THE CONCEPT OF POLY (DINUCLEOTIDES): NOVEL NUCLEIC ACID HELICES WITH DINUCLEOTIDES AS HELIX REPEATS AND THE CONFORMATIONAL DYNAMICS OF DNA DUPLEXES*

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ABSTRACT

The idea of "dinucleotide residues" instead of the mononucleotides, serving as possible helical repeats in nucleic acids and polynucleotides is proposed. The nucleotide moieties in the repeating "dinucleotide units" are conformationally heterogenous by virtue of their differences in conformations which may arise either from different sugar puckers or backbone torsions. Stereochemical feasibility of such poly (dinucleotide) helices has been examined by conformational calculations and model building procedures. A novel poly (dinucleotide) helical structure with a "dinucleotide repeat" possessing alternate C3′ *endo*- C2′ *endo* sugars concomitant with a *trans-gauche*$^-$(*tg*$^-$) conformation for the phosphodiester linking the repeating units is proposed. The importance of this helical structure (i) as a possible candidate for certain highly specific base sequence regions of DNA and synthetic polynucleotides as well as RNA–DNA hybrid structures; (ii) as an intermediate in the dynamics of DNA duplexes; and (iii) conformational recognition in protein-nucleic acid interactions is highlighted.

INTRODUCTION

X-RAY diffraction patterns of DNA, RNA and a variety of synthetic polynucleotides have consistently been interpreted as conforming to right handed helices of either A- or B-genus[1]. The most significant difference between the two classes lies in the nature of the sugar ring geometry, the successive mononucleotide repeating units have the C3′ *endo* geometry in the A-genus and the C2′ *endo* geometry in the B-genus. The conformations around the other bonds including the phosphodiester are very similar and conform to their energetically preferred states. In fact, the four helical segments of yeast $tRNA^{Phe}$ have also been shown[2–4] to belong to A-genus thus providing an evidence for the intertwined character of helices. In continuation of our studies[5,6] to probe the possible varieties of helical structures in nucleic acids and polynucleotides, we report here our initial results on possible helical structures comprising "dinucleotides" as repeating units, instead of the usual mononucleotide considered so far. The nucleotide moieties in the repeating "dinucleotides" are conformationally heterogeneous in that they may possess either different sugar puckers or different backbone torsions. Such analyses are of significant interest from the point of view of other possible helical structures for nucleic acids and also understanding conformational dynamics of DNA duplexes as well as aspects of molecular basis of biological recognition of nucleic acids by proteins.

It is known that DNA exhibits structural alterations[7,9] under the influence of salt and temperature variations and also when interacting with certain proteins. There are also indications[10–12] that certain highly sequence specific regions in DNA may adopt conformations different from the classical B–DNA and may co-exist with the regions possessing B–DNA conformation. A "dinucleotide residue" comprising conformationally heterogeneous nucleotide backbone may well represent the base or base sequence induced effect and helical structures generated using them may further induce some novel conformational features with regard to phosphodiester conformation, helix grooves, base separation, etc., that may be different from nucleic acid helices studied so far. These modifications caused primarily by changes in the sugar-phosphate-sugar backbone of a polynucleotide chain may serve as additional unique sites for protein recognition in addition to possible recognition through base pairs or base sequences commonly implicated. The sugar-phosphate-sugar backbone with its greater conformational variability should be considered as possible recognition sites to proteins. The present studies are also relevant to conformations of intercalated DNA since the latter is to be necessarily treated in terms of "dinucleotide" repeats because of neighbour exclusion effect[13]. An analysis of poly (dinucleotide) helices, with appropriate intercalated geometry for the repeating dinucleotide moieties would aid in delineating the preferred conformations of intercalated DNA. Although X ray[14] and NMR[15] studies have provided important clues to the stereochemical specificity of intercalation, information relating to stereochemistry at the non-intercalating site are lacking. X-ray analysis of at least a deoxytetranucleotide-drug complex

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is necessary to conclusively establish the near neighbour exclusion principle and fill the stereochemical gap (see also Ref. 16). While our studies include "dinucleotide" repeats with and without sugar pucker changes, we present here our preliminary studies obtained with mixed sugar pucker combination. The results clearly demonstrate the stereochemical feasibility of regular polynucleotide helices with mixed sugar puckers and point out some interesting differences compared to conformationally homogeneous, polynucleotide backbone helices studied thus far. The observations also provide interesting clues to the possible conformational pathways during conformational transitions and dynamics of DNA in solution.

Structural Models

A polynucleotide backbone comprising alternate C3′ *endo*-C2′ *endo* (3_E–2_E) or C2′ *endo*- C3′ *endo* (2_E–3_E) sugar puckers can be conformationally represented as poly (3_E–2_E) or poly (2_E–3_E) in which only every other nucleotide residue is conformationally equivalent. For example the polynucleotide backbone poly *d*(A–T). Poly *d*(A–T) could be represented in terms of conformational repeats as Poly *d*(3_E–2_E). Poly *d*(3_E–2_E). The ordered helical conformation of such a polynucleotide chain can be treated[16] as a sequence of "extended single virtual bonds" which span the successive repeating dinucleotide triphosphate residues (Fig. 1). The length of these virtual bonds nucleotides. In the present calculations (ω', ω) have been assigned the values of (290°, 290°) since they are intrinsically the most favoured besides being the only conformation that would lead to "stacking" between adjacent bases. The structural parameters and the other backbone nucleotide torsions adopted are the same as used earlier[5]. Adoption of the above virtual bond scheme not only simplifies the analyses of helical conformation of poly (dinucleotide) chains but provides a conceptual basis for understanding and interpreting nucleic acid helical structures. The helical parameters, namely, *n* the number of repeating dinucleotides turn and *h*, the residue height along the helical axis have been computed as a function of ω'_v and ω_v which link the successive repeating units using the procedures reported earlier[5,6]. Using lab quip molecular modelling kit, several helical models were built to check for the conformity of stereochemical aspects which govern nucleic acid helices.

Results and Discussion

The helical parameters computed for a poly (dinucleotide) chain comprising alternate (3_E–2_E) sugar puckers are shown in Fig. 2. Curves of constant *n*-values (dotted lines) are super-imposed on curves of constant *h*-values (continuous lines) and the points of intersection define possible helical structures with the corresponding n_h geometry. The most interesting observation in the plot is the occurrence of the helix

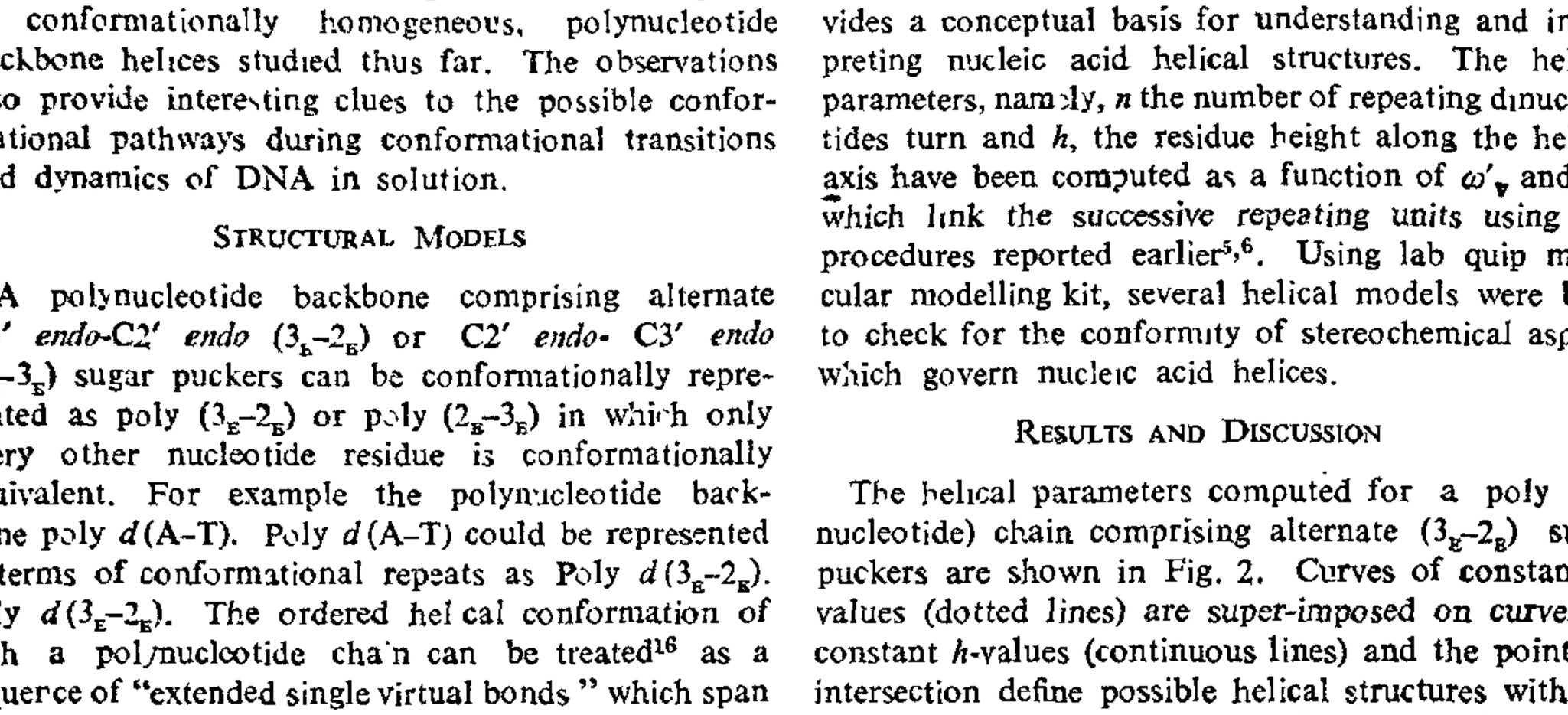

FIG. 1. An "extended virtual bond" (——) scheme in a poly (dinucleotide) chain comprising dinucleotides as repeating units. The repeating units have alternate C3′ endo (3_E) and C2′ endo (2_E) sugar puckers. (ω', ω) and (ω'_v, ω_v) represent the phosphodiester conformation *within* and at the *junction* of the repeating units. l_{vi} and l_{vi+1} represent the virtual bonds. Pu and Py represent purine and pyrimidine base respectively.

are constant for a given nucleotide geometry (ϕ, ψ and ϕ', ψ') and the phosphodiester conformation (ω', ω) which bridges the two conformationally heterogeneous forming domain at (ω'_v, ω_v) (180°, 300°) tg^- region in sharp contrast to g^-g^- (300°, 300°) found for poly (C3′-endo) nucleotides (A-DNA type) but somewhat

similar to those obtained for C2′ endo polynucleotides (B-DNA type). This means that the phosphodiester P–O3′ (ω_v') torsion at the junction of the repeating dinucleotide units exhibits a conformational change (180°) compared to that occurring within the repeating unit (300°). This phosphodiester conformation (tg^-) has not been found to be part of any di-, oligo or polynucleotide helical structures. It would seem that this distinction in the conformation of the phosphodiesters at the *junction* (tg^-) and *within* the repeating units (g^-g^-), correlated to the sugar puckers and their sequence may represent an important backbone structural modification of nucleic acid helical structures. Considering the well-known correlation that C3′ *endo* sugars favour lower *anti* (χ) values and C2′ *endo* sugars favour higher *anti* (χ) values, it would seem that poly (dinucleotide) helices with dinucleotide repeats (3_E–2_E) concomitant with the tg^- phosphodiester may represent a new type of conformational structure of nucleic acids and polynucleotides especially those comprising alternating base sequences. Also they may find relevance as an important intermediate conformation when DNA is undergoing dynamical conformational changes or helix ⇄ helix transitions

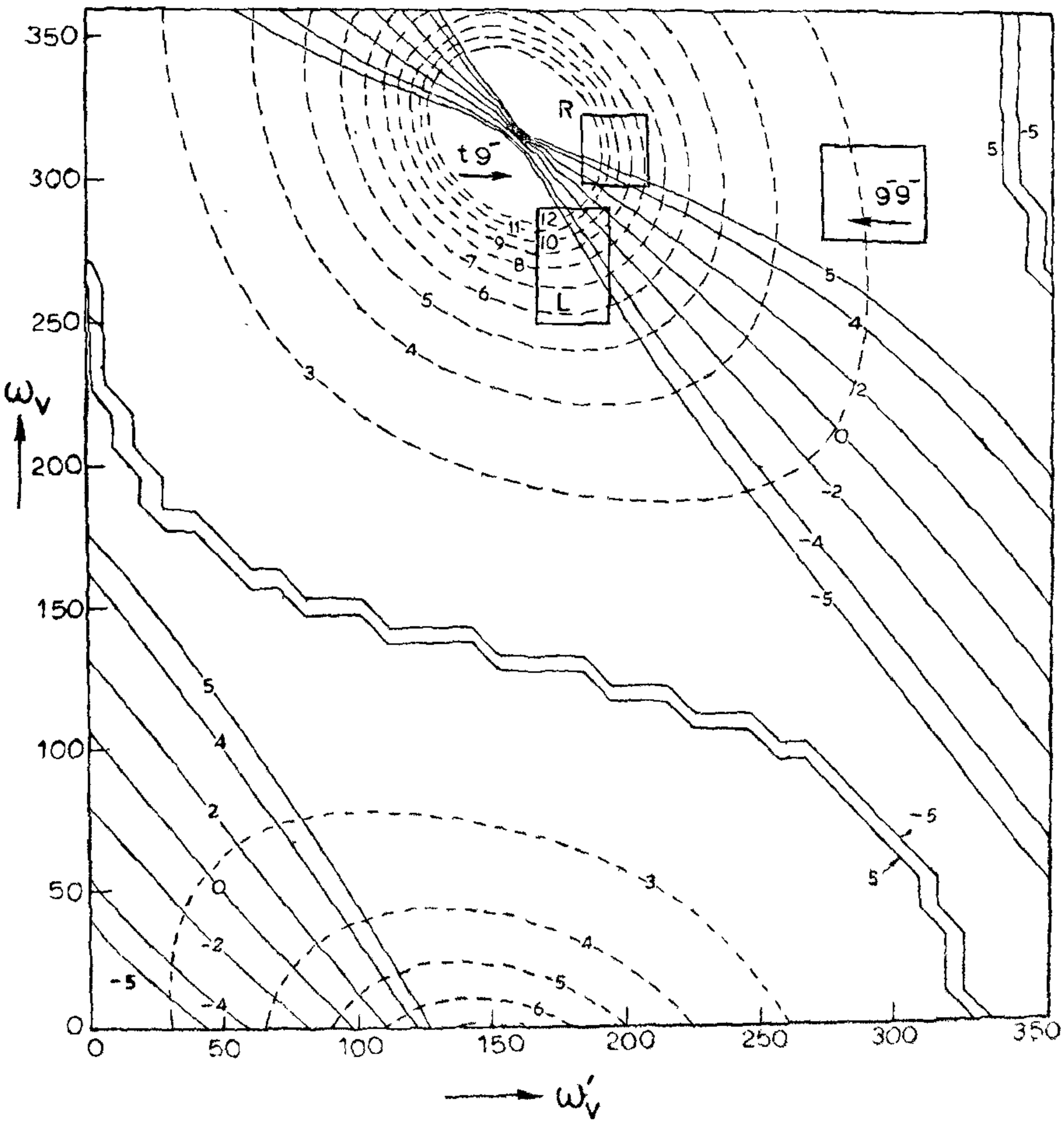

FIG. 2. Curves of iso n (— — — —) and iso h (— —) for poly (dinucleotide) helices having alternate C3′ endo —— C(2′) endo conformations for the sugars. The helix forming domain is confined to tg^- domain. Note that both left (L) and right (R) handed helices are possible.

Interestingly the tg^- phosphodiester conformation is found to be a less favored conformation compared to the stacked g^-g^- conformation by an energy approximately equivalent to stacking interactions. Nevertheless this conformation is found in certain dinucleosides like UpA[17, 18], dpTpT[19], dpTpAp (of dpApTp ApT)[20,21] and $pC_{48}pm^5C_{49}$ (of yeast $tRNA^{Phe}$)[2–4] wherein the 3′-base is always a pyrimidine or both the 3′- and 5′-bases are pyrimidines. Stacking interactions in such situations are known to be significantly weaker which might have induced the phosphodiester to adopt the extended tg^- conformation. Occurrence of a C2′ endo for 3′-sugar pucker would further promote the tg^- phosphodiester (destabilising g^-g^- in the case of ribodimers[22]) and reduce the energy barrier for $g^-g^- \rightleftarrows tg^-$ interconversions.

Molecular modelling have shown that double helical poly (dinucleotide) structures could, indeed, be built with alternating C3′ *endo* and C2′ *endo* nucleotide units satisfying the Watson–Crick base pairing scheme as well as "stacking" interactions between the base pairs at the *junction* as well as *within* the repeating units in conformity with the above theoretical deductions. Figure 3 shows a lab quip model of a typical right handed double helical segment comprising repeating dinucleotide moieties. The base separation and the tilt *within* the helix repeat and at the *junction* are very sensitive to backbone nucleotide torsions and also orientation of the bases (χ). Several models having for example larger base separation *between* the repeating units and smaller base separation *within* the repeating unit and *vice-versa* or nearly same separation within and between the repeating units could be obtained by appropriate adjustments in the torsion angles. The average base separations are in the range of 3 to 6 Å and base tilts $< 30°$ depending on the model. Detailed calculations are in progress to ascertain the energetic preference of various models. The interesting feature is that in all these models the phosphodiester possesses the tg^- conformation. These and the concomitant topological changes of the helix may serve as possible recognition sites to proteins. It is possible that short segments of such helices may occur at specific base sequence regions of DNA and may coexist with the B–DNA conformation possessed by the rest of the molecule. Similarly it is conceivable that the above helical structure with the alternate sugar puckers with a tg^- phosphodiester may represent an important intermediate conformation during $A \rightleftarrows B$ cooperative helical transitions since the DNA with mixed sugar puckers possesses some features of *A* as well as *B* forms of DNA. In solution, at least some DNAs, may exhibit a dynamic equilibrium between a DNA of "homogeneous" and "heterogeneous" conformational repeats.

While only right handed structures are discussed, it is clear from (*n–h*) plot (Fig. 2) that left-handed poly

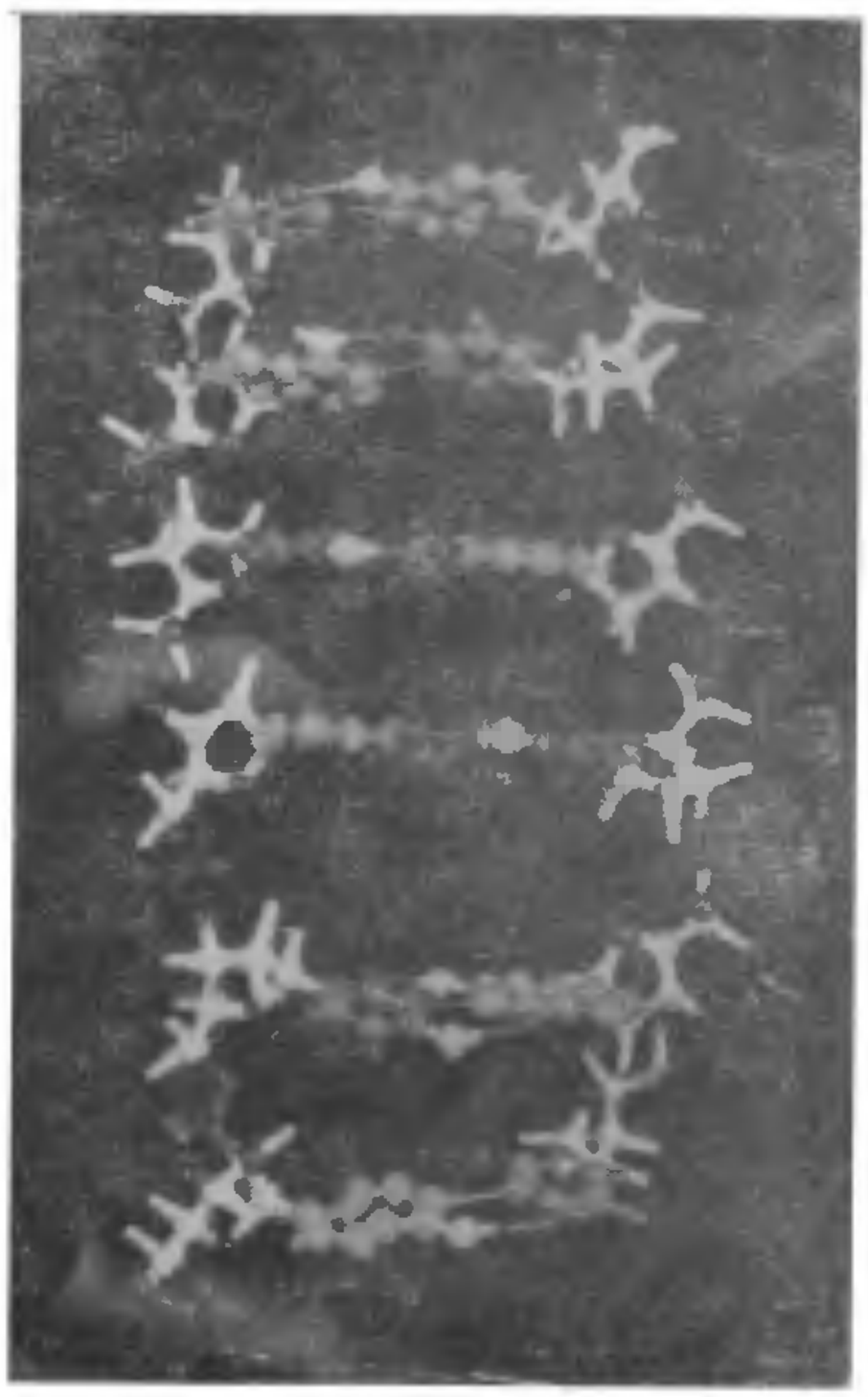

FIG. 3. A molecular model of a right handed poly (dinucleotide) double helix comprising C3′ endo and C2′ endo nucleotides and the tg^- phosphodiesters for (ω'_v, ω_v) and g^-g^- for (ω', ω) (see Fig. 2).

(dinucleotides) as well are possible with only minor torsional variations around the P–O bonds. This unique feature has been recently interpreted[24] as a necessity in nucleic acids rather than a coincidence in view of the left handed tertiary supercoiling of the right handed DNA secondary structure.

CONCLUSIONS

Poly (dinucleotide) helices with mixed sugar puckers in the repeating dinucleotide residues are stereochemically feasible and are found to have several similar and dissimilar features compared to the classical A and B DNA helices. These could as well arise as induced effects due to interaction of DNA with molecules like proteins. Such helical conformations are not energetically improbable. Indirect experimental support for such models can be seen in the crystal of dpApTpApT, a deoxy tetranucleotide tetraphosphate structure[20,21] wherein the sugars in successive residues exhibit 3_E and 2_E conformation to be regarded as a dimer of $(3_E–2_E)$. $(3_E–2_E)$. Most interestingly the phosphodiester conformation linking the dinucleotide

repeat exhibits the *tg*⁻ conformation similar to discussed above although the molecule does not form a duplex structure in the crystal. However, based on the crystal structure, the possibility of using alternate sugar puckers for double helical structure of poly (dA–dT) and DNA–protein recognition has also been suggested[20,21]. Experimental evidence for mixed sugar also comes from the crystal structures of several intercalated dinucleoside monophosphates[14]. Mixed sugar puckers have earlier been used to generate kinked superhelical DNA structures[14]. The concept of poly (dinucleotide) helices in which the nucleotide residues are conformationally heterogeneous deals with regular helical structures within the frame work of Watson–Crick hydrogen bonding scheme but with modifications in the sugar-phosphate-sugar backbone geometry. This offers an important probe for understanding the base sequence dependent backbone structural modifications of nucleic acids which may be relevant in understanding molecular aspects of nucleic acids-protein recognition and interactions. It has come to the attention of authors recently that models comprising alternate sugar puckers have also been proposed based on nmr[25] and X-ray data[26] for polynucleotides possessing alternate purines and pyrimidines supporting our theoretical deductions. Helical structures and their properties obtained for other combinations of sugar pucker, base orientations and backbone torsions are the subject of future communications.

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