The double helical model of poly (dA–dT)·poly (dA–dT), obtained using the crystal structure dimer as the repeating unit, has sequence dependent alternation of both deoxyribose pucker and phosphodiester bridge conformation. If such characteristic backbone structures are present in specific regions of DNA such as operator sequences, the backbone itself can provide a specific locus for discriminating such regions from the rest of the DNA in sequence specific DNA–protein interactions, in addition to the discrimination resulting from selective interactions at the level of the bases.

MANY important steps in genetic expression require selective recognition and binding of proteins (repressors, RNA polymerase, etc. to specific regions of DNA. The conventional models of DNA double helix do not throw much light on the origin of this specificity at a molecular level as in these models the DNA is generally assumed to have the same uniform or standard geometry without any specific conformational changes brought about by specific base-pairs or base sequences.

We have recently solved the crystal structure of the deoxyoligonucleotide d(A–T)$_2$ by high resolution X-raly diffraction methods (Viswamitra *et al.* 1978)[1]. In this study we have been concerned in finding out evidence of any dependence of DNA double helix conformation on base sequence. The study shows (dA–dT)·(dA–dT) dimer double-helix fragments in which the A and T nucleotides have different conformational features. When the crystallographic dimer unit is repeated suitably by model building, it is found to generate a possible sequence specific double helix conformation for poly(dA–dT)·poly(dA–dT). Unlike the well established classical DNA models (Arnott *et al.* 1969)[2], this double helix has periodic changes of conformation in the molecule. The sugar pucker alternates between C3′–endo geometry for A and C2′–endo for T residues. The base-sugar rotation χ . about the glycosidic bond is 0° for A and 70° for T. Also the A–T and T–A sequences have their own characteristic phosphodiester bridge conformations. In particular the torsional angle ω' about the P–O3′ bond is significantly different for the two cases ($\omega'=-70°$ for A–T, and about −125° for T–A). The helical turn per base pair is also different for the two sequences (31° between A and T and about 40° between T and A). Thus the A–T and T–A regions in this structure can be separately recognised because of their distinct conformations.

Many biochemical studies also seem to prefer the unique double helical structures over local regions of DNA, in DNA–protein recognition mechanisms. Scheffler *et al.* (1968)[3] have found that poly d(A–T) is cleaved by pancreatic DNAse at every other phosphodiester bond to give oligonucleotides of the type d(T–A) with T bearing a 5′ P and A a 3′ OH group. The periodic structure of poly(dA–dT) can provide a structural basis for this finding as every other phosphodiester has an alternate conformation particularly about the P–O3′ bond. Recently Chan and Wells (1974)[4] have found that the single stranded DNA specific S_1 and mung bean nucleases cleaved selectively in or near the lac operator region of λ_{pl} lac–DNA. They have considered the result in terms of the unique local DNA structures, including those which are totally helical but with a non-DNA–B geometry as compared to the great part of DNA.

More recently Richmond and Steitz (1976)[5] have studied repressor binding to poly d(A–U–HgX), the structural analogs of poly d(A–T). They find that the modified DNA which has significant occlusion of the major groove by bulky groups, still binds to lac repressor as well as or better than poly d(A–T). They suggest repressor interaction with atypical or unusual DNA conformation with sequence dependent changes in deoxyribose-phosphate backbone, as one of the possible explanations. Lin and Riggs (1971)[6]

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have earlier found that poly d(A–BrU) binds about 40 times more effectively for repressor than poly d(A–T) whereas poly d(A–U) itself competes 20 fold less effectively. The mechanism by which these changes of repressor affinity are brought about is not clear. In the alternating model of poly (dA–dT), the conformation of the deoxyribose–phosphate backbone is base-sequence dependent. These chemical changes, because of stereochemical differences such as stacking can bring about specific conformational changes of both sugar pucker and phosphodiester bridge and thus contribute largely to changes in repressor affinity.

Crystal Structure Results:

d–pApTpApT was crystallised as NH_4, salt at pH 6·5 from water-acetone solutions. The space group is $P2_1$ with a=21·24, b=21·294, c=8·77 Å, β=97·8° =1·53. The crystals were very hygroscopic and the 3 d Mo Kα diffractometer data were collected to a resolution of 1·04 A. The structure was solved by a combination of model building, Patterson methods and application of tangent formula for partial structure (Karle 1968)[7]. The conventional reliability factor at present is R=15·3%.

Molecular Geometry:

The deoxyribose moiety in the backbone of d–pApTpApT molecule (Figs. 1 and 2) alternates between C3′–endo and C2′–endo pucker, depending on whether it belongs to A or T. The glycosidic torsion angle also alternates likewise: O1′–C1′–N9–C3 =5° (A1) and –8·7° (A3) and O1′–C1′–N1–C6= 68·5° (T2) and 74·8° (T4). All the four nucleotide units have the common gauche-gauche (gg) conformation about the exocylic C4′–C5′ sugar bond. The conformation of the phosphodiester bridge about the P–O3′ and P–O5′ bonds is however significantly different: gauche-gauche (gg) between A1 and T2 (also between A3 and T4), trans-gauche (tg⁻) between T2 and A3. The conformation between A and T is that of a right-handed helix and that between T and A is extended. Yathindra[8] has however recently shown from theoretical and model studies that polynucleotides having alternate (C3′ endo–C2′ endo) sugars can have tg⁻ favoured helical structures. (For nomenclature and conventions see Sundaralingam[9]).

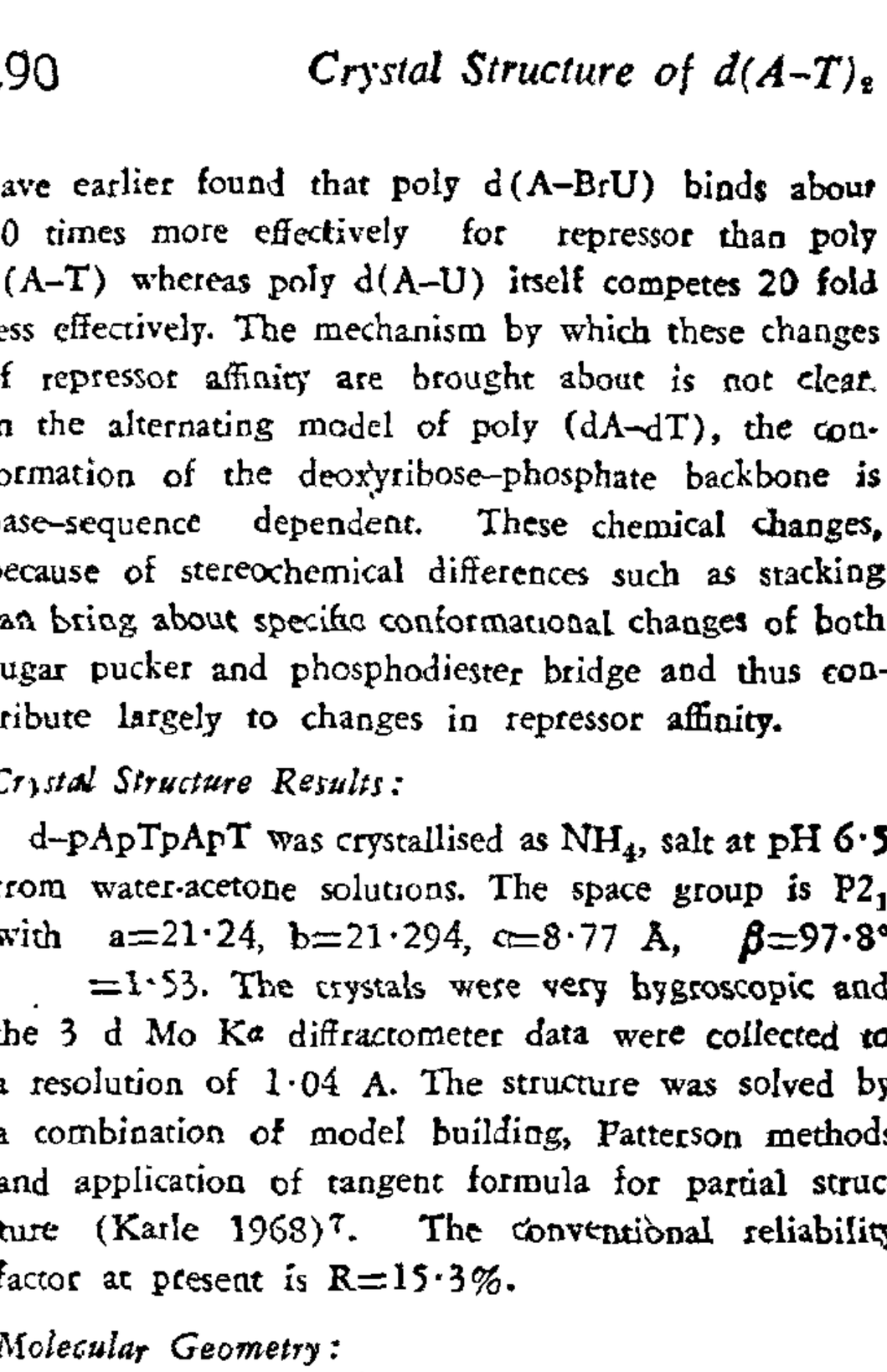

FIG. 1. A view of the deoxytetranucleotide molecule parallel to a–axis. (5′–p–Acelnyly–(3′–5′)–Thymilyl–(3′–5′)–Adenylyl–(3′–5′)–Thymidine (d–pApTpApT).

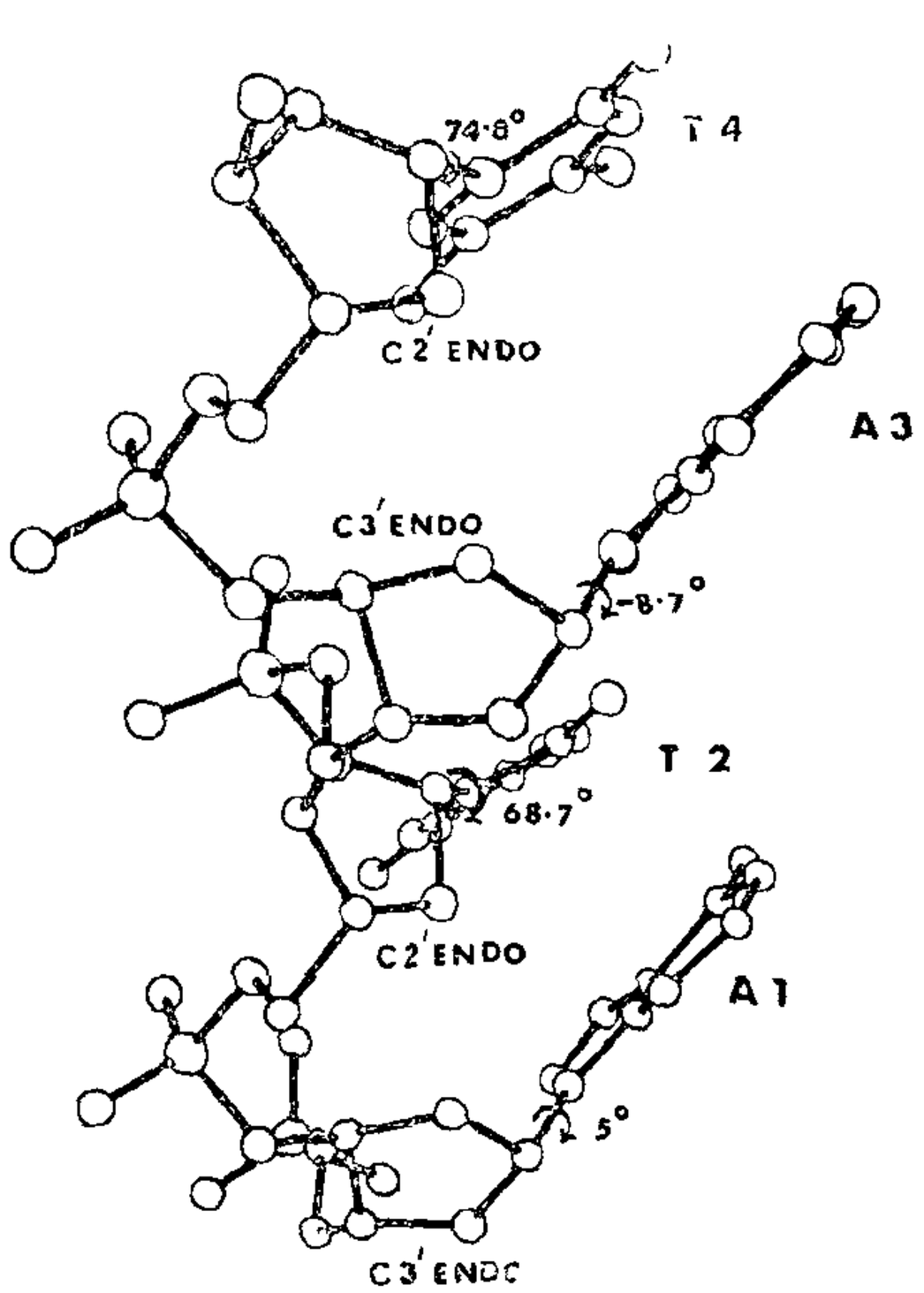

FIG. 2. b–axis view of d–pApTpApT.

The (dA–dT) segments form mini double helix:

The tetranucleotide molecules do not form self-complementary duplexes at the tetranucleotide level. (dA–dT)·(dA–dT) dinucleotide double helix units are however formed with classical Watson–Crick hydrogen bonding between adenine and thymine bases from two molecules related by 2_1 screw axis [N6(A) –O4(T)=3·04 Å av., and N1(A)–N3(T)=2·82 Å av.] (Figs. 3 and 4). *These dimer units are right-handed* and anti-parallel. Within each base pair, the pyrimidine and purine bases are inclined to each other (13°). The mean planes of the two base pairs are however nearly parallel (3°) with a vertical separation of 3·34 Å. The C1′–C1′ (interstrand) separation is 10·2 Å and the helical turn between the two base pairs is 31°. Sudh mini helix units have been observed from

single crystal studies of RNA double helical fragments, but this is the first description of Watson–Crick base pairs observed in a DNA fragment at atomic resolution.

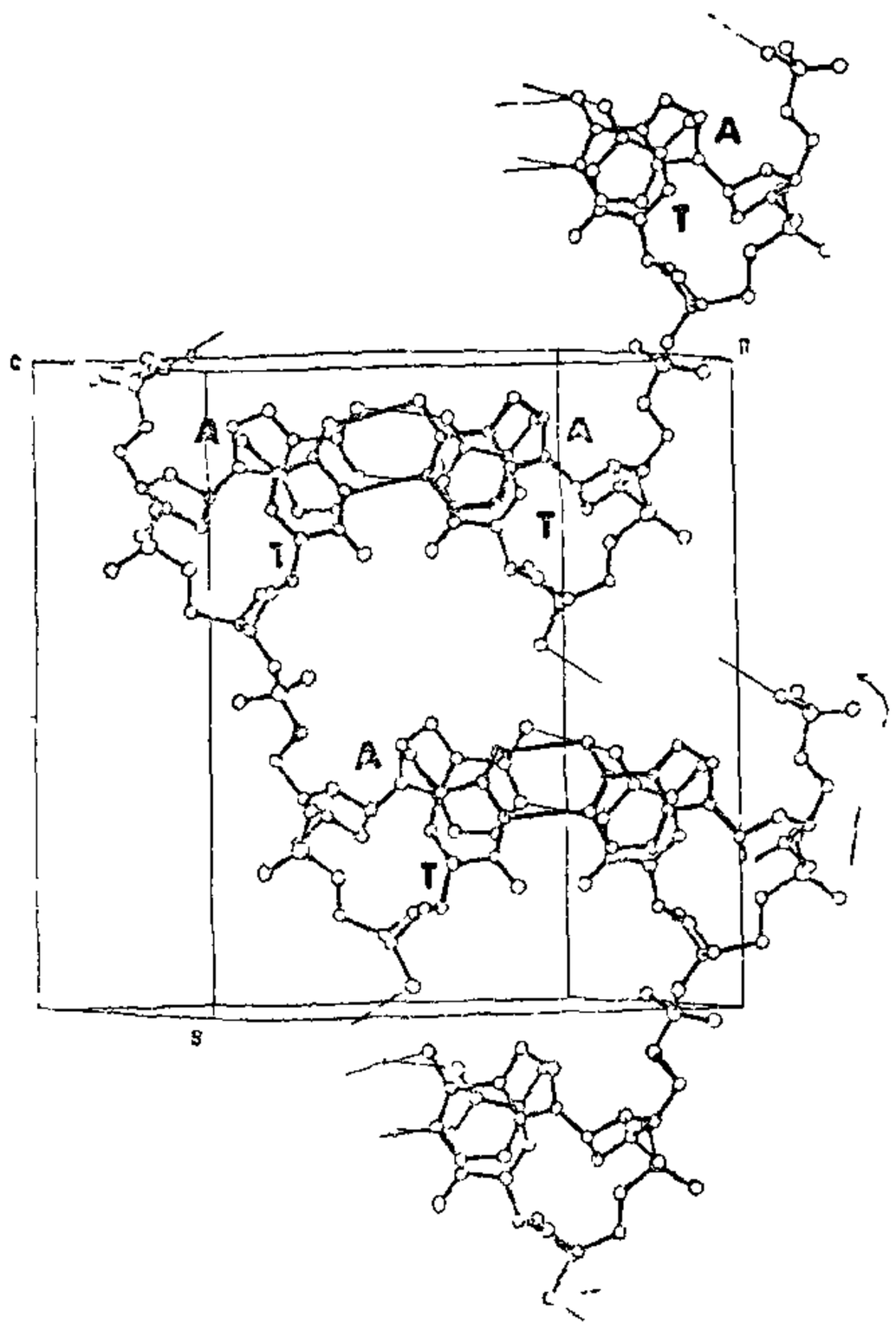

FIG. 3. The (dA–dT(·(dA–dT) mini helix viewed perpendicular to base pairs.

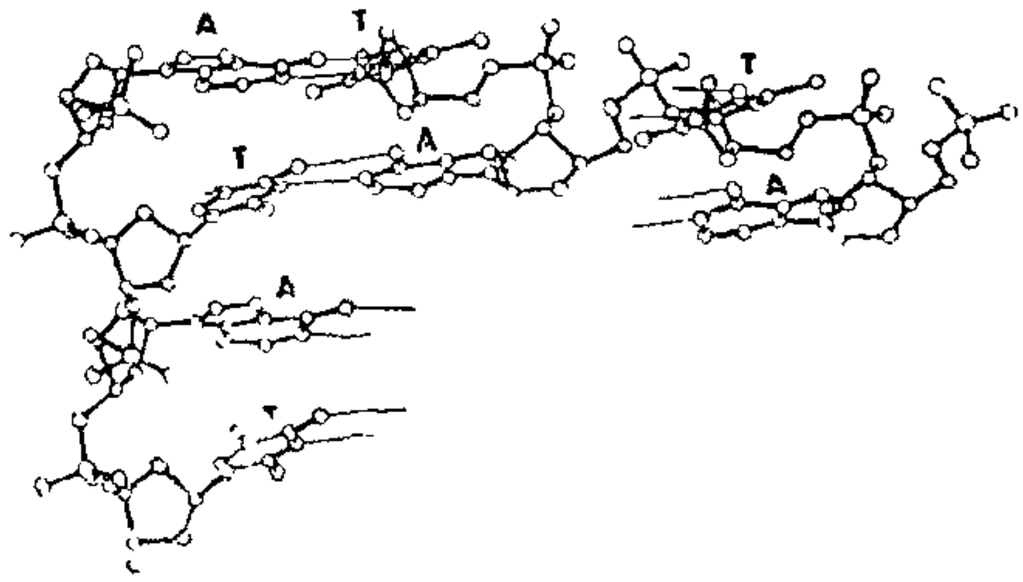

FIG. 4 The (dA–dT)·(dA–dT) mini helix viewed nearly parallel to base pairs.

In the present structure there is considerable overlap between adenine and thymine in the A–T region of the same molecule and also between adenine bases (3·5 Å) of molecules related by the 2_1 axis. The extended structure is further stabilised by base-sugar interactions of the type discussed by Bugg *et al.* (1971)[10]. There is an equivalent of 31 water molecules per tetranucleotide, some of them in positions to form direct hydrogen bonds with phosphate and base O and N atoms. Most of them however make indirect contacts through water-water bridges simulating an almost bulk-water like environment for the nucleotides. The results of the crystal structure may therefore be relevant to solution structures.

A sequence dependent double helical structure for poly(dA–dT) and its relation to sequence specific DNA-protein interactions:

We have used the (dA–dT)·(dA–dT) dimer of the crystal sttucture as the repeating unit to generate a double helical model for the alternating copolymer poly (dA–dT)·lpoly (dA–dT). As pointed out in the beginning, the most interesting property of the present model is that its conformational features also alternate. In the sugar phosphate backbone, the deoxyribose conformation alternates between C3′–endo for A and C2′–endo for T residues and the pohsphodiester bridge geometry is significantly different for the A–T and the T–A regions. The glycosidic angles for A and T residues are also different (0° and 70°). The model is a qualitative one, as its various torsional angles are still being refined by helix-generating computer programmes and energy minimisation procedures. Some of the average features of the model seem to resemble DNA B structure but a proper comparison with the established forms of DNA (Arnott *et al.* 1969)[2] and of the D–DNA helical structures observed for poly(dA–dT) in fibres [Davies and Baldwin 1963)[1] and Arnott *et al.* 1974)[12]] can be made only after further refinement of the present model. It may be mentioned that Bram (1971)[13] has found that the X-ray scattering pattern of AT rich DNA in solution is different from that of DNAs of low and medium AT content or of Calf Thymus DNA. He has suggested that very rich AT DNA may adopt a conformation different from that of normal DNA–B and that such sequence dependent DNA structure can provide a recognition mechanism in DNA–protein interaction.

We have already described the relevance of the alternating structure for poly(dA–dT) to some biochemical findings involving DNAse cleavage and lac repressor interactions[3-6]. In conclusion the present study suggests sequence specific changes of deoxyribose pucker and phosphodiester bridge conformation in poly(dA–dT)·poly(dA–dT). If such characteristic backbone structures can exist in specific regions of DNA such as operator sequences, the backbone itself can provide a specific locus for discriminating such regions

from the rest of the DNA in sequence-specific DNA-protein interactions, in addition to the discrimination resulting from selective interactions involving the bases.

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SOME RECENT OBSERVATIONS ON THE SYSTEMIC MODE OF ACTION OF VITAMIN A

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ABSTRACT

Some of the important observations made in recent years regarding the systemic mode of action of vitamin A are summarised. It is pointed out that regeneration of liver following partial hepatectomy is markedly less in vitamin A deficient rats. Similarly division and differentiation of the primitive epithelial cells of the oviduct of oestrogen-treated chicks are significantly arrested on deprivation of vitamin A. It is discussed that vitamin A is required for controlled division and differentiation of cells.

UNLIKE many of the water-soluble vitamins, proper understanding of the mode of action of the fat-soluble vitamins has been rather challenging, and vitamin A has not proved to be an exception in this respect. Although it was one of the earliest vitamins to be discovered, its systemic mode of action is still not properly understood, and completely new approach has become necessary for unravelling the prerise mechanism through which it functions. Recently Ganguly[1] made such a radically new approach and pointed out that the classical symptoms of deficiency of vitamin A described by numerous workers can be explained on the basis of a general interpretation that vitamin A is required for division and differentiation of cells of higher animals. While arriving at such an interpretation it was assumed that a particular compound should act at a single fundamental point rather than at several areas of physiology. According to Ganguly[1] such an interpretation would readily explain the diverse effects of deficiency of vitamin A described in the literature. Since then we have made several attempts to produce *evidence in support of* such a hypothesis by using regenerating rat liver and oestrogen-primed chick oviduct as typical model systems for rapid growth and differentiation of cells and are summarising here some of our salient observations in this regard.

Regenerating rat liver: Regenerating rat liver, following partial hepatectomy, has been widely recognised as a typical model system for rapid cell division[2]. By using vitamin A-depleted and normal rats Jayaram *et al.*[3] have shown that regeneration of the liver in terms of net increase in tissue weight as well as in terms of DNA, RNA and protein contents is markedly lower in the vitamin A-deprived rats which could be restored to near normal levels by supplementation of the deprived rats with retinyl acetate *immediately after* partial hepatectomy.