The Interaction of Fe(III) and Fe(II) with Deoxyribonucleic Acid

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THE INTERACTION OF Fe(III) AND Fe(II) WITH DEOXYRIBONUCLEIC ACID

by

WILLIAM WARREN MARTZ

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF LOYOLA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

JUNE 1969

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LIFE

William Warren Martz was born March 15, 1940 in Chicago, Illinois. He was graduated from Bremen Community High School, Midlothian, Illinois, in June, 1958. He attended Southern Illinois University, Carbondale, Illinois from 1958 to 1961. From 1961 to 1965, the author attended Northwestern University, Chicago, Illinois, from which he received a degree of Bachelor of Philosophy in August, 1965 with a major in Chemistry.

In September, 1966, the author began his advanced studies in the Graduate School of Loyola University, Department of Biochemistry and Biophysics.

Mr. Martz married the former Nancy Joy Campbell on August 20, 1960. Mr. and Mrs. Martz have two daughters, Jan Renae, age 7 and Heidi Lyn, age 3.
ACKNOWLEDGEMENT

The author is indebted to Dr. Stelios Aktipis for his guidance in the preparation of this thesis and the research contained herein, and to the other staff members and fellow graduate students who have contributed to the author's work by exchange of ideas.

I also wish to thank my wife Nancy Joy and my two daughters, Jan Renae and Heidi Lyn for seemingly limitless patience and understanding.
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CHAPTER I
INTRODUCTION

Nucleic acids isolated from biological sources have been found to contain metals. Magnesium, Calcium, Zinc and Copper, in addition to Iron, are present in considerable amounts in DNA and RNA isolated from various biological sources (1). Generally, these metals are strongly associated with the polynucleotides and although some can be partly removed by dialysis, others appear to be irreversibly bound. Iron, in particular, remained bound to DNA and RNA even when dialyzed against such effective iron complexing agents as EDTA and orthophenanthroline. Beef liver DNA, for example, was found to contain 330-400 microgram of iron per gram of DNA.

Several investigations on the interaction of iron ions with DNA have been recently published. Kriss and Yatsimirskii (2) studied the kinetics of the interaction of Fe(III) ions with calf thymus DNA. They found an equilibrium constant for the formation of the DNA-Fe(III) complex of $1.3 \pm 0.2 \times 10^5$. Ilina and co-workers (3) examined the same interaction by studying the effect of Fe(III) ions on the intrinsic viscosity of DNA and postulated that Fe(III) ions increase the rigidity of the polynucleotide.

Ivanov (4, 5), on the basis of melting profiles of Fe(III) and Fe(II) complexes of DNA has made the proposal that iron may be one of the controlling factors in the "unwinding" and "rewinding" of the helices of the double stranded structure of
DNA. This hypothesis postulates that iron, in the strongly bound to DNA ferric state, promotes the dissociation of DNA strands while the same metal in the weakly bound ferrous state may not interfere with the process of DNA strand "rewinding". An oxidation-reduction step could, therefore, be implicated in the control of the mechanism of DNA strand dissociation. The location of Fe(III) binding in complexes with DNA has been investigated with infrared and ultraviolet spectroscopy (6). It is indicated that the interaction of Fe(III) and DNA results in formation of a charge-transfer complex of Fe(III) with the bases. An apparent stability constant of $2 \times 10^3$ was calculated from the spectral data.

Another aspect of the role of metals interaction with nucleic acids is the recent finding that the amount of DNA-bound iron in the liver and intestinal mucosa of albino rats increased with the age of the animal. This is a possible implication that iron-DNA complexes may be involved in the aging process.

In investigations of the interactions of iron II and iron III ions with nucleic acids, a number of questions may be asked among which: At which polynucleotide site do the ions bind? What effect does the binding of metal ions have on the conformation of the macromolecule? Are these ions irreversibly bound or can they be removed by agents with which they are capable of forming strongly associated complexes.
It is generally accepted that mercury(II) and silver(I) ions bind to the purine and pyrimidine nitrogen (7, 8). Metal substitution experiments with metal complexes of RNA have shown that Fe(III) can displace as much as one-half of the maximum mercury bound to RNA (9). However, when only one-half of the maximum amount of mercury is present, only a minor amount of displacement occurs. With Fe(II), the situation is quite different. Fe(II) does not displace mercury while mercury can completely displace Fe(III). This has been interpreted as an indication that Fe(III) competes with mercury for the nitrogen of purine and pyrimidine bases while Fe(II) which does not appear to compete for these sites, may associate only with the phosphate groups. Similar interactions probably occur between iron and DNA. The results obtained from proton relaxation enhancement brought about by the presence of Fe(III) and Fe(II) in DNA (10), and the results obtained from Nuclear Magnetic Resonance (NMR) studies of iron-DNA complexes are also consistent with the proposal that Fe(III) binds primarily to the bases in DNA. The proton relaxation studies indicated that Fe(III) was located at the interior of the DNA molecule whereas the Fe(II) was located at the exterior of the helix.

A structure for the adenine Fe(III) complex has been proposed (5) in which Fe(III) is associated with the phosphate and the N7 nitrogen of adenine. In this structure, the 6-amino group of adenine may function as a coordinating iron ligand.
If a similar structure is involved in the interaction of Fe(III) ions with nucleic acids, the interaction of Fe(III) with DNA might be expected to result in a loss of complementarity and probably a modification of the conformation of the double stranded DNA helix. Circular dichroism is a useful tool for examining conformational changes in many complex macromolecules and biopolymers including steroids, proteins and nucleic acids (12, 13).

An iron induced change in conformation of DNA such as one resulting from disruption of hydrogen bonding between complementary strands would be expected to result in a change in the circular dichroism of the DNA. In this thesis, the interaction of Fe(III) and Fe(II) with DNA and various synthetic polynucleotides is examined by circular dichroism and temperature-optical density profiles. The reversibility of the association of these metal ions with polynucleotides is also investigated.
CHAPTER II
MATERIALS, PROCEDURES AND EXPERIMENTAL METHODS

A. MATERIALS

Calf Thymus Deoxyribonucleic Acid, Worthington Biochemicals
Ethylenediaminetetraacetic Acid, Fisher Scientific Company
Ferric Chloride, J. T. Baker Chemical Company
Ferrous Chloride, Fisher Scientific Company
Hydroxylamine Hydrochloride, Eastman Organic Chemicals
1, 10-Phenanthroline, Eastman Organic Chemicals
Polyadenylic Acid, Sigma Chemical Company
Polyctidylic Acid, Sigma Chemical Company
Polydeoxyadenylate-Thymidylate, Miles Laboratories
Sodium Acetate, J. T. Baker Chemical Company
Sodium Borohydride, Fisher Scientific Company
Sodium Chloride, Mallinckrodt Chemical Works
Sodium Oxalate, Merck and Company, Inc.
Sodium Pyrophosphate, Merck and Company, Inc.
Tris (Hydroxymethyl) Amino Methane, Fisher Scientific Company
B. PROCEDURES

1. Melting Profiles

The effects of Fe(III) and Fe(II) on the melting profile of DNA were determined using a Beckman TM Analyzer. The TM analyzer consists of an electrically heated sample compartment and a linear temperature programmer. The heated cell compartment consists of a heating jacket, cooling coils, platinum resistance temperature probe and a sample sensing probe, which is inserted into the sample cuvette. The sample compartment of the Cary 15 spectrophotometer was modified to accept the Beckman TM heated cell compartment. A custom made sensitivity-zero suppression unit was used to allow external recording of optical density by use of a Beckman Helipot attached to the pen drive shaft of the Cary 15.

The TM programmer may be adjusted for several temperature spans and program times. For these measurements, the program time was set for twenty-five minutes and the temperature span adjusted from 25°C-125°C. Silica cells (Standard Beckman, 10mm path length) were used.

Melting profiles were recorded on a Hewlett-Packard model 7035B X-Y recorder. The curves consisted of recording temperature on the X-axis versus optical density on the Y-axis.

The temperature scale was standardized by calibrating the temperature bridge versus ice-water at 0°C. The Y-axis of the recorder was calibrated to give the same optical density readings as the Cary 15 recorder, i.e. 0.10 optical density units per inch.
All samples were run against a properly prepared blank in the Cary 15 reference cell compartment. The results are replotted as \( O.D._{260} \) at \( t^\circ C./O.D._{260} \) at 25\(^\circ\)C. versus temperature. The temperature at which one-half of the total increase in optical density is reached upon heating of a sample is defined as the melting temperature (Tm) of a particular sample.

2. Circular Dichroism Measurements

A Jasco ORD/CD/UV-5 Recording Spectropolarimeter was used for all circular dichroism measurements. A demountable cell with quartz windows (Jasco, 10mm path length) of low volume (0.40ml) and a low volume (0.40ml) fused silica cell (Opticell Company) were used. The circular dichroism scale was standardized with a 1.0mg/ml solution of d-10-Camphorsulfonic Acid. Differences in optical densities for right and left circularly polarized light (\( \Delta O.D. \)) are recorded versus wavelength. The standard solution has a \( \Delta O.D. \) of 0.0093 at 290\( \mu \)m. This causes +9.3cm deflection on the 0.01 scale of the spectropolarimeter.

Measurements of circular dichroism were carried out at a polynucleotide concentration of 0.20\( \mu \)m/ml. At this concentration, the optical densities of the polynucleotide solution were 1.34 for DNA and poly dAT, 1.80 for poly A, and 1.26 for poly C. Addition of iron and other reagents increased optical density readings, but all measurements were confined at optical densities below 2.5 unless otherwise specified.
The circular dichroism measurements were run at chart speeds of 2µm per minute for DNA and poly dAT at scale sensitivity settings of 0.002 and a speed of 4µm per minute at a scale sensitivity of 0.005 for poly A and poly C. The data were re-plotted by transferring the ΔO.D. readings with a pair of dividers. Points were generally plotted every 5µm and at certain areas every 2µm intervals. The difference of extinction coefficient between left and right circularly polarized light (E_L - E_r) were obtained from the following equation.

\[ E_L - E_r = \frac{\Delta O.D.}{C l} \]

ΔO.D. is read directly from the recorder. The concentration of the polynucleotide expressed as moles per liter of phosphate is indicated as C and the cell path length in centimeters as l.

3. Dialysis Experiments

The amount of iron remaining bound to DNA after addition of NaBH_4, Ascorbic Acid, and EDTA, pyrophosphate or oxalate was determined by the orthophenanthroline method after dialysis. Samples were prepared identically to those used for circular dichroism measurements, but at larger volumes. Each sample (10ml) was placed in a dialysis bag (5/8 inch diameter) and dialyzed three times versus 250ml of the appropriate buffer with slow stirring at 3°C.
4. Iron Determination

Samples were analyzed for iron content using a modification of the orthophenanthroline method (14). The solution to be analyzed (3.0ml) was placed in a 10ml test tube. The tube content was evaporated to dryness in a glycerine bath at approximately 120°C. After cooling, 0.10ml of concentrated H₂SO₄ and a few drops of 30 percent hydrogen peroxide were added to the tube. Heating at 120°C. was continued for about one hour or until all traces of carbon residue were eliminated. A few drops of hydrogen peroxide were added at five to ten minute intervals to assure complete oxidation of the carbon residue and of the iron to the Fe(III) state. The tube was subsequently cooled and sodium acetate solution (3.0ml-4.0M pH 5.5), orthophenanthroline (5.0ml-0.025%), and hydroxylamine hydrochloride (0.10ml-0.88M) were added. This solution was stored for 15 minutes before optical density measurement at 510mμ. The iron content of the sample was determined from a calibration curve (Figure 1) constructed from optical density readings on Table I.
**TABLE I**

Determination of Iron Content of DNA

<table>
<thead>
<tr>
<th>Sample</th>
<th>Optical Density at 510(\mu)</th>
<th>Corrected Optical Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.021</td>
<td>0.00</td>
</tr>
<tr>
<td>Standard 0.10 Fe/P*</td>
<td>0.102</td>
<td>0.081</td>
</tr>
<tr>
<td>Standard 0.20 Fe/P</td>
<td>0.182</td>
<td>0.161</td>
</tr>
<tr>
<td>Standard 0.30 Fe/P</td>
<td>0.267</td>
<td>0.246</td>
</tr>
</tbody>
</table>

* Designated as iron atoms per nucleotide residue.
Figure 1

Calibration Curve for Iron Determination

Iron Content as Iron Atoms Per Nucleotide Residue

Optical Density at 510 mµ
C. EXPERIMENTAL METHODS

1. Preparation of Polynucleotide Solutions
   
a. Preparation of Stock Solutions

   DNA calf thymus was dissolved (2mg/ml) in NaCl (10⁻³M) -tris (10⁻⁴M) buffer, pH 6.6 by slow stirring for about 48 hours. The solution was centrifuged and dialyzed twice versus buffer of the same concentration.

   The synthetic polynucleotides were purchased as lyophilized powders. The stock solutions of polyadenylic and polycytidylic acids were prepared in the same manner as the DNA solution. Polydeoxyadenylate-thymidylate samples were prepared by diluting the powder directly to the concentration to be used in these studies.

b. Determination of Concentration of Stock Solutions

   The concentrations of the polynucleotide stock solutions were determined spectrophotometrically from the following extinction coefficients at 260mμ.

<table>
<thead>
<tr>
<th>Polynucleotide</th>
<th>Molar Extinction Coefficient at 260mμ</th>
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<tr>
<td>Calf Thymus DNA</td>
<td>6700 (15)</td>
</tr>
<tr>
<td>Polyriboadenylic Acid (poly A)</td>
<td>9000 (15)</td>
</tr>
<tr>
<td>Polyribocytidylic Acid (poly C)</td>
<td>6300 (15)</td>
</tr>
<tr>
<td>Polydeoxyadenylate-thymidylate (poly dAT)</td>
<td>6650 (15)</td>
</tr>
</tbody>
</table>

   The spectra were obtained in buffer (0.10M NaCl-0.10M Tris) pH 7.5 at 1:100 dilution.
c. Ferric and Ferrous Chloride Stock Solutions

These solutions (13.45µm/ml) were prepared by dissolving ferric chloride (FeCl₃·6H₂O, 0.364g.) and ferrous chloride (FeCl₂·4H₂O, 0.267g.) respectively in 100ml of distilled and deionized water. The solutions were freshly made every three weeks and stored at 3°C.

2. Circular Dichroism Measurements

Experimental DNA iron solutions were prepared by mixing aliquots of DNA and iron stock solutions.

a. DNA-Ferric Chloride at pH 4.5

A 0.40µm/ml DNA solution in 0.15M NaCl-10⁻²M acetate buffer at pH 4.5 and a 0.25µm/ml ferric chloride solution in the same buffer were prepared by dilutions of the corresponding stock solutions.

To a portion of the DNA solution (1.00ml), 0.80ml of the dilute Fe(III) solution and 0.20ml of the dilute Fe(III) solution and 0.20ml of the buffer (pH 4.5) were added. The resulting final concentrations were 0.20µm/ml for the DNA and 0.10µm/ml for the iron. A DNA sample without iron was prepared by adding 1.00ml of the buffer to 1.00ml of the DNA solution (0.40µm/ml).

b. DNA-Ferric Chloride at pH 6.6

For studies at pH 6.6, DNA was diluted to a concentration of 0.40µm/ml in 0.15M NaCl-10⁻³M tris buffer, pH 6.6. This iron solution (0.25µm/ml) was prepared by diluting the stock
solution in 0.15M NaCl. To a portion (1.00ml) of the DNA solution iron (0.80ml) and sodium chloride (0.20ml) were added. The final concentrations were DNA (calculated as nucleotide residues), 0.20µm/ml; iron, 0.10µm/ml; in 0.15M NaCl-5 x 10^-4 tris buffer, pH 6.6. A DNA sample of the same concentration (0.20µm/ml) without iron was prepared by adding 1.00ml of the DNA solution to 1.00ml of 0.15M sodium chloride. 

**c. DNA-Ferrous Chloride at pH 6.6**

To a portion (1.00ml) or DNA solution (0.40µm/ml) used for the DNA-Fe(III) studies at pH 6.6, 0.80ml of Fe(II) solution (0.25µm/ml in 0.15M NaCl) and 0.20ml of 0.15M NaCl were added. The final concentrations were DNA, 0.20µm/ml; iron 0.10µm/ml; in 0.15M NaCl-5 x 10^-4M tris buffer, pH 6.6.

3. **Preparation of EDTA, Pyrophosphate and Oxalate Solutions**

Solutions (0.10M) of each of these compounds were prepared by dissolving disodium EDTA (2.923g.), sodium pyrophosphate (4.462g.), and sodium oxalate (1.340g.) respectively in 100ml of distilled and deionized water. Each solution was divided into two parts. The pH of the first part was adjusted to 4.5 and the pH of the other part to 6.6 by addition of hydrochloric acid.

**a. DNA-Ferric Chloride in Presence of EDTA, Pyrophosphate and Oxalate**

Ferric chloride (0.80ml-0.25µm/ml) was added to 1.00ml of DNA solution (0.40µm/ml). Five minutes after mixing, a
solution (0.20ml-0.10M) of either EDTA, pyrophosphate, or oxalate adjusted to the pH of the buffer (4.5 or 6.6) was added. Final concentrations were 0.20µm/ml DNA, 0.10µm/ml iron, and 10.0µm/ml of the respective iron complexing anion.

b. DNA-Ferrous Chloride in the Presence of EDTA Pyrophosphate or Oxalate

Preparation of these solutions was carried out in a manner identical to that used for DNA-Fe(III) in the presence of EDTA, pyrophosphate or oxalate with dilute (0.25µm/ml) Fe(II) solution used in place of the Fe(III) solution. The final concentrations were identical.

4. Circular Dichroism at Low Iron to Nucleotide Ratios

a. DNA-Ferric Chloride

To an aliquot (1.00ml) of DNA solution (0.40µm/ml) 0.80ml of Fe(III) solution (0.125µm/ml) and 0.20ml of 0.15M sodium chloride were added. Final concentrations were 0.20µm/ml DNA and 0.05µm/ml iron in 0.15M NaCl-5 x 10^-4 tris buffer, pH 6.6

b. DNA-Ferric Chloride in the Presence of EDTA

Iron (0.80ml-0.125µm/ml) was added to 1.00ml of DNA (0.40µm/ml). Five minutes after mixing, EDTA (0.20ml of 0.10M) at pH 6.6 was added. Final concentrations were 0.20µm/ml DNA, 0.05µm/ml iron and 10.0µm/ml EDTA.

5. Measurement in the Presence of Sodium Borohydride

a. DNA-Ferric Chloride

Sodium borohydride (NaBH₄) (0.01ml-0.10M) was added to an
Fe(III)-DNA solution (2.00ml) containing 0.20µm/ml DNA, and 0.10µm/ml Fe(III). The final concentration of NaBH₄ was 0.50µm/ml.

b. DNA-Ferric Chloride in the Presence of EDTA

Iron (0.80ml-0.25µm/ml) was added to 1.00ml of DNA (0.40µm/ml in 0.15M NaCl-10⁻³ tris buffer, pH 6.6). After five minutes NaBH₄ (0.01ml-0.10M in 0.15M NaCl) was added. EDTA solution (0.20ml-0.10M in 0.15 NaCl, pH 6.6) was added ten minutes later. The final concentrations were 0.20µm/ml DNA, 0.10µm/ml iron, 0.50µm/ml NaBH₄, and 10.0µm/ml EDTA in buffer pH 6.6. This solution was stored for one-half hour before measurement of the circular dichroism.
CHAPTER III

RESULTS

A. CIRCULAR DICHROISM

The effect of addition of ferric chloride on the circular dichroism of calf thymus DNA at pH 6.6 is shown in Figure 2. The presence of either 0.25 or 0.50 Fe(III) per nucleotide resulted in reduced elipticity throughout the region from 220 to 300mλ. The addition of either EDTA, pyrophosphate or oxalate caused considerable reversal of this decrease in elipticity for the Fe(III) per nucleotide ratios of 0.5, but no appreciable reversal at a ratio of 0.25 Fe(III) per nucleotide as seen in Figure 4.

A similar decrease in circular dichroism is observed upon addition of 0.5 Fe(III) per nucleotide at pH 4.5. The effect of added EDTA to this DNA-Fe(III) is seen in Figure 3. In contrast to the results obtained at pH 6.6, EDTA or pyrophosphate addition caused only a slight restoration of the circular dichroism.

The effect of 0.5 Fe(III) per nucleotide on the circular dichroism of heat denatured DNA at pH 6.6 is shown in Figure 5. A considerable decrease in elipticity is indicated as observed with native DNA. However, unlike the results obtained with the latter at this pH, a complete restoration of the elipticity is observed upon addition of pyrophosphate ion.

The effect of 0.25 Fe(III) per nucleotide on the circular dichroism of polyriboadenylic acid (poly A) and polyribocytidylic acid (poly C) is shown in Figures 6 and 7 respectively. For both
EFFECT OF FERRIC CHLORIDE ON THE CIRCULAR DICHLROISM OF DNA AT pH 6.6

- DNA
- DNA containing 0.50 Fe(III) per nucleotide
- DNA containing 0.50 Fe(III) per nucleotide plus EDTA
FIGURE 3

EFFECT OF FERRIC CHLORIDE ON THE CIRCULAR DICHRONISM OF DNA AT pH 4.5

DNA

***DNA containing 0.50 Fe(III) per nucleotide

---DNA containing 0.50 Fe(III) per nucleotide plus EDTA

λ, mμ

220 230 240 250 260 270 280 290 300 310
FIGURE 4

EFFECT OF FERRIC CHLORIDE ON THE CIRCULAR DICHROISM OF DNA AT LOW IRON TO NUCLEOTIDE RATIOS AT pH 6.6

- - - - - DNA
- - - - - DNA containing 0.25 Fe(III) per nucleotide
- - - - - DNA containing 0.25 Fe(III) per nucleotide plus EDTA
EFFECT OF FERRIC CHLORIDE ON THE CIRCULAR DICHROISM OF DENATURED DNA AT pH 6.6

FIGURE 5

- Denatured DNA OR Denatured DNA containing 0.5 Fe(III) per nucleotide plus pyrophosphate
- Denatured DNA containing 0.5 Fe(III) per nucleotide
FIGURE 6

EFFECT OF FERRIC CHLORIDE ON THE CIRCULAR
DICHROISM OF POLY A

POLY A OR POLY A containing 0.25 Fe(III) per nucleotide plus EDTA

--- POLY A containing 0.25 Fe(III) per nucleotide

PH 4.5

PH 6.6

$\lambda$, m$\mu$
FIGURE 7

EFFECT OF FERRIC CHLORIDE ON THE CIRCULAR
DICROISM OF POLY C

PH 4.5

PH 6.6

POLY C OR POLY C containing 0.25 Fe(III) per nucleotide plus EDTA

--- POLY C containing 0.25 Fe(III) per nucleotide
polynucleotides in the presence of Fe(III) and at both pH 4.5 and 6.6 there is a decrease in the elipticity values. The elipticity is restored upon addition of EDTA. These polynucleotides are single stranded at pH 6.6 (16,17), and exist as double stranded protonated structures at acid pH.

The effect of 0.25 Fe(III) per nucleotide on the circular dichroism of the double stranded synthetic polynucleotide poly dAT at pH 6.6 is shown in Figure 8. A small decrease in elipticity is observed in the presence of Fe(III) and complete restoration of the circular dichroism occurs upon addition of EDTA.

The effect of ferrous chloride on the circular dichroism of native calf thymus DNA is shown in Figure 9. The presence of 0.5 Fe(II) per nucleotide causes a smaller decrease in elipticity values than the decreases resulting from the same amount of Fe(III). Addition of EDTA to the DNA-Fe(II) restores the circular dichroism to the original values observed for DNA. The effect of sodium borohydride on DNA-Fe(III) is also shown in Figure 9. The NaBH₄ causes the circular dichroism of DNA-Fe(III) to coincide with the elipticity values for DNA-Fe(II). However, in contrast to the results observed with DNA-Fe(II), the addition of EDTA to the sodium borohydride treated DNA-Fe(III) does not result in complete restoration of the circular dichroism to that observed for DNA.

The circular dichroism of DNA and DNA-Fe(III) at low ionic strength (10⁻²M NaCl-10⁻³M tris buffer, pH 6.6) is shown in
FIGURE 8

EFFECT OF FERRIC CHLORIDE ON THE CIRCULAR DICHOISM OF POLY dAT AT pH 6.6

PolydAT OR PolydAT containing 0.25 Fe(III) per nucleotide plus EDTA

PolydAT containing 0.25 Fe(III) per nucleotide
FIGURE 9

EFFECT OF FERRIC AND FERROUS CHLORIDE ON THE
CIRCULAR DICHLROISM OF DNA AT pH 6.6

--DNA or DNA containing 0.50 Fe(II) per nucleotide plus EDTA

---DNA containing 0.50 Fe(III) per nucleotide

....DNA containing 0.50 Fe(II) per nucleotide

OR DNA containing 0.50 Fe(III) per nucleotide plus NaBH₄

OR DNA containing 0.50 Fe(III) per nucleotide plus NaBH₄ plus EDTA
Figure 10. There is a decrease in ellipticity similar to that observed at the higher ionic strength. In contrast to the results obtained at high ionic strength, the addition of pyrophosphate ion to the DNA-Fe(III) at the low ionic strength restores the ellipticity.

B. DIALYSIS EXPERIMENTS

The amount of iron remaining bound to DNA after addition of NaBH₄, EDTA, P₂O₇⁻³ or C₂O₄⁻² and dialysis was determined by the orthophenanthroline method. The following samples (10ml) were dialyzed three times versus 250ml of buffer as indicated in Table II.

The dialysis results indicate that the addition of 0.5 Fe(III) per nucleotide to DNA followed by addition of EDTA, or oxalate and dialysis removes most of the added iron. There also appears to be more iron removed at pH 4.5 than at pH 6.6. It is interesting to note that at pH 6.6, there is still one Fe(II) ion per every twenty nucleotides which indicates that both Fe(III) and Fe(II) may both be bound irreversibly to DNA at this pH and ionic strength (0.15M NaCl). The results at low ionic strength (0.015M NaCl) shows that Fe(III) is not strongly bound to DNA under these conditions.

C. MELTING PROFILES

Melting profile curves are shown in Figure 11. Numerical values obtained from these curves are tabulated in Table III. The presence of iron still remaining after dialysis at pH 4.5 and 6.6 does not have a significant effect on the melting profile of
FIGURE 10

EFFECT OF FERRIC CHLORIDE ON THE CIRCULAR
DICHROISM OF DNA AT LOW IONIC STRENGTH

- DNA OR DNA containing 0.50 Fe(III) per nucleotide plus EDTA
- DNA containing 0.50 Fe(III) per nucleotide
FIGURE II

MELTING PROFILES

Numbers refer to Table III

PH 4.5
DIALYZED

PH 4.5
UNDIALYZED

PH 6.6
DIALYZED

PH 6.6
UNDIALYZED

OD_{260} t./ OD_{260} 25°

TEMPERATURE °C

75 80 85 90 95
75 80 85 90 95
80 90 100 80 90 10
TABLE II
TABULATION OF RESULTS OF DIALYSIS EXPERIMENTS

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Iron Remaining After Dialysis</th>
<th>Contents</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe/P*</td>
<td>µm/ml</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.043</td>
<td>DNA (0.20)</td>
<td>0.15M NaCl-10⁻²M acetate,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
<td>pH 4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.088</td>
<td>DNA (0.20)</td>
<td>0.15M NaCl-5 x 10⁻⁴ tris,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
<td>pH 6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.018</td>
<td>DNA (0.20)</td>
<td>Same as No. 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
<td>pH 4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.018</td>
<td>DNA (0.20)</td>
<td>Same as No. 1</td>
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<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
<td>pH 4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxalate (10.0)</td>
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<tr>
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<td>Same as No. 2</td>
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<td>FeCl₃ (0.10)</td>
<td>pH 6.6</td>
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<td></td>
<td>EDTA (10.0)</td>
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</tr>
<tr>
<td>6</td>
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<td>DNA (0.20)</td>
<td>Same as No. 2</td>
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<tr>
<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
<td>pH 6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxalate (10.0)</td>
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<tr>
<td>7</td>
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<td>FeCl₃ (0.10)</td>
<td>pH 6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NaBH₄ (0.50)</td>
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</tr>
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<td></td>
<td>EDTA (10.0)</td>
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<tr>
<td>8</td>
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<td></td>
<td>FeCl₃ (0.10)</td>
<td>pH 6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NaBH₄ (0.50)</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>EDTA (10.0)</td>
<td></td>
</tr>
<tr>
<td>Sample No.</td>
<td>Iron Remaining After Dialysis</td>
<td>Contents</td>
<td>Buffer</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>Fe/P* µm/ml</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>0.103</td>
<td>DNA (0.20)</td>
<td>Same as No. 2</td>
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<tr>
<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td>pH 6.6</td>
</tr>
<tr>
<td>10</td>
<td>0.057</td>
<td>DNA (0.20)</td>
<td>Same as No. 2</td>
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<td></td>
<td></td>
<td>FeCl₂ (0.10)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td>pH 6.6</td>
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<td>11</td>
<td>0.031</td>
<td>DNA (0.20)</td>
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<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascorbic Acid (10.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td>pH 6.6</td>
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<tr>
<td>12</td>
<td>0.048</td>
<td>DNA (0.20)</td>
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<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
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<td></td>
<td>EDTA (10.0)</td>
<td>pH 4.5</td>
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<tr>
<td>13</td>
<td>0.005</td>
<td>DNA (0.20)</td>
<td>Same as No. 1</td>
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<td></td>
<td></td>
<td>FeCl₂ (0.10)</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>EDTA (10.0)</td>
<td>pH 4.5</td>
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<tr>
<td>14</td>
<td>0.038</td>
<td>DNA (0.20)</td>
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<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Ascorbic Acid (10.0)</td>
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<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td>pH 4.5</td>
</tr>
<tr>
<td>15</td>
<td>0.000</td>
<td>DNA (0.20)</td>
<td>Low ionic</td>
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<td>FeCl₃ (0.10)</td>
<td>Strength 0.015M</td>
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<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td>NaCl-10⁻³ tris, pH 6.6</td>
</tr>
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</table>
### TABLE II CONTINUED

**TABULATION OF RESULTS OF DIALYSIS EXPERIMENTS**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Iron Remaining After Dialysis</th>
<th>Contents</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe/P*</td>
<td>µm/ml</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.000</td>
<td>DNA (0.20)</td>
<td>Same as No. 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FeCl₂ (0.10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td>pH 6.6</td>
</tr>
<tr>
<td>17</td>
<td>0.000</td>
<td>DNA (0.20)</td>
<td>Same as No. 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FeCl₃ (0.10)</td>
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</tr>
<tr>
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<td></td>
<td>Ascorbic Acid (10.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA (10.0)</td>
<td>pH 6.6</td>
</tr>
</tbody>
</table>

* Expressed as moles of iron per mole of nucleotide.
### TABLE III
SUMMARY OF NUMERICAL VALUES OBTAINED FROM MELTING PROFILES

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hyperchromicity</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA + 0.5 Fe(III)/P + EDTA pH 6.6 dialyzed</td>
<td>1.38</td>
<td>87.7°C</td>
</tr>
<tr>
<td>1. (Table II Sample 9) (0.10 Fe/P remaining)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA + 0.5 Fe(II)/P + EDTA pH 6.6 dialyzed</td>
<td>1.39</td>
<td>85.7</td>
</tr>
<tr>
<td>2. (Table II Sample 10) (0.057 Fe/P remaining)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA + 0.5 Fe(III)/P + Ascorbic Acid + EDTA pH 6.6 dialyzed (Table II Sample 11) (0.031 Fe/P remaining)</td>
<td>1.29</td>
<td>81.4</td>
</tr>
<tr>
<td>DNA + 0.5 Fe(III)/P + EDTA pH 4.5 dialyzed</td>
<td>1.37</td>
<td>81.5</td>
</tr>
<tr>
<td>4. (Table II Sample 12) (0.048 Fe/P remaining)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA + 0.5 Fe(II)/P + EDTA pH 4.5 dialyzed</td>
<td>1.35</td>
<td>81.4</td>
</tr>
<tr>
<td>5. (Table II Sample 13) (0.005 Fe/P remaining)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA + 0.5 Fe(III)/P + Ascorbic Acid + EDTA pH 4.5 dialyzed</td>
<td>1.31</td>
<td>79.5</td>
</tr>
<tr>
<td>6. pH 4.5 dialyzed (Table II Sample 14) (0.038 Fe/P remaining)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA + EDTA pH 6.6 dialyzed</td>
<td>1.37</td>
<td>87.7</td>
</tr>
<tr>
<td>7. DNA + EDTA pH 6.6 dialyzed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA + EDTA pH 4.5 dialyzed</td>
<td>1.37</td>
<td>80.4</td>
</tr>
<tr>
<td>8. DNA + EDTA pH 4.5 dialyzed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA + 0.10 Fe(III)/P undialyzed pH 6.6</td>
<td>1.36</td>
<td>85.7</td>
</tr>
<tr>
<td>9. DNA + 0.10 Fe(II)/P undialyzed pH 6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA + 0.10 Fe(II)/P undialyzed pH 6.6</td>
<td>1.39</td>
<td>85.9</td>
</tr>
<tr>
<td>10. DNA + 0.10 Fe(II)/P undialyzed pH 6.6</td>
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<td></td>
</tr>
</tbody>
</table>
### TABLE III CONTINUED

**SUMMARY OF NUMBERICAL VALUES OBTAINED FROM MELTING PROFILES**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hyperchromicity</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. DNA undialyzed pH 6.6</td>
<td>1.39</td>
<td>85.7</td>
</tr>
<tr>
<td>12. DNA undialyzed pH 4.5</td>
<td>1.38</td>
<td>82.6</td>
</tr>
<tr>
<td>13. DNA + 0.10 Fe(III)/P undialyzed pH 4.5</td>
<td>1.33</td>
<td>81.8</td>
</tr>
<tr>
<td>14. DNA + 0.10 Fe(II)/P undialyzed pH 4.5</td>
<td>1.38</td>
<td>81.6</td>
</tr>
</tbody>
</table>

* Expressed as moles of iron per mole of nucleotide
DNA. The presence of 0.1 Fe(III) per nucleotide in samples that were not dialyzed results in a significant decrease in the hyperchromicity of DNA while the mid transition temperature (Tm) remains essentially unchanged. At pH 4.5, the hyperchromicity of DNA is decreased from 38.3 percent to 33.4 percent by the presence of the Fe(III) and at pH 6.6, it is lowered from 39.3 percent to 35.9 percent. This indicates that the presence of the added Fe(III) cause a disruption of certain hydrogen bonds without causing a major decrease in the stability of the rest of the hydrogen bonds in the base paired double stranded helical structure of DNA. The presence of added Fe(II) before or after dialysis does not alter the melting profile significantly. The addition of ascorbic acid alters the melting profile of DNA Fe(III) by decreasing the hyperchromicity and lowering the Tm. The ascorbic acid may reduce the Fe(III) to Fe(II) as it was intended to do, but its strong effect of the melting profile of the DNA-Fe(III) may indicate that the ascorbic acid also in some way modifies the DNA aside from its effect on the iron.
CHAPTER IV
DISCUSSION

The circular dichroism measurements indicate that the presence of added ferric or ferrous iron alters the conformation of DNA which is consistent with previous observations (18) that the intrinsic viscosity of DNA is reduced by ferric ions. Conformational changes induced by ferric ions are observed with other polynucleotides as indicated by decreases in ellipticities of poly A, poly C and poly dAT. This decrease in circular dichroism was also observed for heat denatured DNA. It appears that a native structure is not required for these ferric ion induced changes in polynucleotides.

With the important exception of native DNA, the conformational change induced by the addition of ferric ions may be reversed upon addition of EDTA or pyrophosphate. Complete reversibility was observed for denatured DNA, poly A, poly C and poly dAT at both pH 4.5 and 6.6.

The addition of ferrous ions to native DNA also resulted in decreased ellipticity values. However, in contrast to the results obtained with ferric ions, the addition of EDTA to the native DNA-ferrous ion complex completely restored the ellipticity to values characteristic of native DNA.

Comparison of the results obtained from dialysis and melting profiles indicate that even though both ferrous and ferric ions
may bind irreversibly to native DNA, their presence does not alter the stability of the double stranded DNA helix. The presence of as much as one irreversibly bound ferric ion per every ten nucleotides or one irreversibly bound ferrous ion remaining associated with DNA after dialysis per every twenty nucleotides does not influence the melting profile of DNA. By contrast, the presence of one added ferric ion per every ten nucleotides results in a decrease in the hyperchromicity of the DNA from 39 percent to 36 percent at pH 6.6 and from 38 percent to 33 percent at pH 4.5. The same amount of added ferrous iron does not alter the melting profile of DNA. Thus, the polynucleotide sites for reversible binding of ferric are probably different in native DNA from the sites of ferrous binding. These results are in agreement with the proton relaxation and NMR studies, which indicated that ferric and ferrous ions are bound to different DNA sites (10, 19). The metal substitution experiments previously discussed indicate that ferric iron binds to the DNA bases. If ferric iron does bind to the bases as is also suggested by NMR data (19), the imino form of adenine would be expected to be stabilized by iron in the adenine-ferric ion complex. Thus, the binding of ferric iron to purines would be expected to lead to a partial loss of complementarity between DNA strands as shown in Figure 12. This disruption of complementarity would be expected to result in conformational reorganizations of the polynucleotide and a decrease in the stability of the double
FIGURE 12
EFFECT OF pH AND FERRIC CHLORIDE ON THE
COMPLEMENTARITY OF ADENINE WITH THYMINE
AND CYTOSINE
helix. The decrease in the hyperchromicity of DNA observed in the presence of one added ferric ion per every ten nucleotides is consistent with this expectation.

The metal substitution experiments and proton relaxation studies indicate that ferrous iron may not bind to the bases but is probably associated with phosphate groups of DNA (10, 9). The fact that one added ferrous ion per every ten nucleotides does not alter the hyperchromicity of DNA is evidence that the binding of ferrous ions to DNA does not disrupt hydrogen binding between complementary strands. This indicates that the binding of ferrous ions to DNA is different ferric ion binding in nature or binding site. The ferric iron bound to the DNA bases is probably removed by addition of EDTA and dialysis under these conditions, the hyperchromicity of DNA is fully restored. The dialysis results also indicates that there is some iron still remaining associated with the DNA. This may indicate the presence of another type of binding site since neither the irreversibly bound ferric or ferrous iron effects the hyperchromicity of DNA.

If addition of EDTA to a DNA-ferric iron complex removes all ferric ions bound to the bases and leading to hydrogen binding disruption as indicated by the temperature-optical density profiles, the question arises why the circular dichroism is not restored. It is probable that the loss of complementarity resulting from the complexing of ferric ion with adenine results in a partial "slippage" between the two strands. This "slippage" may
result in formation of a more thermodynamically stable structure not very different from that resulting from thermal denaturation.

The DNA-ferric ion structure could be partially stabilized by the formation of new hydrogen bonds between the adenine-ferric moiety and cytosine rather than thymine. Addition of EDTA would result in removal of the iron and reformation of only part of the original hydrogen bonds. This stabilization of the slippage by formation of new hydrogen bonds would be expected to result in an irreversible change in the circular dichroism.

The loss of complementarity upon addition of ferric ions would also be expected to occur in denatured DNA, poly A, poly C and poly dAT. Poly A and poly C are single stranded at pH 6.6 and form double stranded protonated polynucleotides at pH 4.5 (16,17). An irreversible slippage of the type described for native DNA would not be expected to occur with these synthetic homopolymers. The interaction of ferric ions with poly dAT is shown in Figure 13. Disruption of the adenine-thymine hydrogen bonded base pairs may occur but since there is no cytosine present to form new hydrogen bonds with the adenine-ferric ion complex, removal of the iron would be expected to result in reformation of the original hydrogen bonds. Denatured DNA has already attained a thermodynamically-stable structure. Structure stabilizing shifts promoted by ferric ions are therefore not likely to occur in denatured DNA. The reversibility of the ferric ion induced change in ellipticity is consistent with this
## FIGURE 13

**INTERACTION OF POLY dAT WITH Fe(III)**

<table>
<thead>
<tr>
<th>Poly dAT</th>
<th>Addition of Fe(III)</th>
<th>Removal of Fe(III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully Complementary Strands in Poly dAT</td>
<td>Partial Loss of Complementarity in the Poly dAT Fe(III) Complex</td>
<td>Restoration of Complementarity in Poly dAT</td>
</tr>
</tbody>
</table>

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A --- T</td>
<td>Fe(III)AH T</td>
<td></td>
</tr>
<tr>
<td>T --- A</td>
<td>T --- A</td>
<td></td>
</tr>
<tr>
<td>A --- T</td>
<td>Fe(III)AH T</td>
<td></td>
</tr>
<tr>
<td>T --- A</td>
<td>T --- A</td>
<td></td>
</tr>
</tbody>
</table>
reasoning.

The imino form of adenine which is assumed to be stabilized by ferric ion would be further stabilized at low pH (20). The circular dichroism data which indicate that the ferric ion induced change in conformation is irreversible at pH 4.5 and may be considerably reversed at pH 6.6 support this hypothesis.

The reversible binding of both ferric and ferrous iron is influenced by ionic strength and pH as indicated by the dialysis studies. A greater amount of iron remains bound to the DNA at pH 6.6, than at pH 4.5 in 0.15M sodium chloride. At pH 6.6 in 0.015M sodium chloride, no detectable iron remains after dialysis. This effect of pH may be due to a higher degree of protonation of phosphate groups and the nitrogen of the bases thereby, decreasing the ability of these groups to bind iron ions irreversibly. It must be noted that the ability of EDTA, pyrophosphate and oxalate to bind iron increases at decreasing pH (21). However, the stability constant for the oxalate-iron complex does not appreciably increase within the investigated pH range as shown in Table IV. Nevertheless, oxalate was shown to remove iron more effectively at the lower pH. In fact, the oxalate-iron complex with a lower stability constant than EDTA-iron complex removes an even greater amount of iron than EDTA. It would therefore, seem that the differences in irreversibility between the two pH's does not reflect differences in behavior of the iron complexing agents.
The effect of pH on the ferric ion might effect the ability of the iron to form a complex with DNA. In Table V, the effect of pH on ferric ion is shown (3). It is unlikely that the pH effect on the iron would interfere with the ability of the ferric ion to form complexes with DNA since at both pH 4.5 and 6.6, the ferric ion is almost completely in the Fe(OH)\(^{2+}\) form.

The ability of the DNA to irreversibly bind iron may depend on the stability of the double stranded helix. The DNA helix has greater stability at higher ionic strength and neutral pH (23). The dialysis data indicates that the ability of DNA to irreversibly bind iron is greater at neutral pH and at 0.15M sodium chloride. Less iron is irreversibly bound to the DNA at pH 6.6 in 0.015M sodium chloride and at pH 4.5 in 0.15M sodium chloride. Under these conditions, the DNA double stranded helix is known to be partly destabilized.

The circular dichroism measurements of samples in which sodium borohydride was employed, were attempted in an effort to test whether the reduction of ferric to ferrous ion may occur while ferric ion is bound to DNA. This type of reduction is a step in the postulated mechanism by which iron might influence the process of DNA "unwinding" proceeding the process of replication (5). It was observed that addition of sodium borohydride to DNA-ferric ion complex resulted in appearance of a circular dichroism similar to that obtained for the DNA-ferrous ion complex, but in contrast to the results obtained by adding
### TABLE IV

**EFFECT OF pH ON THE STABILITY CONSTANTS OF SOME Fe(III) COMPLEXING AGENTS**

<table>
<thead>
<tr>
<th>Complexing Agent</th>
<th>Log Stability Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 4.5</td>
</tr>
<tr>
<td>EDTA</td>
<td>15.2</td>
</tr>
<tr>
<td>Pyrophosphate</td>
<td>17.0</td>
</tr>
<tr>
<td>Oxalate</td>
<td>15.0</td>
</tr>
</tbody>
</table>

### TABLE V

**EFFECT OF pH ON THE STRUCTURE OF Fe(III) ION IN AQUEOUS SOLUTION**

<table>
<thead>
<tr>
<th>pH</th>
<th>Structure of Fe(III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>95% Fe(OH)²⁺, 5% Fe(OH)₂⁺</td>
</tr>
<tr>
<td>Above 5.0</td>
<td>100% Fe(OH)²⁺</td>
</tr>
</tbody>
</table>
EDTA to the DNA-ferrous ion complex, the addition of EDTA to the borohydride-treated DNA-ferric ion complex did not restore the ellipticity to values characteristic of DNA. This may be further indication that the ferric ion induced circular dichroism change is not due to irreversibly bound iron but rather that once a conformational change occurs it may not be reversed by the removal of the bound iron. The dialysis experiments indicate that the iron content of the sodium borohydride-treated samples is not less and may even be higher than that of samples that were not treated with sodium borohydride. Ascorbic acid was incorporated in the dialysis experiments to reduce ferric to ferrous ion. Melting profiles on the dialyzed samples indicate that the ascorbic acid-treated samples have a broader transition, lower hyperchromicity and decreased Tm than the samples not treated with ascorbic acid. It is, therefore, possible that the addition of reducing agents may have chemically or conformationally altered DNA, in addition to any effect that these agents may have had on the iron.

The addition of ferric or ferrous ions causes a change in the conformation of DNA as indicated by the circular dichroism results. The conformational change induced by ferrous ion is small and reversible upon addition of EDTA. In contrast, the conformational change induced by ferric ion is larger and only partly reversible. The melting profiles indicate that the presence of one added ferric ion per every ten nucleotides decreases the stability
of the double stranded DNA helix resulting in reduced hyperchromicity. The presence of the same amount of ferric ion after dialysis does not appear to significantly alter the melting profile. The presence of ferrous ions before or after dialysis does not alter the melting profile of DNA. The addition of ascorbic acid or sodium borohydride to the DNA-ferric ion complex appeared to alter either the chemical or conformational structure of DNA as indicated by results of dialysis and melting profile experiments.

The results of these studies may be interpreted as consistent with the theory (5) that ferric ions bound to DNA promote strand separation required in DNA replication while ferrous ions do not interfere with the process of strand "rewinding". An oxidation-reduction step could therefore, be implicated as a control of the mechanism of DNA strand dissociation.
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The final copies of the thesis, submitted by William W. Martz, have been examined and approved by the director of the thesis. The signature which appears below verifies the fact that any necessary changes have been incorporated, and that it is now given final approval with reference to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 26, 1969  Signature of Adviser