Alterations in the Ultraviolet Absorption Spectra of Steroids upon Binding to Serum Proteins†

Stephen D. Stroupe‡ and U. Westphal*

ABSTRACT: Difference spectra of progesterone-binding globulin (PBG) complexes with progesterone and testosterone were measured. The contributions of steroid and protein to the difference spectra were resolved by use of 5α-pregnane-3,20-dione and dihydrotestosterone to compensate for the perturbation of PBG. The absorption spectra of seven bound steroids all showed increased extinction coefficients, sharpened absorption bands, a small blue shift, and an increased area implying an enhanced transition moment. This is in contrast to the steroid complexes with the low affinity binders, human serum albumin, and α_1-acid glycoprotein, which exhibit decreased extinction coefficients and reduced transition moments.

Alterations in the ultraviolet absorption spectra of steroids when bound to proteins were first reported two decades ago (Westphal, 1957). It was found that the extinction at the absorption maximum of Δ^4-3-ketosteroids (248–249 nm in aqueous systems) was diminished up to about 15% when the steroids were bound to serum albumin. Accompanying the decrease in extinction was a small 2–3-nm shift of the absorption maximum toward shorter wavelengths. This phenomenon was used to measure the interaction of a wide variety of steroids to serum albumin and β-lactoglobulin (Westphal, 1957; Westphal & Ashley, 1958, 1959, 1962). More recent studies on the interaction of steroids with albumin (Ryan, 1968; Ryan & Gibbs, 1970) have confirmed the earlier studies of Westphal and have expanded observations to include information on the perturbation of protein structure upon binding steroids.

Studies from our laboratory on the progesterone-binding globulin–progesterone complex (Stroupe & Westphal, 1975a) represent, as far as we know, the only work on alterations in spectral properties of proteins and steroids associated with high affinity. Extension of the studies on PBG[1] has confirmed and expanded the initial observations; however, it also became clear that our earlier explanation of the origin of the perturbed steroid spectrum was overly simplistic. The previous report (Stroupe & Westphal, 1975a) suggested that the large negative difference signal at 268 nm in the PBG·progesterone complex resulted from a simple shift in the absorption maximum of the bound progesterone from 249 nm to 240 nm. More detailed data will be presented below which require a more complex interpretation. The difference signal is due to a pronounced alteration in steroid absorption with the absorption band sharpening significantly and increasing in intensity, as well as undergoing a small blue shift.

Materials and Methods

PBG was prepared from pregnant guinea pig serum by the affinity chromatography method of Cheng et al. (1976). Binding site concentrations were measured using the fluorescence quenching method (Stroupe et al., 1975). HSA from Sigma was charcoal defatted (Chen, 1967) and fractionated on Sephadex G-200 to yield the monomer fraction. AAG was obtained from the American Red Cross and was delipidated with ethanol and acetone (Ganguly et al., 1967) prior to use. Concentrations of HSA and AAG were determined spectrophotometrically using extinction coefficients of ϵ_{279} = 35 200 M^{-1} cm^{-1} (Eisenberg & Edsall, 1963; Franglen, 1974) and ϵ_{278} = 38 700 M^{-1} cm^{-1} (Kute & Westphal, 1976), respectively. A 10 mM Tris-Cl buffer, pH 7.4, containing 0.1 M NaCl was used. Steroids were commercial products or gifts from research laboratories.[2] Stock steroid solutions (about 2 mM in alcohol) were prepared by weight and the concentrations verified by UV absorption.

Difference spectra were obtained with a Cary 15 scanning spectrophotometer utilizing the expanded scale. The accuracy of the absorption and wavelength scales were verified with a standard potassium dichromate solution. A tandem cell technique was used with protein solutions in the front cuvettes and buffer in the rear cuvettes. A baseline was recorded with all four cuvettes appropriately filled with 1.0 mL of solution. Difference spectra were induced by adding a small volume (typically 6 to 8 μL by means of a Hamilton 10-μL syringe) of steroid stock solution to the protein solution in the sample beam. An equal volume of steroid solution was added to the buffer cuvette in the reference beam, with the same volume of alcohol added to the protein cuvette in the reference beam. All solutions were thoroughly mixed after addition of alcohol or alcoholic steroid solutions. For PBG spectra, 1 mol of steroid per mol of protein was added. The concentration of steroid in the reference buffer solution was below the solubility limits for all measurements (see Westphal, 1957).

Absorption spectra of steroids bound to proteins were obtained using identical protein solutions in both the sample and reference beams of the Cary 15 spectrophotometer. A baseline

† From the Department of Biochemistry, University of Louisville School of Medicine, Health Sciences Center, Louisville, Kentucky 40232. *Received September 13, 1977.* This is report No. 39 in the series Steroid-Protein Interactions; for the preceding paper, see Blanford et al. (1978). This work was supported by a grant from the National Institute of Arthritis, Metabolism and Digestive Diseases (AM-06369) and a research career award (U.W.) from the Division of General Medical Sciences (GM-K6-14,138) of the U.S. Public Health Service.

‡ Present address: Abbott Laboratories, Abbott Park, North Chicago, Illinois 60064.

[1] Abbreviations used: PBG, progesterone-binding globulin; AAG, α_1-acid glycoprotein or orosomucoid; HSA, human serum albumin; DHT, 17β-hydroxy-5α-androstan-3-one; R-5020, 17,21-dimethyl-19-norpregna-4,9-diene-3,20-dione; medrogestone, 6,17-dimethyl-4,6-pregnadiene-3,20-dione.

[2] Acknowledgment has been made in previous papers.

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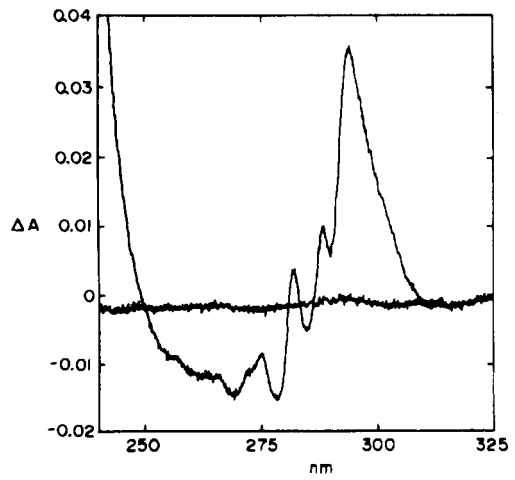

FIGURE 1: Difference spectrum of PBG·5α-pregnanedione complex. Complex concentration = 13.9 μM.

was recorded using the direct scale. For HSA and AAG, alcoholic steroid solution was added to the protein solution in the sample beam with an equal volume of alcohol added to the reference cuvette. In the case of PBG, complex formation is accompanied by significant changes in the protein absorption spectrum (Stroupe & Westphal, 1975a); therefore, a different protocol was followed. Equal volumes (1.0 mL) of the same PBG solution were placed in cuvettes in both the sample and reference beams. An equimolar amount of steroid in a concentrated alcoholic solution was added to the sample cuvette. To compensate for absorption changes due to the altered protein absorption in the steroid- PBG complex, an equimolar amount of 5α-pregnane-3,20-dione was added to the reference sample. Stock solutions of the steroids under study and of 5α-pregnanedione were adjusted to the same concentration so that equal volumes of ethanol were added to both sample and reference cuvettes. For the testosterone measurements, DHT was used instead of 5α-pregnanedione. The rationale for this protocol will be discussed. All spectral measurements were performed at 22 ± 2 °C.

Results

The change in the absorption spectrum of PBG upon complex formation is given in Figure 1. Since 5α-pregnanedione is essentially transparent in the ultraviolet region of interest (ϵ_{max} = 65 M^{-1} cm^{-1} at 285 nm), all observed signal changes obtained at 13.9 μM complex must be due to alterations in the absorbance of PBG. The difference spectrum displays a large positive signal at 294 nm with $\Delta\epsilon_{294}$ = 2600 M^{-1} cm^{-1}. At shorter wavelengths the change becomes negative ($\Delta\epsilon_{270}$ = −800 M^{-1} cm^{-1}). Farther into the UV, the signal becomes positive with $\Delta\epsilon_{233}$ = 12 000 M^{-1} cm^{-1} (not shown).

When the steroid has a chromophore the difference spectrum reflects perturbations of the absorption spectra of both the steroid and PBG; Figure 2 illustrates this point. In the difference spectrum between the PBG·progesterone complex and the unmixed components there is a strong minimum at 268 nm ($\Delta\epsilon_{268}$ = −3850 M^{-1} cm^{-1}) due to perturbation of the progesterone chromophore. The near-UV portion of the difference spectrum for the PBG·progesterone complex is essentially identical with that of the 5α-pregnanedione complex, both spectra having $\Delta\epsilon_{294}$ = 2600 M^{-1} cm^{-1}. The spectrum shown in Figure 2 gives details unavailable in the spectrum obtained previously (Stroupe & Westphal, 1975a) with a single-beam instrument; however, the $\Delta\epsilon$ values are essentially the same.

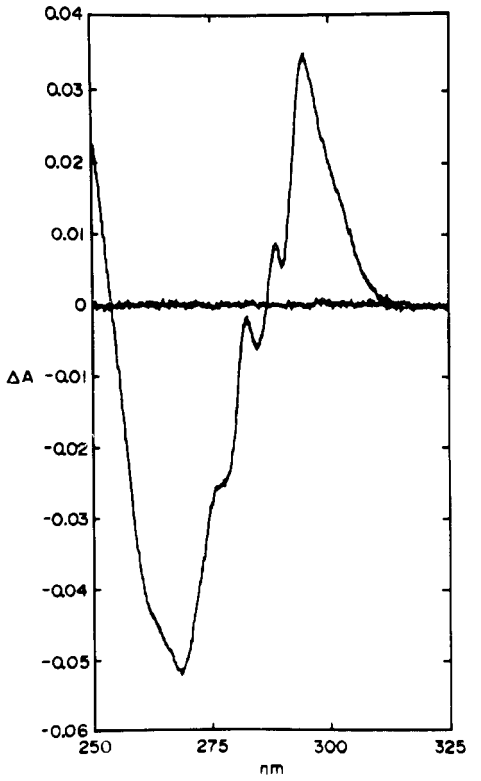

FIGURE 2: Difference spectrum of PBG·progesterone complex. Complex concentration = 13.3 μM.

The identity of the protein contribution to the difference spectra of 5α-pregnanedione and progesterone complexes with PBG is not unexpected. The two steroids differ only at the bond between C(4) and C(5) of ring A, and both are bound to PBG with the same high affinity, the K_a value (by equilibrium dialysis, 4 °C) of the progesterone complex being 2.2 × 10^9 M^{-1} (Stroupe & Westphal, 1975b), and that of the 5α-pregnane-3,20-dione complex 2.1 × 10^9 M^{-1} (Blanford et al., 1978). The spectrum in Figure 3 was obtained utilizing the fact that both the transparent steroid and progesterone produce the same conformational changes in PBG. In the first of tandem cuvettes in the sample beam, 13.3 μM PBG was complexed with equimolar progesterone; buffer was placed in the second sample cuvette. The first reference cuvette contained 13.3 μM PBG complexed with equimolar 5α-pregnanedione; progesterone at 13.3 μM in buffer was in the second reference cuvette. The resultant spectrum displayed in Figure 3 is a difference spectrum between PBG-bound progesterone and progesterone free in buffer. Note the absence of a peak of 294 nm indicating the identical effects of 5α-pregnanedione and progesterone on PBG conformation. The spectrum exhibits a minimum at 268 nm with $\Delta\epsilon$ = −3100 M^{-1} cm^{-1} and a maximum at 241 nm with $\Delta\epsilon$ = +3300 M^{-1} cm^{-1}. Mathematical addition of the difference spectrum of PBG-bound progesterone to that of free progesterone indicates an alteration in the progesterone absorption spectrum more drastic than the simple shift in λ_{max} suggested earlier (Stroupe & Westphal, 1975a).

Figure 4 gives the result of a similar experiment for testosterone bound to PBG using dihydrotestosterone (DHT) as the transparent steroid. The standard difference spectrum is very similar to that for progesterone with $\Delta\epsilon_{294}$ = +2100 M^{-1} cm^{-1} and $\Delta\epsilon_{268}$ = −2900 M^{-1} cm^{-1}. With PBG complexed with DHT in the reference beam, the resultant spectrum reflects only the perturbed steroid chromophore giving $\Delta\epsilon_{268}$ = −2100 M^{-1} cm^{-1} and $\Delta\epsilon_{240}$ = +2300 M^{-1} cm^{-1}. Again, mathe-

TABLE I: Spectroscopic Parameters of Steroids Bound to PBG.

Steroid	K_a[a] ($M^{-1} \times 10^{-8}$)	λ_{max} (nm) Bound	λ_{max} (nm) Free	ϵ_{max} (M^{-1} cm^{-1}) Bound	ϵ_{max} (M^{-1} cm^{-1}) Free	θ (cm^{-1}) Bound	θ (cm^{-1}) Free	Area[b] (M^{-1} $cm^{-2} \times 10^{-7}$) Bound	Area[b] (M^{-1} $cm^{-2} \times 10^{-7}$) Free
Progesterone[c]	20	245.5	249.0	19 600 ± 100	16 500 ± 200	2620 ± 60	2760 ± 30	9.08 ± 0.27	8.00 ± 0.01
Deoxycorticosterone	10	245.5	248.5	18 400	16 000	2610	2760	8.52	7.83
Corticosterone	0.2	246.1	247.5	18 000	15 800	2726	2840	8.71	7.97
Cortisol	0.02	248.5	247.5	17 500	15 900	2718	2750	8.43	7.76
Testosterone	2.9	246.0	248.8	18 200	15 900	2660	2740	8.58	7.72
Testosterone acetate	9.2	245.0	248.0	19 100	16 200	2650	2800	8.97	8.04
Medrogestone[d]	45.5	297.5	298.0	32 400	24 800	2050	2450	11.79	10.78

[a] Stroupe and Westphal, 1975b. [b] Area = $\sqrt{\pi}\epsilon_{max}\theta$. Assuming the absorption band to be Gaussian in shape, the area of the absorption band is proportional to the transition moment (Sandorfy, 1964). [c] Values given for progesterone are the means of three determinations ± standard deviations. Values for all other steroids represent single determinations. [d] 6,17-Dimethyl-4,6-pregnadiene-3,20-dione.

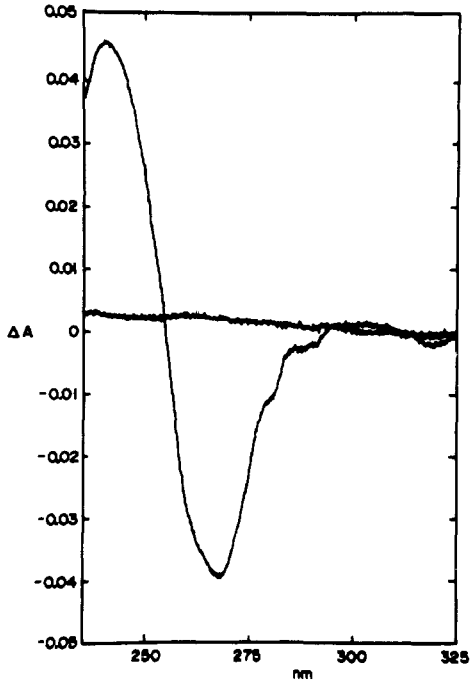

FIGURE 3: Difference spectrum of progesterone bound to PBG against free progesterone, corrected for PBG perturbations as described under Materials and Methods. Concentration of bound progesterone = 13.3 μM.

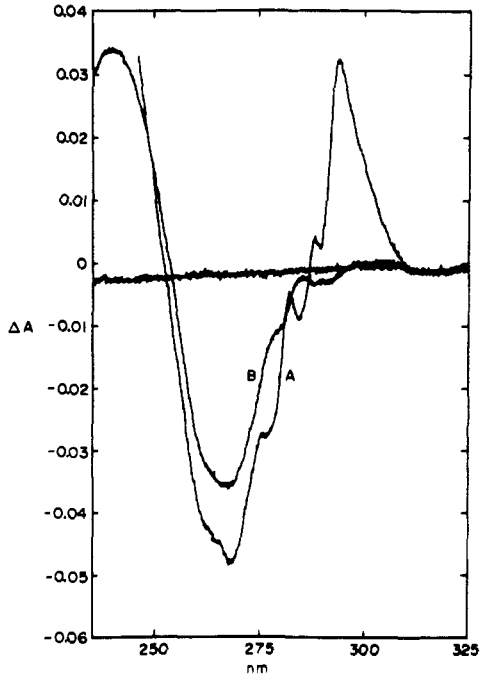

FIGURE 4: Difference spectrum of PBG-testosterone complex. After recording the difference spectrum of the complex (A), the PBG perturbations were compensated for by adding DHT to the reference PBG sample. The difference spectrum of testosterone bound to PBG was then recorded (B).

matical addition of the difference spectrum to the absorption spectrum of free testosterone indicates that the bound chromophore had undergone an alteration more complex than a simple shift in λ_{max}.

The nature of the alteration in progesterone absorption when bound to PBG was further explored utilizing direct spectra of progesterone bound to PBG and free in solution. Figure 5 gives direct spectra of the identical concentration (13.6 μM) of progesterone bound to PBG and free in buffer. Immediately apparent is the significant increase in absorbance of progesterone bound to PBG. Accompanying this absorbance increase is a slight blue shift of λ_{max} to 246 nm and a pronounced sharpening of the absorption band. Table I summarizes several parameters of the absorption bands of steroids bound to PBG and free in solution. Note that, in every case including the weakly bound cortisol, the molar extinction coefficient is increased. Also in every instance θ, the difference of the frequency between ϵ_{max} and ϵ_{max}/e (Sandorfy, 1964) is smaller for the bound than for the free steroid. θ is used as a measure of the spectral band width. For the bound steroids, the increase in ϵ_{max} and the decrease of θ are proportionate to the affinity

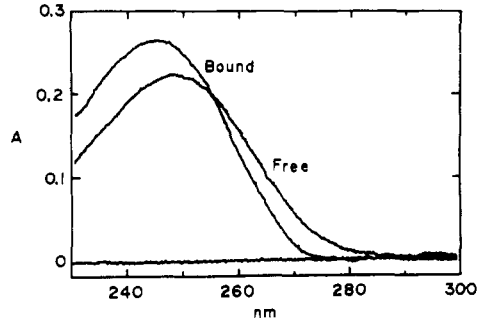

FIGURE 5: Absorption spectrum of progesterone bound to PBG, and free in aqueous solution. The spectrum of bound progesterone was corrected for PBG perturbations as described under Materials and Methods. Concentration of PBG and progesterone = 13.6 μM.

constants with the more tightly bound steroids exhibiting the greater effects.

The dramatic enhancement of the extinction coefficient for bound medrogestone, a 4,6-dien-3-one, is documented in

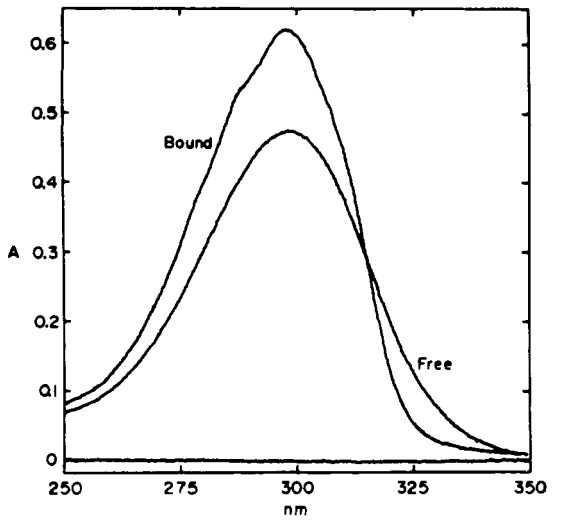

FIGURE 6: Absorption spectrum of medrogestone bound to PBG and free in aqueous solution corrected as described under Materials and Methods. Concentration of PBG and of medrogestone = 19.1 μM.

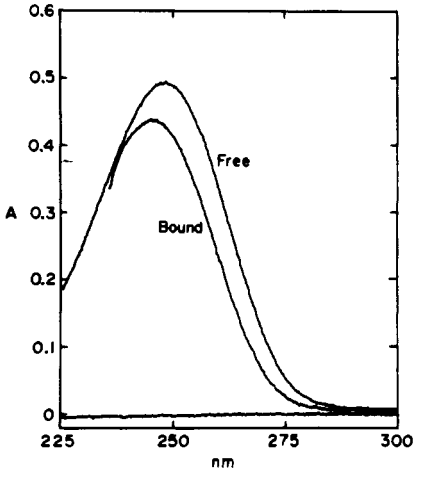

FIGURE 7: Absorption spectrum of progesterone bound to HSA and free in aqueous solution. Concentration of progesterone = 30 μM; concentration of HSA = 37 μM.

Figure 6. In addition to the increase in extinction and sharpening seen with the other steroids in Table I, there is evidence of fine structure with the two $\pi \rightarrow \pi^*$ transitions sharpened and partially resolved.

The enhanced extinction coefficient of steroids bound to PBG is in contrast to the results obtained when steroids are bound to the lower affinity serum binding proteins, HSA and AAG. As illustrated in Figures 7 and 8, progesterone has a higher extinction coefficient free in buffer than when bound to HSA or AAG, with AAG exhibiting the greater effect. Table II summarizes the spectral properties of the two complexes of Figures 7 and 8 as well as of the testosterone–HSA complex. In every case the extinction coefficients of the bound steroids are decreased.

Discussion

As indicated in our earlier report (Stroupe & Westphal, 1975a) the binding of steroids to PBG is accompanied by marked alterations in both the protein and steroid absorption spectra. Perturbations in the protein absorption result from a conformational change induced by steroid binding; however, the observed changes in steroid absorption do not lend themselves to such a facile explanation.

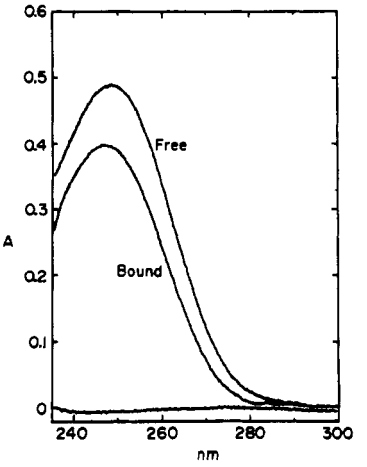

FIGURE 8: Absorption spectrum of progesterone bound to AAG and free in aqueous solution. Concentration of progesterone = 30 μM; concentration of AAG = 39 μM.

TABLE II: Spectroscopic Parameters of Steroids Bound to Serum Proteins.

Complex	λ_{max} (nm)	ϵ_{max} (M^{-1} cm^{-1})	θ (cm^{-1})	Area (M^{-1} cm^{-2} × 10^{-7})	$-\Delta\epsilon_{249}$ (%)[a]
Progesterone·HSA	245.5	14600	2782	7.231	13.6
Progesterone·AAG	247	13300	2694	6.351	20.2
Testosterone·HSA	247	14300	2870	7.274	11.2

[a] $\Delta\epsilon_{249} = 100(\epsilon_{free} - \epsilon_{bound})/\epsilon_{free}$ (Westphal, 1957).

Recording accurate absorption spectra of steroids bound to PBG necessitated the use of PBG complexes of UV transparent steroids in order to compensate for the protein contribution to the difference spectra. If the transparent steroid is not added to the PBG sample in the reference cuvette a complicated spectrum is obtained (see Figure 6, Stroupe et al., 1975) with overlapping contributions from both protein and steroid. Use of transparent steroids, such as 5α-pregnanedione or DHT, allows acquisition of spectra of steroids bound to PBG but corrected for PBG perturbations.

Conversely, the two low-affinity serum proteins, HSA and AAG, exhibit only weak perturbations of absorption spectra upon binding steroids. Ryan & Gibbs (1970) have reported $\Delta\epsilon_{292} = -270$ M^{-1} cm^{-1} for testosterone binding to HSA; no mention of signal changes at shorter wavelengths was made. Also, no such signal changes in the 240–260-nm range were observed in the present study. Therefore, correction for a protein contribution to the observed spectrum of a steroid bound to HSA is unnecessary. Control experiments indicate that the AAG absorption spectrum changes less than $\Delta\epsilon = 50$ M^{-1} cm^{-1} (the limit of detectability with AAG at 65 μM saturated with progesterone or 5α-pregnanedione) over the wavelengths scanned. A circular dichroism spectrum of the AAG·R-5020 complex compared with that of AAG also indicated no conformational change in AAG upon steroid binding (Wittliff et al., 1977). Thus, no correction for protein perturbation is required for the absorption spectra of steroids bound to AAG.

The spectra and associated data were obtained under conditions where instrumental artifacts should not interfere (Donovan, 1973). The total absorbance was below 2.0 in all

difference spectra over the wavelength range of interest; at wavelengths shorter than 240 nm, sample absorptions were too great to allow meaningful measurements. An additional check on the accuracy of the Cary 15 measurements was made with a Zeiss PMQ II. The single beam instrument gave a molar extinction coefficient of 19 200 M^{-1} cm^{-1} for progesterone bound to PBG, in agreement with the value determined with the double beam instrument. The agreement between single and double beam spectrophotometers precludes instrumental artifacts.

The absorption spectrum of PBG-bound progesterone (Figure 3) reveals the source of the strong minimum at 268 nm seen in Figure 2. The trough at 268 nm results primarily from a sharpening of the absorption band of progesterone associated with PBG, and only to a lesser extent from the shift in λ_{max} from 249 to 245.5 nm. Our earlier assignment of the trough to a large blue shift of λ_{max} to 240 nm (Stroupe & Westphal, 1975a) was incorrect. The sharpening of the absorption band is also responsible at least in part for the increase in ϵ_{max} for all steroids bound to PBG. The chromophore must have a restricted number of vibrational levels available for occupancy when bound to PBG, resulting in a sharpened absorption spectrum (Laskowski, 1970).

The observed sharpening (decrease in θ) cannot, however, explain the total increase in ϵ_{max} since in that case the area of the absorption band would not change. However, this area increases about 10% for all steroids bound to PBG (Table I). The calculated area may be in error due to the assumption of Gaussian shape and because only the front half of the absorption band is experimentally available for measurement. Obviously, the spectrum of medrogestone bound to PBG is not Gaussian (Figure 6), but rather consists of two partially resolved absorption bands. Within these limitations, it appears that in addition to a sharpening of the absorption band, the transition moment for steroid chromophores is increased when bound to PBG.

The increased extinction coefficient and sharpened absorption band are not due to simple solvent effects. It has been reported previously that steroids have the same extinction coefficient in alcohol and water (Fieser & Fieser, 1959), the only difference being an 8-nm red shift for the steroids in water. Using water, ethanol, and heptane as solvents in control experiments, we found that progesterone exhibits the same values for ϵ_{max} and θ, with λ_{max} shifting from 249 to 241 to 230 nm, respectively. This result for the $\pi \rightarrow \pi^*$ absorption band is in contrast to the results for the solvent-sensitive $n \rightarrow \pi^*$ absorption band of progesterone in the same solvents (Stroupe et al., 1975).

The spectral effects on the bound steroid chromophores in the complexes of all three proteins studied are probably not due to hydrogen bonding as suggested earlier (Ryan & Gibbs, 1970). In a hydrogen-bonded progesterone molecule the 3-oxo group would share its unpaired electrons with a loosely bound proton donated by the protein. An extreme model for a hydrogen bonded steroid is the steroid dissolved in a strong mineral acid in which the 3- and 20-oxo groups are protonated. As reviewed by Smith & Bernstein (1963) and confirmed by us,[3] dissolution of progesterone in concentrated sulfuric acid results in only a shift of λ_{max} to the red (about 40 nm) with no change in ϵ_{max}. Use of phenolic compounds as models for 4-ene-3-oxo steroids (Ryan & Gibbs, 1970) is considered invalid because these two classes of chromophores are different. Indeed, a hydrogen-bonded phenolic compound has an increased electron density in the π electron cloud while the converse is true for 4-ene-3-oxo steroids.

The results with HSA and AAG confirm earlier reports on the decrease in extinction coefficient of steroids bound to HSA (Westphal, 1957; Ryan, 1968; Ryan & Gibbs, 1970) and AAG (Ganguly & Westphal, 1968). No ready explanation of the extinction coefficient decrease can be offered. One simple interpretation of the reduced ϵ_{max} would be the assumption that HSA and AAG have heterogeneous binding sites resulting in an increase of θ. Tables I and II show that, for progesterone·HSA, θ bound is equal to θ free; in fact, progesterone bound to AAG exhibits a significantly reduced value of θ. Therefore, the depression of ϵ_{max} cannot be due to a broadening of the absorption spectrum.

A unified explanation of the alterations in ϵ_{max} for steroids bound to protein may be found in the exciton theory of hypochromism (Tinoco, 1960; Rhodes, 1961; Kasha, 1963). Although originally proposed to explain the decreased absorption of polynucleotides, the theory can be used to explain qualitatively the spectral observations for steroid-binding proteins. We suggest that hypochromism in the case of HSA and AAG is the result of a randomly oriented chromophore (the steroid) becoming oriented (bound) with its transition electric dipole moment aligned parallel to the dipole moment of a chromophore in the protein. The hyperchromism of steroids bound to PBG may then be explained by postulating that the bound steroid has its transition moment aligned head to tail with that of a chromophore in PBG. Dynamic coupling between chromophores in proteins and bound ligands has been observed in other binding systems (Athey & Cathou, 1977).

Regardless of the source of the hyperchromism observed with PBG, the origin of the two major features of the difference spectra in Figures 2 and 4 (curve A) can be explained. The negative signal at 268 nm is due primarily to a sharpening of the absorption band of steroids bound to PBG with lesser contributions from the blue shift of the steroid λ_{max}'s and perturbation of the protein (Figure 1). The prominent peak at 294 nm is thought to be the result of a change in charge in the vicinity of a tryptophan residue in the binding site (Stroupe & Westphal, 1975a).

Binding of steroids to PBG is accompanied by profound alterations in the absorption spectra of both the steroid and the protein. These perturbations reflect strong and specific interactions of the steroid molecules with amino acid residues of the binding site; they contrast with the much smaller effects of steroids on the conformation of HSA and AAG.

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Circular Dichroism Study of the Interaction between T4 Gene 32 Protein and Polynucleotides†

J. Greve,*,‡ M. F. Maestre,§ H. Moise,§ and J. Hosoda§

ABSTRACT: The interaction of bacteriophage T4 gene 32 coded protein (DNA melting- or unwinding protein) and the synthetic polynucleotides poly(dA), poly(dT), poly(dA)·poly(dT), and poly[d(A-T)]·poly[d(A-T)] was examined by means of circular dichroism spectroscopy. The protein induced strand separation of the double-stranded molecules at temperatures far below the regular melting temperatures. This denaturation is reversible for poly[d(A-T)]·poly[d(A-T)] but irreversible for poly(dA)·poly(dT). In the complexes formed with the protein the single polynucleotide has a conformation in which the bases are stacked. This conformation closely resembles that of one strand of a poly[d(A-T)]·poly[d(A-T)] molecule at high LiCl concentration. To arrive at this conclusion qualitative rules for the interpretation of the polynucleotide circular dichroism spectra were derived. These rules are: (a) Upon strand separation the minimum in the CD spectrum near 250 nm shifts by about 3 nm to the red. (b) The degree of base stacking in a single-stranded polynucleotide follows from the depth of the minimum in the circular dichroism spectrum near 250 nm. (c) The type of conformation of the single-stranded polynucleotide can be determined from a comparison of the long wavelength part of the circular dichroism spectra of single- and double-stranded polynucleotides. This part of the spectrum has the same shape for a double-stranded and a single-stranded molecule provided the conformations of the separate strands are equal.

Bacteriophage T4 gene 32 coded protein (gp32) was first isolated by Alberts & co-workers (Alberts et al., 1968; Alberts, 1970; Alberts & Frey, 1970). It binds tightly and cooperatively to single-stranded DNA. It facilitates both the denaturation and the renaturation of DNA. Genetic studies have demonstrated that this protein is essential for genetic recombination (Berger et al., 1969; Tomizawa et al., 1966), DNA replication (Epstein et al., 1963), repair of radiation damaged DNA (Wu & Yeh, 1973), and protection of replicating T4 chromosome from degradative activities (Curtis & Alberts, 1976; Mosig & Bock, 1976). In vitro gp32 enhances the rate at which T4 DNA polymerase utilizes single-stranded DNA temperatures by five- to tenfold (Huberman et al., 1971). gp32 is also one of the essential components of the reconstructed DNA replicating apparatus (Alberts et al., 1975; Morris et al., 1975).

It has been suggested that gp32 binds to the phosphate groups of polynucleotides or DNA rather than to the bases so that the bases in the complexes are in exposed positions (Alberts & Frey, 1970; Huberman et al., 1971; Kelly et al., 1976). Sedimentation behavior of gp32 fd DNA complexes indicated

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‡ Current address: Physics Laboratory, Vrije Universiteit, Amsterdam, The Netherlands.
§ Current address: Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720.

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