for high excess of Pb^{+2}, decrease in T_m values, *e.g.* 69.2°C for P = 50 (Table I), generally indicates the interaction of metal ion with bases of DNA, which destabilizes a double-helical structure [9]. A new phase transition appearing around 40°C may indicate more specific interaction of Pb^{+2} ion with DNA.

Table I
T_m values for Pb^{+2} – DNA solutions

P	T_m [°C]
0	72.4
1	75.7
10	74.6
15	74.3
	$T_{m_1}^{a)} = 37.0$
50	69.2
	$T_{m_1}^{a)} = 41.0$

a) T_{m_1} temperature of the first phase transition.

CONCLUSIONS

Pb^{+2} ions interact with DNA in two different ways, *i.e.* via phosphate chain leading to stabilization of DNA and via base donors destabilizing a double-stranded structure. These results are supported also by the recent X-ray studies on Pb^{+2} interaction with RNA which has proved the involvement of base donors in metal ion binding [11].

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PS5.58 — TH

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USING A TRANSITION METAL COMPLEX TO MAKE AN IMAGE OF THE HELICAL TWIST OF DNA

We have developed a new method for determining the helical twist along a molecule of DNA of particular sequence. In this technique, a transition metal complex produces an image of the helicity of DNA, by fragmenting a DNA molecule bound to a flat inorganic surface. This method offers a rapid way of surveying the structure of any DNA molecule of moderate size, up to a few hundred base pairs in length. Such studies can be used to determine common motifs in the three-dimensional conformation of DNA that are not apparent at the level of simple base sequence. It is possible that regions of DNA important in biological regulation (promoters, for example) exist in conformations that have different helical twists from those of neighboring sequences [1], and could thus be recognized by proteins involved in regulatory processes.

RESULTS AND DISCUSSION

Our experiment is based on the determination by Rhodes and Klug of the helicity of DNA in solution, in which the enzyme deoxyribonuclease I (DNase I) was used to cut random sequence nucleosomal DNA bound to an inorganic surface (crystalline calcium phosphate, for example) [2]. The idea behind this experiment is that only certain bonds in the phosphodiester backbone are accessible to the nuclease; the backbone bonds near the inorganic surface are shielded from the enzyme, and are thus cut with much lower frequency

than the bonds exposed to the solvent. This modulation of enzyme cutting frequency results in a modulation of the band intensities in a gel electrophoretic separation of the reaction products. Such gels are capable of separating DNA molecules different in length by only one base, so all products are visualized. The periodicity of the sinusoidal modulation of cutting frequency (most easily visualized in a densitometer tracing of the autoradiograph of the electrophoresis gel) is directly related to the number of base pairs per turn of the DNA molecule. Rhodes and Klug found by their method that the helical twist of random sequence DNA is 10.6 base pairs per turn.

This method can't be used to map helicity along restriction fragments of particular sequence, though, because of the sequence specificity of DNase I. The many phosphodiester bonds of a particular DNA molecule are each cut to a different extent by DNase I, resulting in a highly non-uniform digestion pattern. We have found that the hydroxyl radical, produced by $Fe(EDTA)^{2-}$ and hydrogen peroxide, is well suited as a DNA cutting reagent in such an experiment. Hydroxyl radical degrades DNA with no regard to sequence, by attacking the sugars of the DNA backbone and thereby breaking the chain [3].

In order to develop our method, we repeated the Rhodes and Klug experiment, using random sequence nucleosomal DNA bound to calcium phosphate crystals, and compared as cutting reagents DNase I and ferrous EDTA. Both reagents gave a sinusoidal modulation pattern of cutting, and both gave a helical periodicity of around 10.5 base pairs per turn. Fig. 1 shows a densitometer tracing of two of the lanes of an autoradiograph of the electrophoresis gel from this experiment, showing the comparison between the two cutting reagents. The iron reagent gives a pattern with shallower peaks and valleys than the DNase I pattern, undoubtedly due to the smaller size of hydroxyl radical. We found that it was critical to use a negatively-charged iron complex in this experiment; ferrous ion alone will fragment DNA bound to a surface, but with no modulation pattern. We think that positively-charged iron complexes can electrostatically associate with DNA, allowing hydroxyl radical production close enough to the DNA that even backbone bonds near the surface can be cut. In contrast, the negatively-charged $Fe(EDTA)^{2-}$ complex can't associate with the DNA, and the hydroxyl radical therefore can react only with the most sterically-accessible sites on the DNA molecule before the radical is quenched.

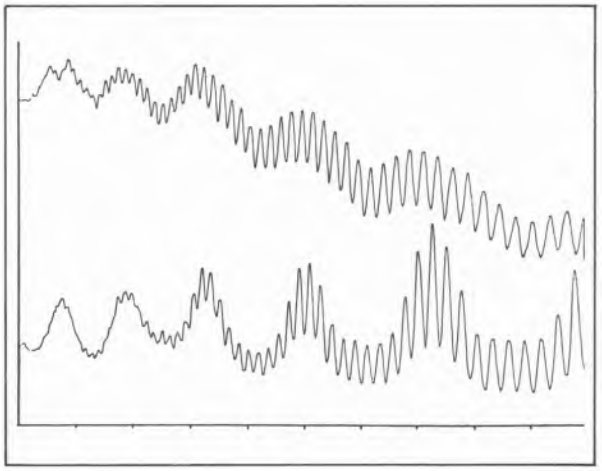

Fig. 1

Densitometer traces of two lanes of an autoradiograph of an electrophoresis gel, showing modulation patterns produced by $Fe(EDTA)^{2-}$ (top) and DNase I (bottom) fragmentation of radioactively labeled nucleosomal DNA bound to a calcium phosphate precipitate

A key feature of our method is the lack of interaction of the cutting reagent with the DNA molecule. During our measurement of helical twist we wish to perturb the DNA structure as little as possible. Several other workers also have developed transition metal-based DNA cutting reagents [3-5], but their complexes each consist of a DNA intercalating group in close conjunction to a transition metal. These reagents, which depend for their utility on binding to DNA by intercalation, would not be suitable for our purpose, because the reagent itself would modify DNA structure upon binding.

Because of the uniformity of DNA backbone cutting by hydroxyl radical produced by $Fe(EDTA)^{2-}$, our method can be used to determine the helical twist along DNA restriction fragments of particular sequence. Preliminary experiments have shown that we can indeed obtain smooth helical modulation patterns for such DNA molecules. These results will be reported in a subsequent publication.

EXPERIMENTAL SECTION

The reaction mixtures initially consisted of 2 μL of carrier DNA (1 μg), 3 μL of nucleosomal DNA (146 base pairs in length) labeled at the 5' ends with radioactive phosphorous, and 65 μL of a suspension of calcium phosphate precipitate. The calcium phosphate precipitate was prepared by mixing solutions of calcium chloride and potassium phosphate, in a Tris-chloride buffer of pH 8.0, to final concentrations of 26 mM phosphate and 16 mM calcium. The DNA was allowed to adsorb to the calcium phosphate precipitate for 1 hour; control experiments show that this is sufficient time for all DNA to be bound. To the mixture was then added the cutting reagent: either 0.2 units of DNase I, or 20 μL of 25 mM ferrous ammonium sulfate, 50 mM EDTA. To the iron-containing mixtures was then added 10 μL of 0.3% H_2O_2 to initiate the reaction. The reaction mixtures were incubated at 37°C for the appropriate times, quenched by addition of excess EDTA and thiourea (a hydroxyl radical scavenger), ethanol precipitated, dissolved in formamide-dye mixture, denatured, and electrophoresed.

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PS5.59 — MO

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SOLVENT-INFLUENCE ON THE INTRAMOLECULAR EQUILIBRIA OCCURRING IN COMPLEXES OF ADENOSINE 5'-TRIPHOSPHATE

Metal ion complexes of nucleotides are substrates for many enzymic reactions. In fact, nucleotides, especially adenosine 5'-triphosphate (ATP^{4-}), are quite versatile ligands [1-3]. Part of this versatility is connected with the occurrence of intramolecular equilibria in their binary and ternary complexes. To learn how the position of these equilibria is influenced by the polarity of the solvent, we have studied the effect of dioxane on the stability and structure of the complexes formed in the Cu^{2+}/1,10-phenanthroline (Phen)/ATP system. Such knowledge is important, because enzymic reactions usually take place in active-site cavities of proteins, and there is evidence [4] that the polarity in these cavities is decreased compared with the polarity of aqueous solutions.

The following results and conclusions are based on potentiometric pH titrations carried out in water and in 30% or 50% (v/v) dioxane-water mixtures (I = 0.1, $NaClO_4$; 25°C) [5]. For the eva-