DNA FLEXIBILITY AND TERTIARY FOLDING. A MODEL FOR SUPERFOLDING OF DNA IN CHROMATIN*

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ABSTRACT

A stereochemical model for superhelical structure of DNA in chromatin is proposed by postulating that chromatin DNA may be regarded as a polymer chain comprising "10 base-paired B-DNA" as repeating units and that the tertiary folding of DNA may be achieved by the flexibility afforded by the P–O ester bonds which link the successive secondary structural segments. The results show that 70 to 100 base-pairs can be accommodated per superhelical turn with a pitch of 28 A and radii varying from 30–50 A. Such smoothly kinked superhelices having more than 100 base-pairs/turn are found to have radii greater than 70A and are not compatible with the known nucleosome dimensions. An interesting feature of this model is its better ability to account for the 10 base-pair repeat indicated by nuclease digestion studies.

INTRODUCTION

THE accumulating evidence that in nucleosomes DNA wraps around the histone core in some kind of a superhelical structure and that DNA essentially conserves its secondary structural B-form have marked a major conceptual advancement in understanding the supermolecular organisation of proteins and nucleic acids and their interactions at the molecular level[1–3]. A stereochemical description of the nature of the possible conformational modifications that may be manifested in the tertiary folding of DNA in chromatin would further enable to visualise the topological arrangement of DNA and histones and furnish the important information concerning the nature of flexibility in DNA molecules and their interaction with proteins. Crick and Klug[4] have proposed a super helical structure for chromatin DNA by introducing sharp bends or kinks at the junctions of 20 base-paired DNA secondary structural units by changing the conformation around the C4′—C5′ bond of the super form the preferred *gauche*$^+$ (60°) to the less favoured, but stereochemically feasible, *trans* (180°) conformation together with the change in the phosphodiester (ω', ω)[5] from the g^-g^- (300°, 300°) to the g^-t (300°, 180°) conformation. Such regularly placed kinks lead to a left handed superhelix with an unstacking of a base-pair at every kink. Sobell *et al.*[6] have introduced kinks similarly by altering the sugar ring conformation from the preferred C(2′) *endo* to the mixed super pucker sequence C3′ *endo*)–C2′ *endo* (3_g–2_g), at the successive junctions of 20 base-pairs. The resulting superhelix also has the left handed screw sense with the helical parameters $n = 9$ ($t = -41{\cdot}1°$) and $h = 5{\cdot}26$ A. Although both these models conform to the molecular dimensions of nucleosome of about 50 A radius[3,7], they involve abrupt conformational changes in the nucleotide backbone C—C bonds (C4′—C3′ or C4′—C5′) from one domain to another at the kinks.

We propose here a possible model for superhelical DNA in chromatin by postulating that chromatin DNA may be regarded as a polymer chain comprising "10 base-paired B-DNA" as the repeating monomer units and that the tertiary folding of DNA in chromatin may be achieved by smooth conformational changes in the ester P—O bonds which link the successive "10 base-paired" secondary structural units. This necessarily implies that the ester P—O bonds torsion (ω'_v, ω_v) at the *junctions* should be different from the P—O torsions (ω', ω) occurring *within* the repeating unit. This distinct dissimilarity, however small it may be, between the P—O torsions at the junctions of the repeating B–DNA units and those within them is sufficient to introduce smooth bends which will facilitate DNA to superfold into a compact structure about a superhelical axis which is different from the local helical axes associated with individual B–DNA secondary structural segments. Adoption of P—O torsions at the junctions identical to those occurring within would result in a rod-like structure for chromatin DNA of 140–200 base-pair length. Our model while preserving the symmetry of the B–DNA structure introduces smooth 'kinks' at intervals of $10n$ phosphodiester (base-pairs) and does not call for any abrupt conformational changes. With this hypothesis we have computed the superhelical parameters (n, the number of repeating B-DNA units per turn and h, the height per residue along the superhelical axis) by using a scheme of "extended single virtual bonds" to investigate the possible superhelical structures for chromatin DNA. The results of the analysis reveal, that both left-handed and right-handed superhelical structures conforming to the dimensions of

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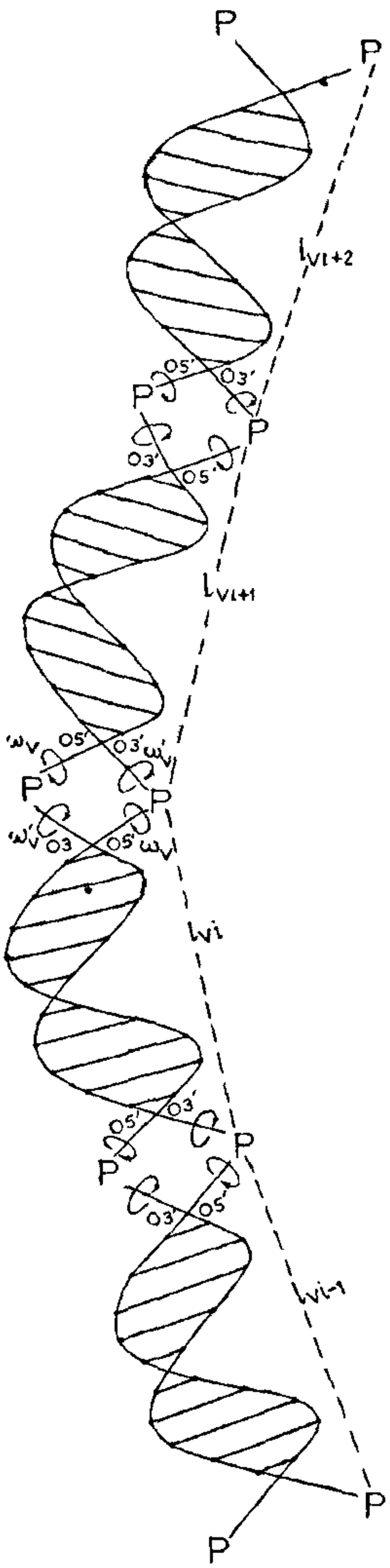

FIG. 1. A section of a poly (DNA) chain in chromatin comprising of 10 base-paired B–DNA secondary structural fragments as the repeating unit. The "extended virtual bonds" shown by dotted lines are formed by joining the phosphorous atoms which link the successive repeating B–DNA secondary structural segments. The rotations around the P–03′ (ω'_v) and P–05′ (ω_v) ester bonds which are flanked on either side by B–DNA repeating units are postulated to produce superfolding of DNA in chromatin and their values are different from (ω', ω) occurring within the repeating secondary structural units.

nucleosowes can be obtained by relatively minor rotations about the P—O bond torsions linking the "10 base-paired B–DNA" secondary structural segments.

METHOD

According to this scheme DNA in chromatin is regarded as made up of the repeating units comprising one full turn of B–DNA (10 base-paired structure) which are linked at the successive phosphodiester as shown in Fig. 1. This permits the description of the conformation of chromatin DNA in terms of what we refer to as the "extended single virtual bonds" which link the successive phosphorous atoms (Fig. 1) of the repeating rigid B-DNA secondary structure. These "extended virtual bonds" are oriented along the local helical axes of the DNA repeating units with a magnitude (l_v) equal to the pitch (34 A) of the B–DNA helix which is unaffected by the P—O torsions at the hinges connecting the secondary structural units. The relative orientations of any two extended virtual bonds are defined by a pair of rotations (ω_v, ω_v) around the ester P—O bonds which provide the necessary flexibility in the chromatin DNA. The specification of the orientation of these extended virtual bonds (l_v) at successive linkages would completely describe the spatial configuration of the chain and hence the conformational structure of chromatin DNA. Identical values for the P—O torsions at every junctions would necessarily generate a helical conformation and in such a structure the successive B—DNA repeating units become readily amenable to a simple description in terms of a rotation ($t = 360/n$) and a *translation* (h) *about an axis*.

EVALUATION OF SUPERHELICAL PARAMETERS

The above description of chromatin DNA in terms of rigid 10 base-paired B–DNA segments with the flexibility at the P—O torsions which link the successive B–DNA segments and the idea of "extended single virtual bond" render the evaluation of superhelical parameters relatively simple and facilitates the adoption methods used earlier for polynucleotides[8,9]. The superhelical parameters n, the number of B–DNA repeating units per turn and h, the projected height of the repeating unit along the superhelical axis have been computed as a function of the P—03′ (ω'_v) and P—05′ (ω_v) torsions at the junction of two B–DNA repeating units for all the values from 0 to 360° at intervals of 10° using the B–DNA coordinates[10]. The constant value n and h curves are represented by dashed and continuous mode respectively in Fig. 2. Intersection of any two of these curves defines a possible

superhelical structure for DNA. Curves of constant value of $n < 10$ are not shown. Similarly h-curves corresponding to values of $+5$, 0, -5 and -30 alone are shown in Fig. 2.

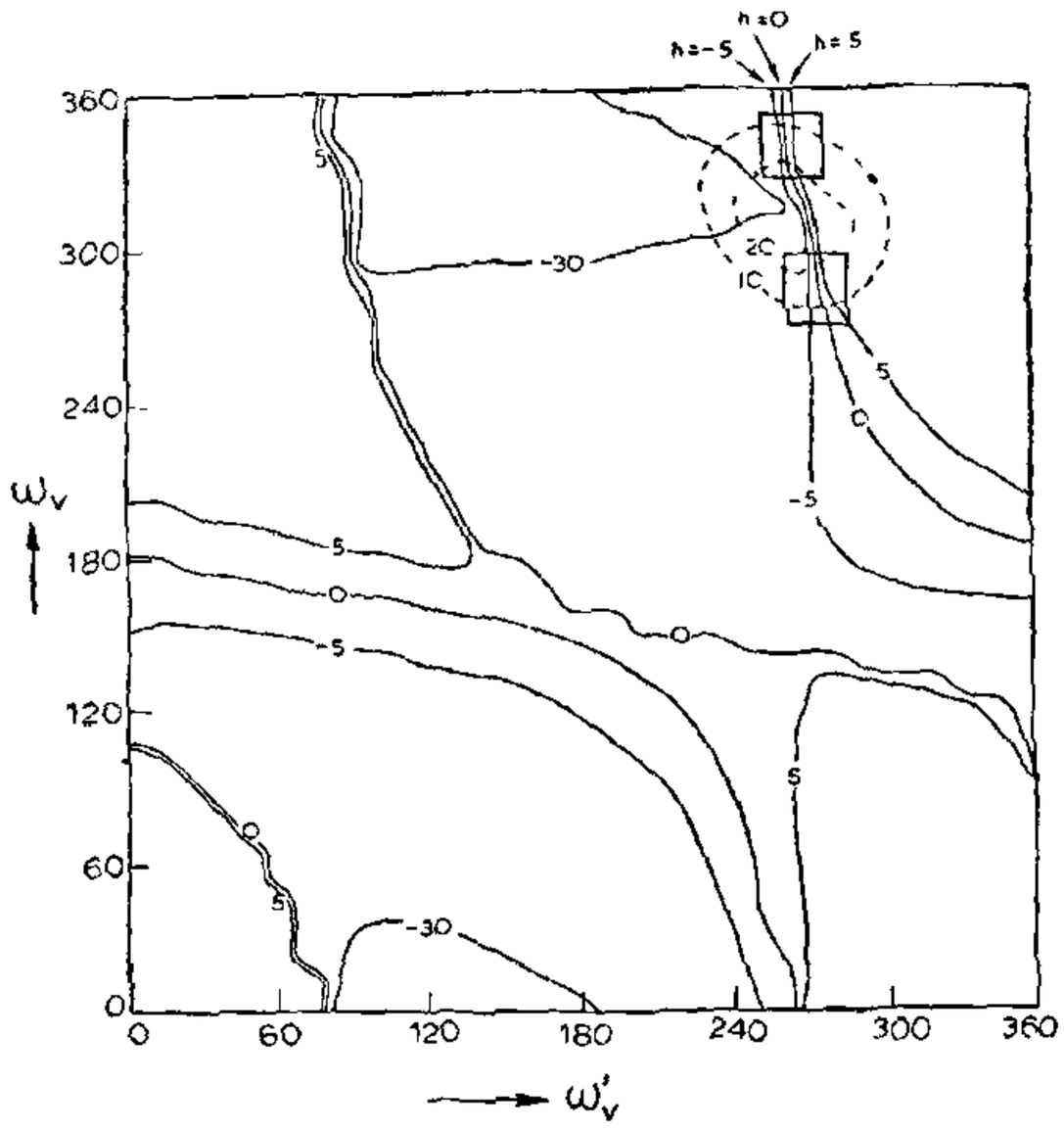

FIG. 2. Superhelical parameter (n, h) plot for poly (DNA) helices obtained as a function of the ester P—O bond rotations ω'_v and ω_v which link the repeating B-DNA units. Curves of iso n, the number of repeating units (base-pairs) per superhelical turn and curves of iso h, the residue height along the superhelical axis are shown by dashed and continuous modes respectively. Curves corresponding to $h = +5, 0, -5$ and -30 and $n = 10$ and 20 alone are shown. Adoption of (ω'_v, ω_v) values at the junctions of the repeating units corresponding to the point of intersection, for instance, $n=10$ and $h=5$, would generate a smooth superhelix having 100 base-pairs per turn.

DISCUSSION

Since the nucleosome core possesses 140 base pairs[2], values of n around 6 to 14 are of interest and only they would generate superhelices comprising around 60 to 140 base pairs per turn. It is most striking that there is only a single phosphodiester domain which satisfies this criterion around $(\omega'_v, \omega_v) \simeq (300°, 300°)$ commonly referred to as g^-g^-. This clearly demonstrates that it is possible to bend or fold the DNA double helix smoothly to generate a family of superhelices by small variations in the P—O torsions alone. Calculations have shown that the helical parameters are extremely sensitive to even minor P—O bond rotations. An interesting consequence is that such small variations in P—O rotations do not result in unstacking at the junctions but are expected to ensure nearly continuous stacking. Furthermore the g^-g^- domains is energetically the most favoured phosphodiester conformation[11] in RNA and DNA and the energy variation within the gg^- domain is uniform. Selection of appropriate phosphodiester (ω'_v, ω_v) torsions corresponding to the superhelical parameter n_h, family of superhelical structures for chromatin DNA can be generated and checked against the molecular dimensions and other criteria.

An analysis of pitch and radius of superhelices obtained as a function of P—O bond rotations ω'_v and ω_v (Fig. 1) suggest that there is only a very narrow region within the g^-g^- phosphodiester conformation domain which permit superhelices of radii between 30–50 A and a pitch between 24–30 A. Furthermore, we find there is a limiting value for n, the number of base pairs/turn which places stringent restrictions on the radius of the nucleosome core. Base pairs between 70–100 can be accommodated per superhelical turn with a pitch of 28A[7] and radii varying 30–50 A. Superhelices having more than 100 bases/turn are found to possess radii greater than 70 A which are not compatible with the known nucleosome dimensions[3,7]. Thus there is definite indication that there has to be more than one superhelical turn in the nucleosome core. Similar conclusions have been arrived at earlier from other experimental evidence[2–7]. A stereo-plot of a superhelical DNA with $n = 71$, $h = -4{\cdot}2$ and $r = 39$A is shown in Fig. 3.

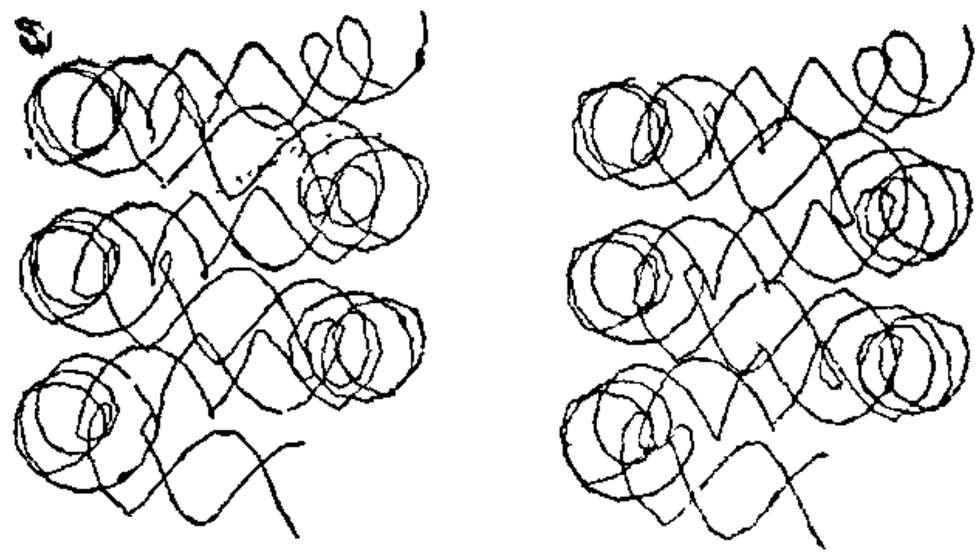

FIG. 3. Stereoplot of a left-handed superhelical model for chromatin DNA possessing $n = 7{\cdot}1$ (71 b.p.) $h = -4{\cdot}2$ and $r = 39$ A.

Table I lists some typical superhelices and their dimensions. It is interesting that in these structures only the ω_v angle is changed slightly to induce superhelical twist. In Fig. 2 the region to the left of $h = 0$ contour represents possible superhelical structures of left handed screw sense while the region of phosphodiesters to the right of $h = 0$ represents possible right handed superhelical structures. This indicates that both left handed as well as right handed superhelical structures could be generated by small changes in P—O rotations within the g^-g^- helix forming domain similar to our earlier results on polynucleotides[8,9]. This unique feature seems a necessity and provides a simple understanding of the conformational path by

which the right-handed secondary helical structure can smoothly superfold into a tertiary structure of opposite screw sense (left-handed) and unfold with only minor changes in the polynucleotide backbone. Curve corresponding to $h = 0$ itself represents helical structures having a zero pitch or in other words closed cyclic structures. This may have important bearing on the possible conformations and structures of circular DNA.

TABLE I

Dimensions of some left-handed and right-handed DNA superhelices

No. of base-pairs/turn (n)	Residue height/10 residues (h A)	Pitch A	Radius (A)
71	−4·2	29·8	39
84	−3·3	27·6	46
110	−2·5	27·8	60
157	−1·7	26·5	86
85	3·4	29·0	47
98	3·5	34·6	54
157	1·8	29·0	86

Conclusions

The result clearly brings out the important finding that DNA can as well be smoothly folded into a superhelical structure conforming to the dimensions found in chromatin[1–3,7] by relatively minor rotation changes around the P—O bonds of the phosphodiester without invoking any abrupt changes in the nucleotide geometry or the phosphodiester. A conformational change of this nature would require minimum stereochemical rearrangement and expected to involve relatively small energies for superfolding. This also implies that a change in the torsions around the other nucleotide backbone bonds or a combination of them may also be able to generate such supersecondary helical structures. Indeed models in which DNA is bent smoothly by minor torsional changes spread over 20 base-paired segments have been recently proposed[12,13]. Since the flexibility in the chromatin DNA is derived from the P—O torsions linking the repeating B-DNA units, this model preserves the local dyad symmetry and all other properties in the DNA repeating units. Most importantly, it has a better ability to account for the 10 base-pair repeat observed in partial nuclease digests of nucleosomes[1,2]. Further the model does not call for unstacking but affords more or less continuous stacking since small rotational changes in the P—O rotation required to induce superfolding do not significantly result in the separation of the bases and their overlap. Although DNA with 10 base-pair unit is chosen as a repeating unit, multiples or sub-multiples of this unit (*e.g.*, 20 base-pair unit or 5 base-pair unit) can also be used as the repeating unit. Similarly, the repeating DNA unit having slightly different geometry within the B-family or A-DNA geometry can also be used if necessary. Calculations are in progress to work out the energetic consequences of these models. Ideas similar to this have also been advanced by Olson[14].

Our model may be regarded as an intermediate between the two possible modes of kinking[1,4] in that the folding here is not achieved by either abrupt changes periodically in the structure or by a continuous distortion distributed throughout the sugar-phosphate backbone but by smooth changes distributed periodically and without any strain along the sugar-phosphate backbone. An interesting stereochemical outcome of this study is that the conformational structures of even superhelical nucleic acids can be stereochemically characterised using the concept of nucleotide rigidity[15].

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