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Report

Drug Binding to α_1-Acid Glycoprotein Studied by Circular Dichroism[1]

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The interactions of acidic and basic drugs with α_1-acid glycoprotein (α_1-AGP) were investigated using circular dichroism (CD) measurements. Extrinsic Cotton effects were generated by the binding of drugs to α_1-AGP. The CD data suggested the presence of a single binding site on the α_1-AGP molecule. The induced ellipticities of the acidic drug–α_1-AGP system decreased with increasing pH, while the ellipticities for the basic drugs increased with pH. The ellipticities for all drugs were reduced by the addition of fatty acids. Furthermore, the induced ellipticities decreased in the presence of cesium chloride for basic drugs bound to α_1-AGP. The extrinsic Cotton effects therefore appear to result from hydrophobic interaction with α_1-AGP for the acidic drugs and from hydrophobic and electrostatic interactions for the basic drugs.

KEY WORDS: circular dichroism; α_1-acid glycoprotein; binding force; binding site; protein binding of drugs; α_1-acid glycoprotein binding of acidic and basic drugs.

INTRODUCTION

The interaction of drugs with α_1-acid glycoprotein (α_1-AGP) can strongly affect the drug's pharmacokinetic behavior. Both basic and acidic drugs are strongly bound to α_1-AGP (1–4). Circular dichroism (CD) spectroscopy represents a powerful tool to study drug protein interaction, because extrinsic Cotton effects may be induced in the wavelength region of drug chromophores when drugs are bound to α_1-AGP in an asymmetric environment. The induced Cotton effects make it possible to determine the drug's binding parameters. In the work reported here CD has been used to investigate the binding of some basic and acidic drugs to α_1-AGP, in order to elucidate the binding mechanism of drugs to α_1-AGP.

MATERIALS AND METHODS

Materials

Flufenamic acid (Taisho Pharmaceutical Co., Tokyo), diazepam (Kaken Pharmaceutical Co., Tokyo), and imipramine (Yoshitomi Pharmaceutical Co., Fukuoka) were used as supplied. Acenocoumarin was a generous gift from Professor L. H. M. Janssen of the University of Utrecht. α_1-AGP (lot No. 36F-9360) was obtained from Sigma Chemical Co. (St. Louis, Mo.). All other materials were of reagent grade and all solutions were prepared in deionized and distilled water. All of the buffers used were prepared with monobasic and dibasic sodium phosphates. The pH values were checked at 25°C using a standardized pH meter.

Apparatus and Methods

CD measurements were made on a Jasco J-50A recording spectropolarimeter (Tokyo) using 10- and 50-mm cells. α_1-AGP solutions of $1–5 \times 10^{-5}$ *M* (MW 44,100) were used. All solutions were scanned from wavelengths at which no induced optical activity was observed. The induced ellipticity is defined as the ellipticity of the drug–α_1-AGP mixture minus the ellipticity of the α_1-AGP alone at the same wavelength and is expressed as degrees.

Anisotropy factors (g values) were calculated following the method of Chignell (5), using the equation

$$g = \frac{[\theta]}{3300 \times \epsilon}$$

where $[\theta]$ is the molar ellipticity at the wavelength of the CD maximum, calculated with respect to the concentration of the complexed drugs, and ϵ is the molar absorptivity of the CD maximum.

Fluorescence measurements were made using a Hitachi 650-60 fluorescence spectrophotometer (Tokyo). The fluorometric titrations were as follows: α_1-AGP solutions of $2.0–10 \times 10^{-6}$ *M* were titrated by successive additions of drugs (to

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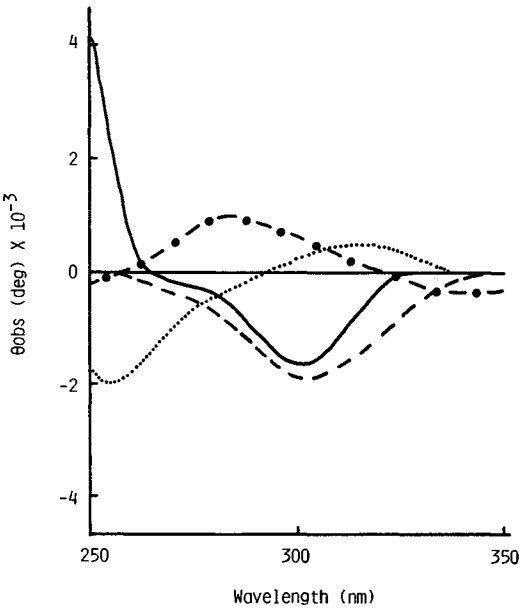

Fig. 1. Induced CD spectra of some drugs (5×10^{-5} *M*) in the presence of α_1-AGP (1×10^{-5} *M*) at 25°C and pH 7.4. (– – – –) Acenocoumarin; (— • —) flufenamic acid; (·····) diazepam; (——)imipramine.

give a final concentration of 0.5–12 $\times$ 10^{-6} *M*), and the fluorescence intensity was measured (excitation at 290 nm and emission at 340 nm). At the selected wavelength, the drugs did not contribute to the fluorescence.

Dialysis experiments were performed using a Sanko Plastic dialysis cell (Fukuoka). α_1-AGP solution (2 ml) was poured into one compartment and 2 ml of drug solution was poured into the opposite compartment. Adsorption of the drugs onto the membrane was negligible. After a 6-hr dialysis, the free concentrations of the drugs were assayed by high-performance liquid chromatography (HPLC).

RESULTS AND DISCUSSION

The induced CD spectra of the binding of the drugs to α_1-AGP are shown in Fig. 1. In order to assess the degree of the optical perturbation of the electronic transition of the drugs studied, the *g* values were determined (Table I), because the molar ellipticity is directly proportional to the molar absorptivity of the drug (6). Imipramine gave the greatest *g* values. The difference of the *g* values for Table I can be explained by the spatial arrangement and the rigidity of the complex.

Table I. CD Characteristics of Some Drugs Bound to α_1-AGP at 25°C and pH 7.4[a]

Drug	λ_{max} (nm)	[θ] ($\times 10^4$)	*g* ($\times 10^{-4}$)
Acenocoumarin	305	−1.96	−2.97
Flufenamic acid	285	1.39	2.34
	340	−0.44	−5.20
Diazepam	255	−2.57	−6.28
	320	0.63	2.27
Imipramine	300	−1.92	−29.1

[a] The values are the averages of three determinations.

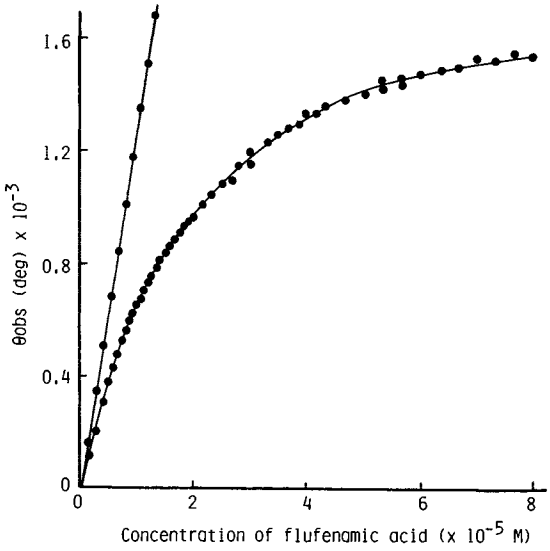

Fig. 2. Estimation of free and bound drug by the method of Rosen (7): [α_1-AGP] = 2×10^{-5} *M*.

Figure 2 shows the induced ellipticity at 330 nm for various ratios of flufenamic acid to α_1-AGP. Following the method of Rosen (7), a tangent to the plot of induced ellipticity against drug to α_1-AGP ratios was drawn, providing an estimate of the free and bound drug concentration. This method allows precise interpretation in terms of binding constants when a single site is involved. When more than one site contributes to the ellipticity, the method is less precise. This problem is shared by other spectroscopic techniques and is not peculiar to the CD technique. Figure 3 shows the Scatchard plot for flufenamic acid to α_1-AGP, using data

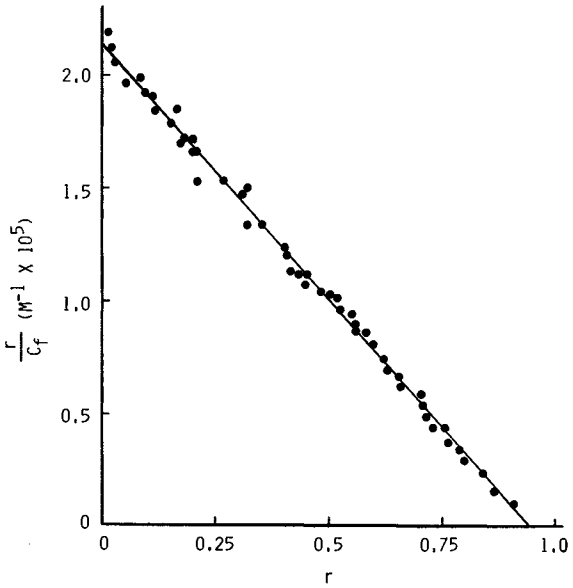

Fig. 3. Scatchard plots of flufenamic acid–α_1-AGP interaction, using data of Fig. 1 (see text).

Table II. Binding Parameters of Some Drugs to α_1-AGP at 25°C and pH 7.4, Obtained from CD and Fluorescence Methods[a]

Drug	CD		Fluorescence	
	N	K ($M^{-1} \times 10^5$)	N	K ($M^{-1} \times 10^5$)
Acenocoumarin	1.0 ± 0.1	2.2 ± 0.3	1.1 ± 0.2	2.2 ± 0.3
Flufenamic acid	0.7 ± 0.2	1.4 ± 0.3	0.8 ± 0.2	0.8 ± 0.2
Diazepam	0.9 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	2.5 ± 0.4
Imipramine	0.6 ± 0.1	8.9 ± 0.9	0.8 ± 0.1	9.3 ± 1.0

[a] The values are the averages of four determinations.

derived in the manner described above, and Table II gives the binding parameters. The number of binding sites and the binding constants K obtained from CD measurements are in good agreement with those obtained from the fluorescence quenching technique as shown in Table II. It should be noted that the acidic drug studied here is bound to α_1-AGP with a high affinity, almost the same as the basic drugs.

The effects of pH on the ellipticities of drug–α_1-AGP complexes were examined. The high drug-to-α_1-AGP ratios compared to those used in the dialysis experiments were necessary to obtain a suitable signal-to-noise ratio for quantitative measurements. As shown in Fig. 4, the induced ellipticities of the basic drugs are increased as the pH is raised to 9.0, whereas the ellipticities of the acidic drugs are slightly decreased by raising the pH. In sharp contrast to the ellipticity, the free concentrations of the basic drugs were decreased by increasing the pH from 6.5 to 8.5, while the free concentrations of the acidic drugs were increased with pH (Table III). The ultraviolet spectra of all the drugs are negligibly or slightly changed between 6.5 and 8.5. Therefore, the pH dependence of the induced ellipticities can be explained on the basis of the conformational changes in α_1-AGP rather than the changes in the degree of ionization of the drug molecule. In fact, the microenvironmental changes

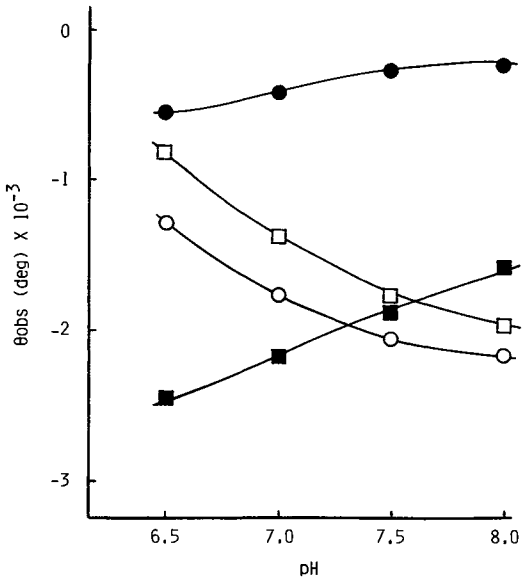

Fig. 4. The pH dependence of the observed ellipticities of drugs (5×10^{-5} M) in the presence of α_1-AGP (1×10^{-5} M). (■) Acenocoumarin system at 305 nm; (●) flufenamic acid system at 340 nm; (□) diazepam system at 260 nm; (○) imipramine system at 300 nm.

in α_1-AGP have recently been found to occur around the physiological pH (8). The hypothesis of the conformational change for the pH profile may also be supported by the finding (9,10) that the drugs studied here were sensitive to the structure transition of human serum albumin, called the N–B transition (11).

To elucidate the nature of binding of acidic and basic drugs, the effects of oleic acid and cesium chloride on the extrinsic Cotton effects of flufenamic acid and imipramine–α_1-AGP complexes were examined. The high drug-to-protein ratios of 5.0 were also chosen to obtain a quantitative signal because of the induced CD generated by the binding of drugs to a single site on the α_1-AGP. As can be seen in Fig. 5, the induced ellipticities of the two α_1-AGP complexes were decreased in the presence of oleic acid, as expected from the previous results of the phenprocoumon–α_1-AGP system (13). Moreover, the induced ellipticities of the imipramine complex were significantly decreased by cesium chloride. The low drug-to-protein ratios of 1.0 were used to look at the primary binding, differing from the ratios of CD experiments. The dialysis experiments indicated that the free concentrations of imipramine were significantly increased in the presence of oleic acid and cesium chloride (Table IV). Thus, the effects of oleic acid and cesium chloride on the ellipticities of the drug–α_1-AGP complexes may be interpreted as displacement rather than a conformational change of the protein.

The present limited data suggest that hydrophobic as well as electrostatic interactions are involved in the binding of basic drugs to α_1-AGP, while ionic forces do not play a crucial role for the acidic drug–α_1-AGP interaction. This inference is consistent with previous suggestions that the high-affinity site of the acidic drug on α_1-AGP is located in a hydrophobic area of the protein (12) and that the binding of the basic drugs is due to the contribution of electrostatic and/or hydrophobic interactions (13,14).

Table III. Effects of pH on the Free Fraction of Flufenamic Acid and Imipramine in α_1-AGP Solution at 25°C[a]

pH	Free fraction (%)	
	Flufenamic acid	Imipramine
6.5	74.6 ± 1.9	60.3 ± 2.0
8.5	94.5 ± 1.6*	39.2 ± 1.6*

[a] α_1-AGP (1×10^{-5} M) and drugs (5×10^{-5} M) were used throughout these experiments. Each value represents the mean ± SD.
* $P < 0.001$; significantly different from the pH 6.5 value.

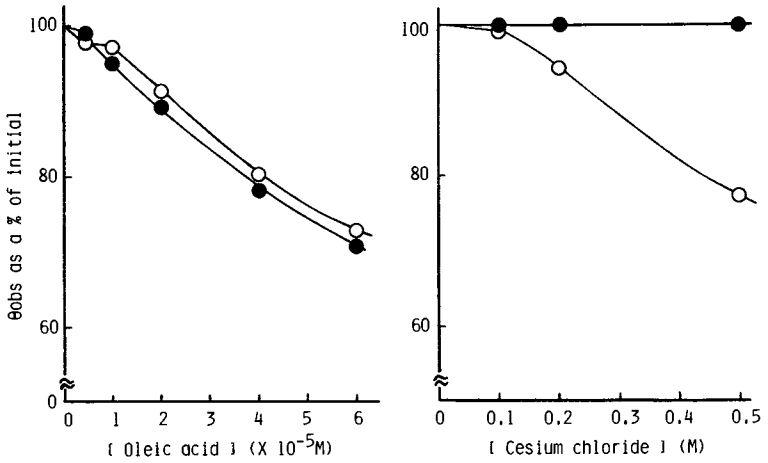

Fig. 5. Effects of oleic acid (left) and cesium chloride (right) on the observed ellipticities of drug–α_1-AGP complexes at 25°C and pH 7.4. (●) Flufenamic acid system; (○) imipramine system. [α_1-AGP] = 1×10^{-5} *M*; [drug] = 5×10^{-5} *M*.

Table IV. Effects of Oleic Acid and Cesium Chloride on the Free Fraction of Flufenamic Acid or Imipramine in α_1-AGP Solution at 25°C and pH 7.4[a]

	Free fraction (%)				
		With oleic acid		With cesium chloride	
Drug	Without displacer	0.03 m*M*	0.06 m*M*	0.2 *M*	0.5 *M*
Flufenamic acid	89.4 ± 0.7	93.1 ± 1.1*	95.6 ± 1.5*	89.5 ± 1.6	89.5 ± 1.6
Imipramine	59.0 ± 1.7	66.7 ± 1.6*	73.0 ± 1.7**	62.1 ± 1.2***	65.0 ± 1.2*

[a] α_1-AGP (1×10^{-5} *M*) and drugs (5×10^{-5} *M*) were used throughout these experiments. Each value represents the mean ± SD.
* $P < 0.01$; significantly different from the value without displacer.
** $P < 0.001$; significantly different from the value without displacer.
*** $P < 0.05$; significantly different from the value without displacer.

REFERENCES

1. S. Urien, E. Albengres, R. Zini, and J. P. Tillement. *Biochem. Pharmacol.* 31:3687–3689 (1982).
2. S. El-Gamal, U. Wollert, and W. E. Müller. *J. Pharm. Sci.* 72:202–205 (1983).
3. P. A. Routledge. *J. Clin. Pharmacol.* 22:499–506 (1986).
4. M. Otagiri, T. Maruyama, T. Imai, A. Suenaga, and Y. Imamura. *J. Pharm. Pharmacol.* 39:416–420 (1986).
5. C. F. Chignell. *Mol. Pharmacol.* 5:455–462 (1969).
6. W. E. Müller and U. Wollert. *Naunyn-Schmiedeberg Arch. Pharmacol.* 278:301–312 (1973).
7. A. Rosen. *Biochem. Pharmacol.* 19:2075–2081 (1970).
8. M. L. Freidman, K. T. Schlueter, T. L. Kirley, and H. B. Halsall. *Biochem. J.* 232:863–867 (1985).
9. J. Wilting, B. J. 'THart, and J. J. DeGier. *Biochim. Biophys. Acta* 626:291–298 (1980).
10. U. Kragh-Hausen. *Pharmacol. Rev.* 33:17–53 (1981).
11. J. Wilting, M. M. Weideman, A. C. Roomer, and J. H. Perrin. *Biochim. Biophys. Acta* 579:469–473 (1979).
12. M. Otagiri, T. Maruyama, T. Imai, and Y. Imamura. *J. Pharm. Sci.* 76:646–649 (1987).
13. S. El-Gamal, U. Wollert, and W. E. Muller. *J. Pharm. Pharmacol.* 34: 152–157 (1981).
14. J. Schley, M. Nundel, E. Reidel, and B. Muller-Oerlinghausen. *Arzneimmittel-Forsch./Drug Res.* 29:106–108 (1979).