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# Interaction of Steroids with Nucleic Acids†

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ABSTRACT: 17β-Estradiol and testosterone bind to both native and denatured DNA, and to RNA and poly(A) · poly(U). Binding affinity depends on the conformation of nucleic acid. Lowering the electrolyte concentration and raising the temperature increase the binding of 17β-estradiol to native DNA and decrease that to denatured DNA. In 0.01 *M* NaCl and at 37°, more 17β-estradiol is bound to native DNA than to denatured DNA. Higher binding of steroid to denatured DNA relative to native DNA at low temperature and high ionic strength is related to larger fraction of binding sites per unit nucleotide in denatured DNA. In addition to 17β-estradiol and testosterone, 17α-estradiol, 17β-estradiol-3-methyl ether and 19-nortestosterone also stabilize the structure of nucleic acids and poly(A) · poly(U) against thermal denaturation. The 17β-estradiol induced elevation of the $T_m$ of DNA is diminished by methanol or high NaCl concentration. These results indicate the involvement of hydrogen bonding and hydrophobic interactions between steroids and nucleic acids. The results of binding isotherms and optical studies suggest a conformational dependence of the binding of steroids to nucleic acids.

Interaction of biologically active molecules with nucleic acids has been studied with a dual purpose: first, to serve as a probe for investigating the conformation which the nucleic acids are capable of adopting, and second, to contribute to our understanding of the mechanism of action of bioactive molecules. Several classes of molecules, such as dyes (Lerman, 1964), antibiotics (Muller and Crothers, 1968; Sobell, 1973), and carcinogenic hydrocarbons (Boyland and Green, 1962; Huggins and Yang, 1962) bind to nucleic acids; they often show preference for a particular conformation and base specificity. Biological effects of some of these molecules frequently can be correlated with their nucleic acid interactions.

Hormonal steroids play many regulatory roles in cellular proliferative and biosynthetic activities. Accumulating evidence suggests that the growth stimulatory effects of some of the steroids in the target tissue are mediated through their binding to a protein receptor(s) (see O'Malley and Means, 1974, for a recent review). Interaction of steroids with nucleic acids may also be involved in the diverse effects attributable to steroid hormones. Ts'o and Lu (1964) proposed that the binding of steroids in nucleic acids depends mainly on hydrophobic forces. Cohen and Kidson (1969), Cohen et al. (1969), and Kidson et al. (1970) concluded that steroids bind to denatured but not to native DNA, which involves both hydrogen bonding and hydrophobic in-

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teractions. Goldberg and Atchley (1966) reported that hormones including steroids destabilize the DNA structure as assessed by thermal transition studies and proposed that hormones promote strand separation as a prelude to activated transcription.

We present our studies demonstrating that steroids can bind with both denatured and native nucleic acids. Nucleic acids show higher affinity for steroids (depending on the temperature and counterion concentration) in native than denatured form. The preferred conformation seems to be neither a compact double helix nor an unordered form but an intermediate one. Thermal transition, optical rotatory dispersion (ORD), and circular dichroism (CD) studies show that steroids stabilize the native structure of nucleic acids against thermal denaturation in medium of low ionic strength. In agreement with Cohen et al. (1969), our studies also suggest the involvement of hydrogen bonding as well as hydrophobic forces in these interactions.

## Experimental Procedures

### *Materials*

*Nucleic Acids.* Calf thymus DNA was purchased from Sigma Chemical Company, yeast tRNA from Calbiochem, and poly(A) and poly(U) were from Miles Laboratories. Crab(dAT) was a gift from Professor N. Davidson and *Mycobacterium tuberculosis* DNA from Professor K. Miura. Nucleic acids were deproteinized either by sodium dodecyl sulfate-chloroform (Marmur, 1961) or phenol (Mandell and Hershey, 1960) extraction. Concentration of nucleic acids (0.001 *M* phosphate-0.01 *M* NaCl (pH 7.0)) was determined spectrophotometrically on the basis of mean residue absorptivities: DNA, $6.6 \times 10^3$ at 260 nm (Ts'o and Lu, 1964); tRNA, $6.8 \times 10^3$ at 260 nm (data of Calbiochem); poly(A), $10 \times 10^3$ at 257 nm; and poly(U), $9.5 \times 10^3$ at 262 nm. The residue absorptivities of polynucleotides were determined by phosphorus analysis (Allen, 1940).

For binding studies with DNA where high concentrations were required, viscosity of the solutions was effectively reduced by shearing a 5 mg/ml solution of DNA in 0.02 *M* phosphate-0.2 *M* NaCl (pH 7.0) in a VirTis "45" mixer (VirTis Co., Gardiner, New York) for 10 min at 4°. The sheared solution was clarified by low-speed centrifugation and dialyzed against appropriate buffer. The hypochromicity of native sheared DNA was 34–36% and that of unsheared DNA was 38–40%. Denatured DNA was obtained by heating DNA solution in 0.5 m*M* phosphate-5 m*M* NaCl (pH 7.0) at 95° for 10 min followed by quick chilling and dialysis against appropriate buffer.

*Steroids.* Unlabeled steroids were obtained from Mann Research Laboratories and recrystallized from methanol-water mixtures. Tritium-labeled 17$\beta$-estradiol (0.5 Ci/mmol) and testosterone (4.18 Ci/mmol) were obtained from Nuclear Chicago Corp. Concentration of steroids in aqueous solution was determined spectrophotometrically; their molar absorptivities are: $2.0 \times 10^3$ at 273 nm for 17$\beta$-estradiol, 17$\alpha$-estradiol, and 17$\beta$-estradiol-3-methyl ether (Wilds and Nelson, 1953); and $1.6 \times 10^4$ at 249 nm for testosterone and 19-nortestosterone (Westphal and Ashley, 1959).

*Binding Studies.* Binding of steroids with nucleic acids was studied by equilibrium dialysis and ultracentrifugation. At least six, usually eight, concentrations of steroid in 0.02 *M* phosphate-0.2 *M* NaCl (pH 7.0) (in duplicate or triplicate) were used, unless stated otherwise. For equilibrium dialysis 1 ml of nucleic acid solution (with or without labeled steroid) in a prewashed dialysis bag was dialyzed against 2–4 ml of a labeled steroid solution in a 5-ml glass-stoppered tube for 10–12 days (Arya, 1968). Concentrations of steroid inside and outside of the bag were determined by counting the radioactivity in aliquots of the respective solutions. Loss of nucleic acids from inside the bag was negligible. Loss of steroids due to adsorption on the bag was 15–20%.

For ultracentrifugation, solutions of DNA containing different amounts of labeled steroids were incubated at 5° for 60 hr and centrifuged at 140,000*g* at 5° for 5 hr in polyallomer tubes. Concentration of unbound steroid was determined by counting the radioactivity of the supernatant. No loss of steroid on polyallomer tubes could be detected.

The binding parameters were computed by plotting the data according the equations (Scatchard, 1949):

$$1/r = 1/n + 1/nK[\mathrm{S}] \quad (1)$$

and

$$r/[\mathrm{S}] = (n - r)K \quad (2)$$

where $r$ is moles of steroid bound per mole of nucleotide, $n$ the number of binding sites per mole of nucleotide, [S] the concentration of unbound steroid, and $K$ apparent binding constant ($M^{-1}$). In experiments where only one or two concentrations of steroid (in triplicate) were used, binding was characterized by a binding coefficient, $f_b$, defined as

$$f_b = [\text{bound steroid}]/[\text{total steroid}][\text{nucleotide}]$$

which equals the fraction of steroid bound per mole of nucleotide.

*Optical Studies.* Thermal transition profiles of nucleic acids with or without steroid were obtained with a Gilford multichannel recording spectrophotometer equipped with thermal sensor, temperature programmer, and circulating water bath. Temperature of the solution, contained in glass-stopped fused quartz cells, was raised 0.5–1° at intervals of 4–5 min. Blanks of steroid alone were also obtained. Any change in the absorbance of steroid with temperature was subtracted from that of nucleic acid plus steroid. The melting point, $T_m$, is the midpoint of thermal transition (Marmur and Doty, 1962).

ORD and CD were measured under a constant flush of dry nitrogen with Cary 60 recording spectropolarimeter and Jasco ORD/UV/CD-5 instrument, respectively. The absorbance of solutions at 260 nm was always less than 2 units for measurement between 350 and 190 nm. Any optical activity due to steroid alone was subtracted from that of nucleic acid plus steroid. Specific rotation, [$\alpha$], and mean residue absorptivity, $\epsilon_L - \epsilon_R$, were calculated (Yang and Samejima, 1969). In the concentration range of steroids ($\leq 1 \times 10^{-4}$ *M*) used, 17$\beta$-estradiol, not testosterone, showed negligible optical activity. The ORD of a $1 \times 10^{-4}$ *M* testosterone solution showed a maximum at 261–262 nm ([$\alpha$] = +6200), a shoulder around 290 nm ([$\alpha$] = +3,000), and a minimum at 323–326 nm ([$\alpha$] = −650) with crossover points at 313–314 nm and 229–230 nm. The corresponding CD showed a positive band at 225 nm ($\epsilon_L - \epsilon_R$ = +11.6), a shoulder around 250 nm ($\epsilon_L - \epsilon_R$ = +7.2), and a negative band above 300 nm ($\epsilon_L - \epsilon_R$ at 300 nm = −1.2) with a crossover point at 277 nm.

Throughout this report, unless stated otherwise, DNA re-

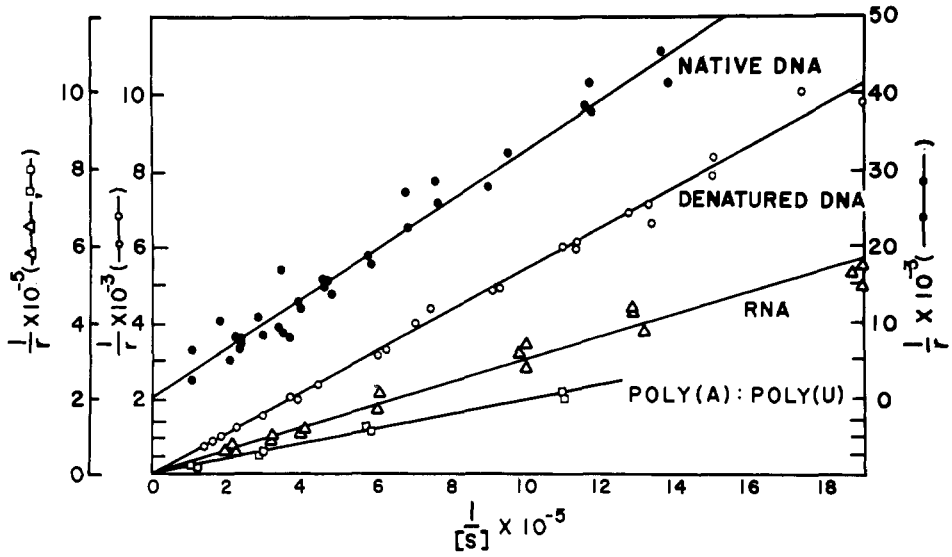


FIGURE 1: Binding isotherms of nucleic acids and 17$\beta$-estradiol at 5° in 0.02 *M* phosphate and 0.2 *M* NaCl (pH 7.0). Data for DNA include that obtained by equilibrium dialysis and ultracentrifugation.

Table I: Parameters of Binding of 17$\beta$-Estradiol and Testosterone to Nucleic Acids at 5°.[a]

| Nucleic Acid | 17$\beta$-Estradiol | | | Testosterone |
|---|---|---|---|---|
| | $nK(M^{-1})$[b] | $K(M^{-1})$[c] | $n$[c] | $nK(M^{-1})$[b] |
| Native calf thymus DNA | 30 | 3500 | $8.3 \times 10^{-3}$ | 25 |
| Denatured calf thymus DNA | 190 | 1200 | $1.6 \times 10^{-1}$ | 300 |
| Yeast tRNA | 3.5 | 4000 | $8.7 \times 10^{-4}$ | 4.0 |
| Poly(A)·poly(U) | 3.5 | | | |

[a] In 0.02 *M* phosphate-0.2 *M* NaCl (pH 7.0). [b] Standard error of measurement and computation is estimated to be ±10% for values less than 100 and ±5% for values more than 100. [c] Values estimated by the Scatchard plot.

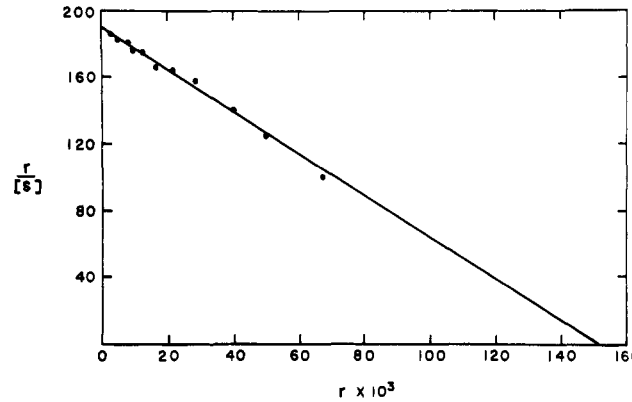


FIGURE 2: Scatchard plot of binding of 17$\beta$-estradiol to denatured calf thymus DNA at 5° in 0.02 *M* phosphate and 0.2 *M* NaCl (pH 7.0).

fers to calf thymus DNA, RNA to yeast tRNA, and *m* to the input molar ratio of steroid to nucleotide in nucleic acid.

## Results

*A. Binding Studies.* Figure 1 presents the binding isotherms of 17$\beta$-estradiol to native DNA, denatured DNA, RNA, and poly(A) · poly(U). Table I lists the slopes, *nK* (eq 1). The values of *r* in these experiments are so low that binding sites are far from saturated. It was not possible to obtain values of *n* and *K* accurately from Figure 1. But they can be estimated by replotting the data according to eq 2. Figure 2 shows such a representative plot. The *n* and *K* values so obtained are listed in Table I. The binding of testosterone to nucleic acids yielded similar isotherms; values of *nK* are also included in Table I.

The *nK* of the 17$\beta$-estradiol in 0.2 *M* NaCl for denatured DNA is about six times that for native DNA. This would seemingly suggest that denatured DNA possesses a higher binding affinity than does native DNA in this case, a conclusion also reported by Ts'o and Lu (1964) and Cohen and Kidson (1969) for binding studies in 0.5 *M* NaCl. Actually, the apparent binding constant, *K*, of 17$\beta$-estradiol for denatured DNA is only one-third that of native DNA. It is the larger fraction of binding sites per unit nucleotide, *r*, in denatured DNA that accounts for the higher *nK* values. A considerably smaller fraction of binding sites in RNA than DNA probably is related to a compact tertiary structure for RNA at low temperature in a medium of high ionic strength (Fresco et al., 1966).

The *nK* of testosterone for denatured DNA is higher than that for native DNA and RNA. By analogy with 17$\beta$-estradiol, we suspect that the apparent binding constant of testosterone for native DNA and RNA is likely to be higher than for denatured DNA. This inference is supported by the effect of steroids on thermal transitions of nucleic acids (see below).

The *nK* of testosterone and 17$\beta$-estradiol for native DNA and RNA are comparable (Table I). However, for denatured DNA, the *nK* of testosterone is significantly higher than that of 17$\beta$-estradiol. This observation suggests that interaction of testosterone with denatured DNA involves some additional binding forces which are not operative in the case of 17$\beta$-estradiol.

*Effect of Temperature and Ionic Strength.* Figure 3 shows that at any given ionic strength, the fraction of 17$\beta$-estradiol bound to native DNA decreases as the temperature is increased. This effect is opposite for denatured DNA and more marked than for native DNA. The van't Hoff plot

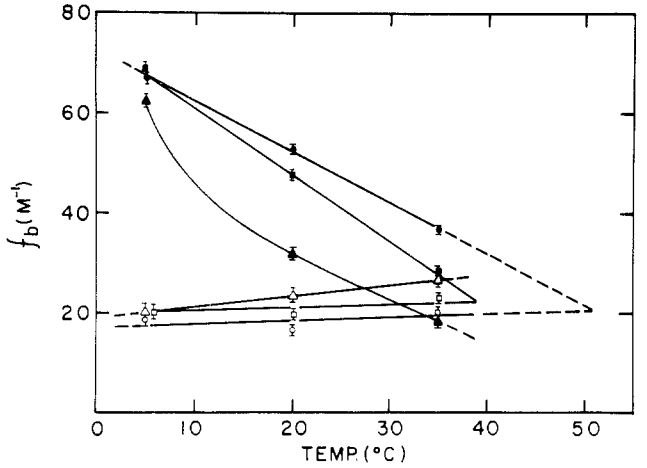


FIGURE 3: Effect of temperature and ionic strength on binding of 17$\beta$-estradiol to calf thymus DNA. Symbols: open, native DNA; filled, denatured DNA. Concentration of NaCl: circles, 0.2 *M*; squares, 0.05 *M*; triangles, 0.01 *M*. $f_b$ = [bound steroid]/[total steroid][nucleotide].

(log $nK$ vs. $1/T$) of these data reveals that binding of 17$\beta$-estradiol to native DNA is accompanied by positive enthalpy and entropy changes and that to denatured DNA by negative changes.

At 5°, changes in salt concentration do not affect the binding significantly. However, at higher temperatures (20 and 37°), lowering the ionic strength (0.1 *M* NaCl and lower) markedly decreases the binding of 17$\beta$-estradiol to denatured DNA and slightly increases the binding to native DNA. Thus at low temperature and high ionic strength, denatured DNA shows a greater degree of binding than native DNA, but the reverse is true at high temperature and low ionic strength. For example, in 0.01 *M* NaCl, the ratio of fractions of 17$\beta$-estradiol bound to native DNA to denatured DNA is 0.72 at 20° but 1.5 at 37°. These results argue for the conformational dependence of binding process.

The concentrations of 17$\beta$-estradiol and testosterone employed in this study were in the range of $1.0 \times 10^{-6}$–$1.2 \times 10^{-5}$ *M* and $5 \times 10^{-9}$–$1.0 \times 10^{-6}$ *M*, respectively. The association constant for stacking dimerization, $K = [S_2]/[S]^2$, is usually small. For example, $K$(stacking) for nucleosides is of the order of one (Ts'o, 1974). Even if $K$ were 1000, the amount of dimer for the highest concentration of steroids used would be about 1% (much less for more dilute solutions). Thus, the possibility of dimerization is remote in our study.

*B. Thermal Transitions.* 17$\beta$-Estradiol, 17$\alpha$-estradiol, 17$\beta$-estradiol-3-methyl ether, testosterone, and 19-nortestosterone all stabilize the structure of nucleic acids against thermal denaturation in a solution of low ionic strength (5 m*M* NaCl). Representative thermal transitions of DNA and RNA in the presence and absence of 17$\beta$-estradiol and testosterone are shown in Figure 4. Table II lists the elevation of the $T_m$'s of nucleic acids in the presence of steroids at various input ratios. (The uv absorption spectra of nucleic acids is not affected by the presence of these steroids.) The width of the thermal transitions of nucleic acids is considerably reduced in the presence of these steroids indicating a more cooperative melting (Dove and Davidson, 1962). We have not observed steroid-induced destabilization of nucleic acid structure at any concentration of steroids studied, as has been reported by Goldberg and Atchley (1966).

These steroids have more pronounced effects on the $T_m$ of RNA than that of DNA (Table II). Some relation between the structure of steroid and its capacity to cause equivalent elevation of the $T_m$ of nucleic acids is discernible from these data. For DNA, 17$\beta$-estradiol is the most effective. Changing the orientation of 17-hydroxy group from $\beta$ to $\alpha$ in estradiol diminishes its effect on the $T_m$ of DNA and also RNA. Methylation of the 3-hydroxy group of estradiol increases its effect on the $T_m$ of RNA. Testosterone is less effective than 17$\beta$-estradiol in causing equivalent elevation of the $T_m$ of DNA but more effective for RNA. 19-Nortestosterone is the least effective of the steroids studied. Our limited data on the effect of steroids on the $T_m$ of nucleic acids of different base compositions show no clear base specificity of nucleic acid–steroid interaction. Cohen et al. (1969) have reported, however, preferential binding of steroids to guanine residues in denatured DNA and Munck et al. (1957) to adenine among nucleosides and nucleotides.

(An anonymous reviewer raised the possibility of ionic contaminants in the steroid samples, which might account for the large changes in $T_m$. The concentration of steroids (recrystallized from methanol or methanol–water) used in Figure 4 was less than or equal to $1 \times 10^{-4}$ *M* and all solutions contained 5 m*M* NaCl and 0.5 m*M* phosphate. Such contaminants, if present, would have been several orders of magnitude greater than the steroids. We have difficulty in justifying this explanation.)

*Solvent Effects.* Addition of a weakly protic solvent,

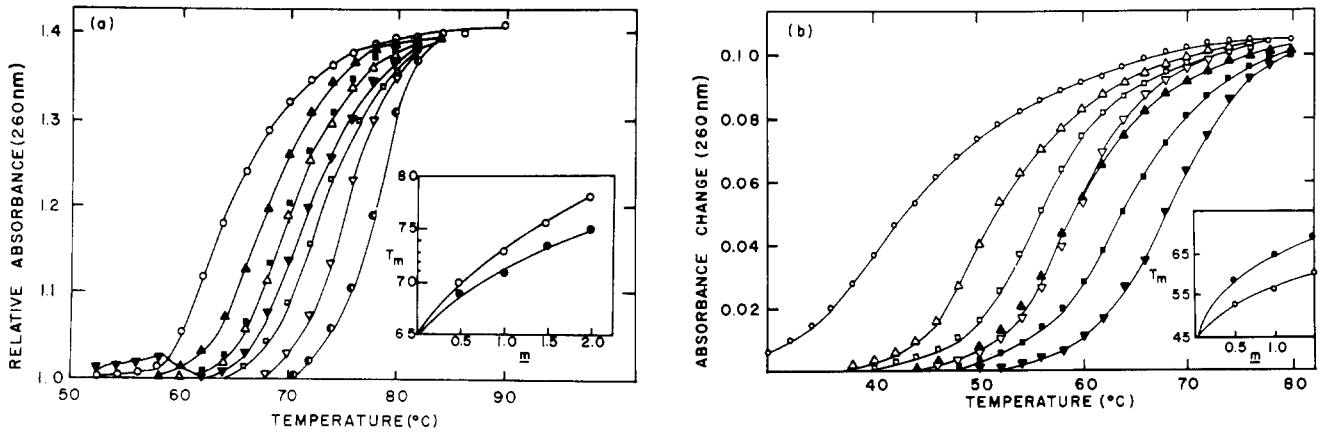


FIGURE 4: Effect of 17$\beta$-estradiol and testosterone on thermal transitions of (a) calf thymus DNA and (b) yeast tRNA in 0.5 m*M* phosphate and 5 m*M* NaCl (pH 7.0). Molar ratios of 17$\beta$-estradiol to nucleotide ($m$): (○) 0; (△) 0.5; (□) 1.0; (▽) 1.5; (◐) 2.0. Molar ratio of testosterone to nucleotide ($m$): (▲) 0.5; (■) 1.0; (▼) 1.5. Inset: $T_m$ of nucleic acids in the presence of 17$\beta$-estradiol (○) and testosterone (●).

Table II: Effect of Steroids on the Melting Temperature Increment, $\Delta T_m$, of Nucleic Acids.[a]

| Steroid | $m$[b] | Calf Thymus DNA[c] (65°)[d] | Yeast tRNA (44°)[d] | Poly-(A)·poly (U) (46°)[d] | Crab-(dAT)[c] (39°)[d] | *M. tuberculosis* DNA[c] (72°)[d] |
|---|---|---|---|---|---|---|
| 17β-Estradiol | 0.5 | 5.0 | 8.0 | 4.0 | | |
| | 1.0 | 8.0 | 12.0 | 12.0 | 6.0 | 5.0 |
| | 1.5 | 10.5 | 15.0 | 16.0 | 9.0 | 7.0 |
| | 2.0 | 15.5 | | | | |
| 17β-Estradiol | 0.5 | 3.0 | 6.0 | | | |
| | 1.0 | 5.5 | 10.0 | | | |
| | 1.5 | 9.0 | 13.0 | | | |
| 17β-Estradiol-3-methyl ether | 0.5 | 4.0 | 10.0 | | | |
| | 1.0 | 6.5 | 14.0 | | | |
| | 1.5 | 9.5 | 18.0 | | | |
| Testosterone | 0.5 | 4.0 | 15.0 | 4.0 | | |
| | 1.0 | 6.0 | 20.0 | 8.0 | | 4.0 |
| | 1.4 | 8.0 | 24.0 | 12.0 | | |
| | 2.0 | 10.0 | | | | 8.0 |
| 19-Nortestosterone | 1.0 | 2.0 | 7.0 | | | |
| | 2.0 | 4.5 | 12.0 | | | |
| | 3.0 | | 15.0 | | | |
| | 4.0 | 7.0 | | | | |

[a] In 0.5 m*M* phosphate–5 m*M* NaCl (pH 7.0). [b] Molar ratio of steroid to nucleotide in nucleic acid. [c] GC contents: calf thymus DNA, 43%; crab (dAT) containing 3.5%; *M. tuberculosis* DNA, 62%. [d] The $T_m$ of nucleic acid in the absence of steroid.

methanol, to the solution decreases the effect of steroids on the thermal transitions of nucleic acids. Table III illustrates that increasing the amount of methanol in the medium progressively diminishes the 17β-estradiol induced $\Delta T_m$ of calf thymus DNA. These results indicate the involvement of hydrophobic interaction in the association of steroids with nucleic acids. Judging from the effects of alcohols on the $T_m$ of DNA (Geiduschek and Herskovits, 1961), the solubility of DNA bases (Herskovits and Harrington, 1972), and the hydrophobic structures such as soap micelles (Emerson and Holtzer, 1967), one would expect this methanol-induced dissociation of the steroid–DNA complexes and thereby the disappearance of the $T_m$ changes by steroids. However, methanol may also affect the conformation of DNA as reported recently by Ivanov et al. (1973) and Girod et al. (1973). In addition, increasing the counterion concentration of the medium also decreases the magnitude of 17β-estradiol induced $T_m$ elevation of DNA. For example, with more than 0.2 *M* NaCl, no measurable effect of 17β-estradiol on the $T_m$ or transition width of DNA was observed. This is consistent with our observation that increasing the ionic strength decreases the binding affinity of steroids for native nucleic acids.

*C. ORD and CD Studies.* All the steroids studied increase the amplitude of Cotton effects of nucleic acids without causing appreciable shifts in their spectral positions. Representative ORD and CD spectra of DNA–17β-estradiol and RNA–testosterone are shown in Figure 5. The interaction of testosterone and RNA results in fairly marked changes in the ORD and CD spectra, whereas the corresponding changes for DNA are small, but measurable. On the other hand, the decrease in the amplitudes of the ORD extrema due to thermal denaturation is significantly greater in the absence of 17β-estradiol than in its presence. For example, the decrease in specific rotation at 290 nm was 39.8% in the absence of 17β-estradiol and 23.4% in its presence when the temperature of the solutions was raised from 25 to 68°.

Table III: Effect of Methanol of 17β-Estradiol-Induced Elevation of $T_m$ of Calf Thymus DNA.[a]

| % Methanol (v/v) | $m$[b] | $T_m$(°C) 0 | 1.0 | 1.5 |
|---|---|---|---|---|
| 0 | | 65 | 73 | 75.5 |
| 5 | | 63 | 68 | 70 |
| 10 | | 61.5 | 63.5 | 65 |
| 25 | | 59 | 59.5 | 60 |
| 50 | | 53.5 | 53.5 | 54 |

[a] In 0.5 m*M* phosphate–5 m*M* NaCl (pH 7.0) plus methanol. [b] $m$ is the molar ratio of steroid to nucleotide in DNA.

## Discussion

Our results demonstrate that steroids bind to both native and denatured DNA, and that native DNA actually has a higher intrinsic binding constant, $K$, than denatured DNA in spite of a larger $nK$ in the later case (Table I). The fact that steroids protect DNA secondary structure against thermal denaturation supports this conclusion. High temperature and low ionic strength which tend to impart a less ordered and open conformation to native DNA (Cavallieri et al., 1956; Ascoli et al., 1959; Marmur et al., 1963) also favor the binding of steroids. That some degree of secondary structure is required for the binding is evident by the decreased affinity of steroids for denatured DNA at higher temperatures and lower ionic strengths, conditions which diminish the residual ordered structure in denatured DNA (Marmur et al., 1963; Eigner and Doty, 1965). These results suggest that the binding of steroids depends on the conformation of DNA in solution. The results of our optical studies show that steroids have larger effects on the $T_m$ of RNA than that of DNA in a medium of low ionic strength

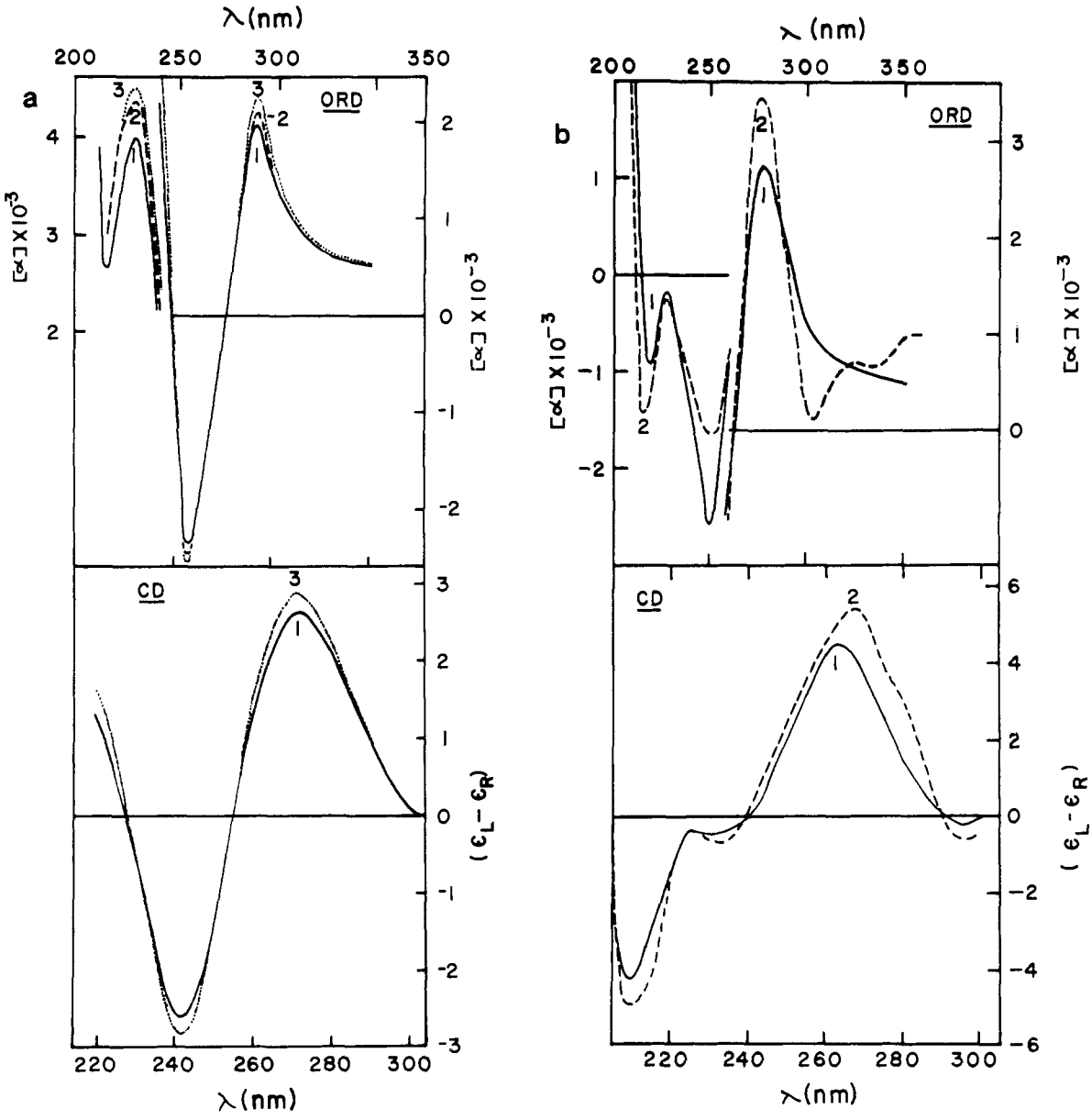


FIGURE 5: Effect of steroids on ORD and CD of nucleic acids at 25° in 0.5 m*M* phosphate and 5 m*M* NaCl (pH 7.0). (a) ORD and CD of calf thymus DNA in the absence (curve 1) and presence (curve 2, $m$ = 1.0; curve 3, m = 1.5) of 17$\beta$-estradiol. (b) ORD and CD of yeast tRNA in the absence (curve 1) and presence (curve 2, $m$ = 1.0) of testosterone. $m$ is the molar ratio of steroid to nucleotide in nucleic acid. The concentration of nucleic acids for these spectra was $1 \times 10^{-4}$ *M* nucleotide.

(Figures 4 and 5). The lower $nK$ of steroid binding to RNA than to DNA in high ionic strength and low temperature (Table I) may be primarily due to a lower number of binding sites. This implies that the compact tertiary structure, which the RNA reportedly adopts in high ionic strength and low temperature (Fresco et al., 1966), is not favorable for steroid binding.

The positive enthalpy and enthropy changes which accompany the binding of 17$\beta$-estradiol to native DNA suggests the involvement of hydrophobic interactions. This is consistent with the diminution of the steroid-induced elevation of the $T_m$ of DNA by methanol. The negative enthalpy and entropy changes observed for the binding of 17$\beta$-estradiol to denatured DNA suggests that such forces as dispersion and dipole–dipole interactions between steroid and bases exposed to the solvent environment may also contribute in this case (Crothers and Ratner, 1968). Hydrogen bonding interactions between steroid and nucleic acid may also be involved as indicated by the effect of modified steroids on the $T_m$ of nucleic acids (Table II).

Modification of polar groups at 3 and 17 positions of the steroid molecule alters the $\Delta T_m$ of nucleic acid–steroid complexes, suggesting the participation of these hydroxy groups in the complex formation. Decreased effectiveness of 17$\alpha$-estradiol relative to 17$\beta$-estradiol indicates the involvement of 17-hydroxy group in hydrogen bonding in its $\beta$ conformation (see Cohen et al., 1969). The possibility that the $\alpha$ orientation of this group introduces steric hindrance in binding cannot, however, be discounted by our results.

The modification of the 3-hydroxy group of estradiol to 3-methoxy in estradiol-3-methyl ether and 3-keto in testosterone results in the decreased effectiveness of estradiol to elevate the $T_m$ of DNA. This indicates a possible involvement of this group in hydrogen bonding as well. However, these modifications augment the steroid-induced elevation of the $T_m$ of RNA. These results point to the possibility of

some differences in the mode or energetics of interaction of steroids with DNA and RNA. A possible explanation as given by Cohen et al. (1969) is that the interaction of steroids with nucleic acids involves, inter alia, hydrophobic and hydrogen bonding interactions. We postulate further that the hydrophobic interactions play a more dominant role in the binding of steroids to RNA than to native DNA so that the structural modifications that increase the hydrophobicity of steroid enhance its affinity for RNA. Regardless of the mode of interaction, 17$\beta$-estradiol evidently shows more pronounced effects on the optical properties of DNA than RNA and the reverse is true for testosterone.

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