is a competitive inhibitor. Furthermore, the internucleotide phosphorothiate bond formed always has the *R* configuration. It seems likely that the inversion of configuration is the result of a simple in-line mechanism where in the transition state the incoming 3′-hydroxy function as well as the leaving phosphate or pyrophosphate group occupies the apical positions of a trigonal bipyramid (Eckstein et al., 1976; Burgers & Eckstein, 1978).

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References

Bollum, F. J. (1966) *Proc. Nucl. Acids Res. 1*, 296–300.
Burgers, P. M. J., & Eckstein, F. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4798–4800.
Eckstein, F., & Goody, R. S. (1976) *Biochemistry 15*, 1685–1691.
Eckstein, F., Armstrong, V. W., & Sternbach, H. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2987–2990.
Eckstein, F., Sternbach, H., & von der Haar, F. (1977) *Biochemistry 16*, 3429–3432.
Gindl, H., & Eckstein, F. (1970) *Eur. J. Biochem. 13*, 558–564.
Godefroy, T., Cohn, M., & Grunberg-Manago, M. (1970) *Eur. J. Biochem. 12*, 236–249.
Lienhard, G. E., & Secemski, I. I. (1973) *J. Biol. Chem. 248*, 1121–1123.
Saenger, W., Suck, D., & Eckstein, F. (1974) *Eur. J. Biochem. 46*, 559–567.
Usher, D., Erenrich, E. S., & Eckstein, F. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 115–118.

Noninteger Pitch and Nuclease Sensitivity of Chromatin DNA†

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ABSTRACT: Assuming that variation of nuclease sensitivity along nucleosomal DNA can basically be attributed to orientations of sugar–phosphate bonds relative to histone core, the pitch of chromatin DNA is estimated to be 10.33–10.40 base pairs. This is in accordance both with the known measured average distance between cleavage sites (10.3–10.4 base pairs) and with published data on variation of relative sensitivities of these sites to nuclease attack. The variation can be explained solely as a result of the systematic change of orientation of sugar–phosphate bonds of sensitive sites without additional suggestions about local steric hindrances by histone molecules. According to the analysis locations of sites least sensitive to nuclease attack should not depend on kind of endonuclease though the stagger could differ. We conclude that the nucleosome core particle is axially symmetrical. The results strongly support the suggestion that DNA is wrapped around the histone octamer smoothly, without interruption of base-stacking interactions.

An important structural feature of chromatin is the limited sensitivity of its DNA to nuclease digestion which is periodically varying along the DNA with a period equal or close to ten base pairs (Noll, 1974, 1977). One suggested explanation of the phenomenon is that the periodical distribution of sensitive sites along the chromatin DNA results from periodical variation of exposure of bonds to the surroundings due to the helical structure of chromatin DNA (Noll, 1974, 1977). According to the nuclease digestion data and to low angle neutron scattering data (Pardon et al., 1975), DNA in the nucleosome is folded around the histone core so that only the outside surface of DNA is accessible to nucleases. The regularity of digestion has led to the suggestion that the folding of DNA is smooth, without disruption of base stacking interactions (Noll, 1974, 1977). The possibility of such smooth folding of DNA was demonstrated recently both from energetical and stereochemical points of view (Sussman & Trifonov, 1978; Levitt, 1978) and supported experimentally (Kallenbach et al., 1978).

An alternative explanation of the periodical variation of nuclease sensitivity of chromatin DNA is a kinked helix (Crick & Klug, 1975) with kinks repeating every ten base pairs.

Recent measurements of lengths of nuclease digestion fragments of nucleosomal DNA using sequenced standards led to the conclusion that the average distance expressed in base pairs between adjacent sensitive sites in chromatin DNA is noninteger: 10.3–10.4 (Prunell et al., 1979). This finding is also supported by recent accurate length determinations of nucleosomal DNA (Tatchell & Van Holde, 1978; Seligy & Poon, 1978) which is found to be 145 rather than 140 base pairs. It leads to the same figure as above.

If the nuclease sensitivity of nucleosomal DNA is determined basically by orientation of base pairs relative to the histone core, then this noninteger number of base pairs should correspond to the pitch of chromatin DNA. An important consequence of nonintegerness of the pitch of DNA is variation of orientations of sugar–phosphate bonds to be disrupted. It should lead to a kind of beating effect, i.e., modulation of periodical sensitivity of nucleosomal DNA. Such modulation was demonstrated indeed by analysis of fragments of 5′-^{32}P-labeled nucleosomal DNA, obtained by nuclease digestion of nucleosomes. Endonucleases tested were of different structure, active in different optimal conditions: DNase I (Simpson & Whitlock, 1976; Whitlock et al., 1977; Noll, 1977; Simpson, 1978), endonuclease of *Aspergillus oryzae*, and DNase II (Whitlock et al., 1977). It was found that sites of nucleosomal DNA positioned at about 30, 110, and, less pronounced, 60 and 80 base pairs from the ends of the DNA are less sensitive to nuclease digestion than, correspondingly, at other multiples of 10 or so base pairs. The variation (modulation) of the sensitivity was ascribed to protection provided by local steric hindrances by histones (Whitlock et al., 1977; Finch et al., 1977).

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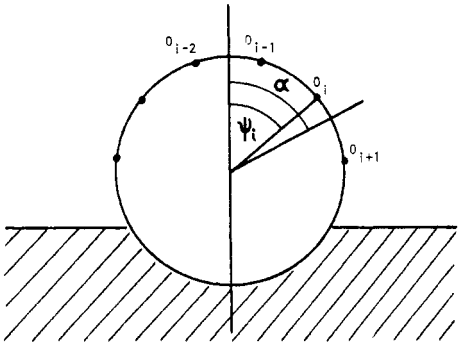

FIGURE 1: Scheme of surface of nucleosome. DNA, its axis being perpendicular to the plane of drawing, is partially buried in histone core of nucleosome (shadowed). (Dots) Consecutive 3′ oxygens of one DNA strand projected into plane of drawing. (ψ) Orientation of the 3′ oxygen relative to perpendicular to histone core surface. (α) Angle of best accessibility for nuclease attack.

As follows from the analysis presented below, the modulation can be explained basically by a beating effect caused by nonintegerness of pitch of chromatin DNA. The pitch is estimated to be between 10.33 and 10.40 base pairs, which coincides with direct lengths measurement as cited above.

Results

Consider nucleosomal DNA as being helically smoothly wrapped around histone core leaving only the outside surface of DNA accessible to nuclease attack. Suppose also that the sugar–phosphate bond is most attackable if the local radius-vector from DNA axis to the 3′ oxygen makes some angle α with the perpendicular to the local surface of the histone core (Figure 1). The angle α does not have to be necessarily zero. It is a function of molecular shape of the nuclease and of relative positions of the DNA-binding site and the active site of the enzyme. As it follows from data on stagger between nuclease cuts in opposite strands of nucleosomal DNA these two functional sites of nuclease might be different (Sollner-Webb & Felsenfeld, 1977).

If the pitch of DNA is integer, say, 10 base pairs, then obviously each 10th 3′ oxygen of the bond to be disrupted will be in the same orientation as the zeroth one. As a result all nuclease-sensitive sites have the same sensitivity. In the case of noninteger pitch $10 + a$ $(0 < a < 1)$ the orientation ψ_n of the nth 3′ oxygen is $\psi_0 + n(360°/10 + a)$, where ψ_0 is orientation of zeroth oxygen.[1] Our main assumption is therefore that the bigger the angular distance $|\psi - \alpha|$, the more "protected" is the sugar–phosphate bond. 3′ oxygens with ψ_n closest to α correspond to nuclease sensitive sites.

The diagram shown in Figure 2 presents as an example orientations $\psi_s - \alpha$ of nuclease sensitive sites for one strand of nucleosomal DNA with noninteger pitch 10.375 base pairs. Each numbered point of the diagram corresponds to orientations $\psi_s - \alpha$ of 3′ oxygens of sensitive sites. The numbers are their positions along the nucleosomal DNA (in base pairs). The middle sensitive site (oxygen number 72) is chosen as a center of axial symmetry of the strand of nucleosomal DNA; therefore, the diagram is symmetrical in respect to this point.

The diagram shows that, in this case, the angular distances from the most attackable orientation α are maximal for n = 31, 62, 82, and 113. These sugar–phosphate bonds are therefore orientationally most protected. Protection of this kind provided by different noninteger periods between 10 and 11 figured on the basis of similar diagrams is presented in Table I. For each interval listed in the table only four positions are indicated (crosses), corresponding to the orientationally most protected sites. It is seen from the table that, for the interval 10–11 base pairs, sites III, VI, VIII, and XI, known as least sensitive (Simpson & Whitlock, 1976; Whitlock et al., 1977; Noll, 1977; Simpson, 1978), are protected only at periods between 10.33 and 10.40 base pairs and 10.60 to 10.67 base pairs. Thus the geometrical approach to the problem formally leads to two solutions for pitch of chromatin DNA. One of them (10.33–10.40) is consistent both with observed variation of nuclease sensitivity along nucleosomal DNA and with experimentally measured average distance between sites sensitive to nucleases which equals to 10.3–10.4 base pairs (Prunell et al., 1979; Tatchell & Van Holde, 1978; Seligy & Poon, 1978).

Table I: Orientational Protection of Nucleosomal DNA

pitch of DNA (base pairs)	orientationally protected sites											
	I	II	III	IV	V	VI	VII	V-III	IX	X	XI	X-XII III
10.000–10.100	+	+										+ +
10.100–10.125		+	+								+	+
10.125–10.167			+	+						+	+	
10.167–10.222				+	+				+	+		
10.222–10.273	+				+				+			+
10.273–10.333		+			+				+			+
10.333–10.400			+			+		+			+	
10.400–10.444	+					+		+				+
10.444–10.556				+		+		+		+		
10.556–10.600	+					+		+				+
10.600–10.667			+			+		+			+	
10.667–10.727		+			+				+			+
10.727–10.778	+				+				+			+
10.778–10.833				+	+				+	+		
10.833–10.875			+	+						+	+	
10.875–10.900		+	+								+	+
10.900–11.000	+	+										+ +

Discussion

The consistence of nuclease digestion data with the simple model described strongly suggests that the measured average distance between cleavage sites (10.3–10.4 base pairs) corresponds to the pitch of chromatin DNA. It is interesting to note that determination of pitch of nonchromatin (plasmid) DNA in solution also came to the figure 10.4 ± 0.1 base pairs (Wang, 1979). Further measurements will show if the pitch of DNA in chromatin is really close to the pitch of DNA freed of histones.

The optimal angle α for digestion by DNase I can be estimated from known data on stagger between two adjacent DNase I cleavage sites in complementary strands of DNA in chromatin. The stagger is 2 nucleotides, 5′-P termini being shorter (Sollner-Webb & Felsenfeld, 1977; Noll, 1977; Lutter, 1977). If the detailed structure of DNA is known, the angle between 3′ oxygens of staggered bases of opposite strands can be taken directly from corresponding angular coordinates. Since both staggered oxygens are attacked by the nuclease, the absolute values of their orientation angles are not far from optimal angle α. Belonging to opposite strands, the orientation angles are of opposite sign. Then α is approximately one-half of the angle between staggered 3′ oxygens. The angular coordinate θ for the 3′ oxygen of a base pair of B-DNA is 96.5° (Arnott et al., 1969). For the symmetrical 3′ oxygen of the same base pair, opposite strand, θ equals −96.5°. So the angular difference between two 3′ oxygens of sugar–phosphate bonds of opposite strands, staggered by two bases, is $2 \times 96.5° - (2 + 1)36° = 85°$. Then $\alpha = 1/2 \times 85° \simeq 42°$.

[1] To restrict orientation ψ_n to the domain −180° to 180°, multiples of 360° must be subtracted.

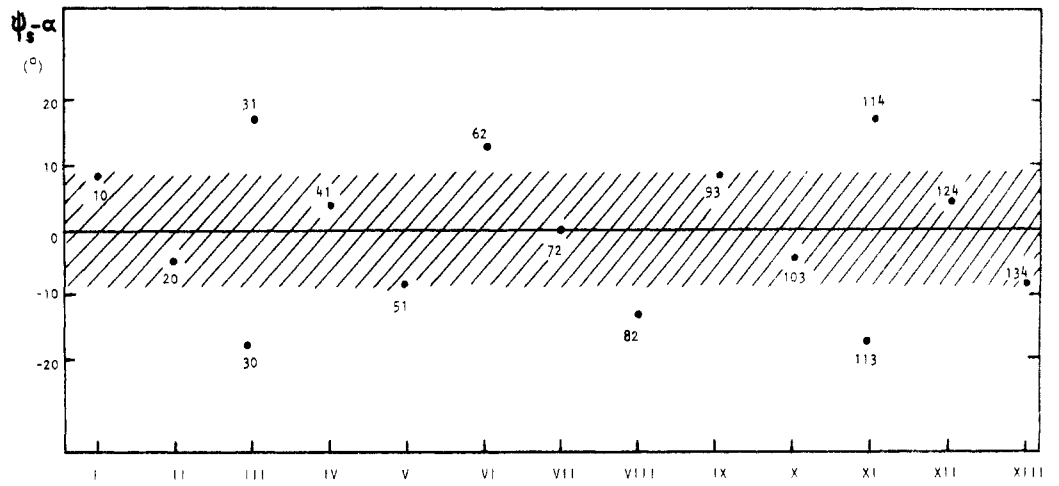

FIGURE 2: Orientations of nuclease sensitive sites of nucleosomal DNA. 3′ oxygens are numbered in arabic. Roman figures are used for numeration of nuclease sensitive sites. Region of maximal accessibility of sugar phosphate bonds is shadowed (arbitrarily) for clarity.

The angular dependence of orientational protection on deviation angle $\psi - \alpha$ from the most attackable angle α should not be exactly symmetrical since $\alpha \neq 0$ and because of asymmetrical position of the sugar–phosphate bond on the DNA strand. This asymmetry, possibly, is responsible for the difference in orientational protection of sites VI and VIII (Whitlock et al., 1977).

Although the modulation pattern (sites ~30, 60, 80, and 110 base pairs preferentially protected) can be explained basically by variation of orientations of sugar–phosphate bonds in DNA with noninteger pitch, other factors should also influence the pattern. First, since probably the structure of nucleosome is not rigid, the pitch of DNA along the molecule might vary to some extent around the average pitch 10.3–10.4 base pairs. It should give rise to some smear of the modulation pattern. On the other hand, the surface of the histone core obviously is not perfectly cylindrical and local relief of the surface may also affect the pattern.

As follows from the analysis presented, locations of sites least sensitive to nuclease attack should not depend on the kind of endonuclease. Since different enzymes possess different molecular shapes and asymmetries the relative accessibilities of the orientationally protected sites should differ as was indeed observed (Whitlock et al., 1977). For the pitch 10.33–10.40 base pairs sites III and XI should be most protected while sites VI and VIII are less pronounced (Figure 2). This is in agreement with experimental data (Simpson & Whitlock, 1976; Whitlock et al., 1977; Noll, 1977; Simpson, 1978). The optimal angle α and staggers as well do not necessarily have to be the same for different nucleases.

Since histones are attached to DNA from only one side, the nonintegerness of pitch of chromatin DNA provides a unique geometry for each histone–DNA contact. From the axial symmetry of nucleosomal DNA relative to its middle point it follows also that any unique DNA–histone binding site in the nucleosome has its symmetrically positioned equivalent. Thus nonintegerness of the pitch of chromatin DNA dictates the axial symmetry of the nucleosome and the idea about the symmetry (Weintraub et al., 1976), based originally on the double representation of four different histones in the nucleosome, gets additional strong support. The order of eight histone molecules along the nucleosomal DNA also has to be symmetrical (Trifonov, 1978).

The periodical variation of nuclease sensitivity of chromatin DNA was the only experimental evidence for proposition of a kinked model of chromatin DNA (Crick & Klug, 1975). Not only periodical variation but also modulation of nuclease sensitivity can be explained solely on the basis of orientational protection. No additional assumptions are necessary such as local protection of DNA by neighboring patches of histones (Whitlock et al., 1977; Finch et al., 1977). The presented concept strongly supports the suggestion (Noll, 1974, 1977; Sussman & Trifonov, 1978; Levitt, 1978) that DNA is wrapped around the histone core smoothly, without interruption of base-stacking interactions.

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References

Arnott, S., Dover, S., & Wonacott, A. (1969) *Acta Crystallogr., Sect. B 25*, 2192-2206.

Crick, F. H. C., & Klug, A. (1975) *Nature (London) 255*, 530–533.

Finch, J., Lutter, L., Rhodes, D., Brown, R., Rushton, B., Levitt, M., & Klug, A. (1977) *Nature (London) 269*, 29–36.

Kallenbach, N., Appleby, D., & Bradley, C. (1978) *Nature (London) 272*, 134–138.

Levitt, M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 640–644.

Lutter, L. (1977) *J. Mol. Biol. 117*, 53–71.

Noll, M. (1974) *Nucleic Acids Res. 1*, 1573–1578.

Noll, M. (1977) *J. Mol. Biol. 116*, 49–71.

Pardon, J., Worcester, D., Wooley, J., Tatchell, K., Van Holde, K., & Richards, B. (1975) *Nucleic Acids Res. 2*, 2163–2176.

Prunell, A., Kornberg, R., Lutter, L., Klug, A., Levitt, M., & Crick, F. H. C. (1979) *Science* (in press).

Seligy, V., & Poon, N. (1978) *Nucleic Acids Res. 5*, 2233–2253.

Simpson, R. (1978) *Cell 13*, 691–700.

Simpson, R., & Whitlock, J. (1976) *Cell 9*, 347–353.

Sollner-Webb, B., & Felsenfeld, G. (1977) *Cell 10*, 537–548.

Sussman, J., & Trifonov, E. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 103–107.

Tatchell, K., & Van Holde, K. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3583–3587.

Trifonov, E. (1978) *Nucleic Acids Res. 5*, 1371–1381.

Wang, J. (1979) *Cold Spring Harbor Symp. Quant. Biol.* (in press).

Weintraub, H., Worcel, A., & Alberts, B. (1976) *Cell 9*, 409–417.

Whitlock, J., Rushizky, G., & Simpson, R. (1977) *J. Biol. Chem. 252*, 3003–3011.