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Thermodynamics of the Binding of Phenothiazines to Human Plasma, Human Serum Albumin and α_1-Acid Glycoprotein: a Calorimetric Study

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Abstract—A flow microcalorimetric study has been carried out to investigate the interactions between phenothiazine derivatives and human plasma, human serum albumin (HSA) and α_1-acid glycoprotein (AGP) at pH 7·4 and 37°C. The direct analyses of enthalpic titration curves allowed the determination of the binding enthalpy change (ΔH), the apparent binding constant (K), and the number of the binding sites (n), as well as the evaluation of the apparent free energy (ΔG), and entropy (ΔS) changes. The overall binding of phenothiazines was exothermic with negative ΔH, which was compensated for by changes in ΔS. The values of ΔG were relatively insensitive to variation in the molecular details of the binding reaction. HSA possessed two classes of binding sites for phenothiazines. The first ($n_1 = 1$), with high affinity ($K_1 = 10^5$–10^6 M^{-1}) was characterized by small negative ΔH and positive ΔS values due to hydrophobic interaction. The second class of sites had a low affinity ($K_2 = 10^3$–10^4 M^{-1}) and high capacity ($n_2 = 3$–8) and contributed to the negative ΔH and ΔS values. The binding and thermodynamic parameters were influenced by the aliphatic side chain moieties on the phenothiazine nucleus. On the other hand, the drugs were bound to AGP at a single common binding site with a binding affinity of the order of 10^4M^{-1}, characterized by negative ΔH and ΔS values, which partially reflected the effect of a van der Waals' interaction. In plasma the drugs were bound as a result of an enthalpy-driven type of reaction having large negative ΔH and a large binding capacity resulting from the presence of AGP and HSA.

The binding of phenothiazines to plasma proteins and erythrocyte cells has been examined to ascertain its possible clinical relevance (Freedberg et al 1979; Lund 1980; Piafsky 1980). Phenothiazines are basic (cationic) compounds, bound more than 90% to plasma proteins (Bickel 1975; Verbeeck et al 1983). As with other drugs, most studies on phenothiazines have been concerned with their binding to albumin (Vallner 1977; Hulshoff & Perrin 1977). Their interactions with albumin tend to be of the high-capacity and low-affinity type, where their binding constants are mostly around 10^3 M^{-1}. On the other hand, a high-affinity but low-capacity type of binding has been reported for many basic drugs with α_1-acid glycoprotein (AGP) (Piafsky & Bórga 1977). For almost all phenothiazines the presence of one common binding site on AGP has been demonstrated by their high association constants (Kornguth et al 1981; Müller & Stillbauer 1983). Quantitative studies of the interactions between phenothiazines and plasma proteins have usually been restricted to estimation of binding constants by equilibrium dialysis (Verbeeck et al 1983; Kornguth et al 1981), fluorescent probes (Sugiyama et al 1985) and some other techniques (El-Gamal et al 1983). However, no information has so far been obtained about the thermodynamics of the binding process in human plasma.

In the present work the thermodynamics and the mechanisms of complex formation between the drugs and plasma proteins have been studied by a microcalorimetric method carried out at 37°C to determine the enthalpy change (ΔH) and the number of binding sites (n) as well as the binding constant (K), without using Scatchard plots. The free energy change (ΔG) and entropy change (ΔS) on formation of the complex have been calculated with the well-known thermodynamic formula: $\Delta G = -RT \ln K = \Delta H - T\Delta S$.

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Materials and Methods

Materials

Human serum albumin (HSA, Fraction V) and α_1-acid glycoprotein (AGP) were purchased from Sigma (St Louis MO, USA) and used without further purification. Human plasma was supplied from the Red Cross Blood Centre (Fukuoka, Japan). These proteins were dissolved in 10 mM isotonic sodium phosphate buffer, pH 7·4, to give the following concentration initially: 10% v/v (plasma), 0·1% w/v (AGP), and 0·25–0·50% w/v (HSA). Chlorpromazine hydrochloride (CPZ), promazine hydrochloride (PMZ) and trifluoperazine dihydrochloride (FPRZ) were purchased from Sigma, and fluphenazine dimaleate (FPZ), perphenazine dimaleate (PPZ), prochlorperazine dimaleate (CPRZ) and perazine dimaleate (PRZ) were from Yoshitomi Pharm. Ind. Ltd. (Osaka, Japan).

Calorimetry

Calorimetric titrations were carried out at 37·0 ± 0·05°C with either a differential flow microcalorimeter (Yamamoto & Aki 1988) or an LKB microcalorimeter (2277 Bio Activity Monitor, Bromma, Sweden) (Suurkuusk & Wadsö 1982), and a Rikadenki chart recorder (Tokyo, Japan). The instruments were calibrated both electrically and chemically. The chemical calibrations was accomplished by the dilution of sucrose solution (Gucker et al 1939). The sensitivity used was

10 μW full scale, and the background noise was less than $\pm 0{\cdot}05$ μW. The reaction solutions were introduced at equal flow rates (0·12 mL min^{-1}) into the calorimeter through Tigon tubing with a peristaltic pump (Gilson minipuls 2, Villers-Le-Bel, France). The basal recorder line was established by the heat of dilution of protein. Solutions of the drugs in different concentrations were then introduced sequentially into the buffer flow. The heat of mixing was proportional to the recorded steady-state value; 2 mL was necessary to reach steady state. The heat of dilution of each drug was measured separately and subtracted from the heat of mixing to obtain the heat of reaction.

All solutions were prepared in a 10 mM aqueous sodium phosphate buffer solution (pH 7·4, made isotonic with sodium chloride). The concentrations of HSA (mol. wt 69 000) and AGP (mol. wt 44 100) in the initial and final calorimetric solutions were determined by the UV absorption using λ_{max} nm (E 1% 1 cm): 278 (5·30) (Cohn et al 1947) and 278 (8·93) (Schmidt 1953), respectively. The protein concentration in plasma was estimated by the Lowry method (Lowry et al 1951) with HSA as a standard and that of AGP was measured with Partigen M plates (Hoechst Japan, Tokyo). The total concentrations of phenothiazines in the final calorimetric solutions were measured by UV absorption.

Data analysis

The heat of binding (ΔQ) is proportional to the amount of drug-protein complex formed with the total protein concentration fixed at P_t, as follows:

$$\Delta Q = \Delta H\ F_r\ D_b \quad (1)$$

where ΔH is the binding enthalpy per mole of a drug and D_b is the bound drug concentration at constant flow rate, F_r. Assuming that a drug interacts with protein by stepwise equilibrium, the binding model derived from the law of mass action is

$$D_b = P_t \sum_{i=1}^{m} (n_i\ K_i\ D_f)/(1 + K_i\ D_f) \quad (2)$$

where m is the number of classes of independent binding sites, n_i the number of binding sites in the ith class with the binding constant K_i, and D_f is the free drug concentration related to the equation,

$$D_t = D_b + D_f \quad (3)$$

In the simplest case of drug binding where only one class of binding sites is involved (m = 1), equation 1 can be expressed as a function of D_t by use of equation 2 and 3, as follows:

$$\Delta Q = \Delta H\ F_r\ (A - \sqrt{A^2 - 4n_1\ P_t\ D_t})/2 \quad (4)$$

where

$$A = 1/K_1 + n_1\ P_t + D_t \quad (5)$$

For a two-class binding model (m = 2), equation 2 can be expressed in the form,

$$(D_t - D_f)/P_t = n_1\ K_1 D_f/(1 + K_1\ D_f) + n_2\ K_2 D_f/(1 + K_2\ D_f) \quad (6)$$

This expression yields,

$$D_f^3 + A\ D_f^2 + B\ D_f + C = 0 \quad (7)$$

where

$$A = P_t\ (n_1 + n_2) + (1/K_1 + 1/K_2) - D_t$$

$$B = P_t\ (n_1/K_2 + n_2/K_1) - D_t(1/K_1 + 1/K_2) + 1/K_1\ K_2$$

$$C = -D_t/K_1\ K_2 \quad (8)$$

The value of D_f can be found by solving the cubic equation 7, ΔQ in equation 1 can be estimated by use of equation 3. The binding and thermodynamic parameters, K_i, n_i and ΔH, were computed from actual measurements with a computer program for the non-linear least squares regression method. An initial value of ΔH was estimated from the slope of the initial linear plots of the titration curve. The program was run on a FACOM M-380R digital computer at Fukuoka University.

Results

Heat of binding of phenothiazines to plasma, AGP and HSA

The results of curve-fitting for the drug binding are shown in Figs 1–3. The calorimetric data are shown best as a plot of the heat produced per mole of protein (ΔQ, J mol^{-1}) versus the total concentration (D_t) of drug in the final calorimetric solution. Points show the values calculated from the measurements and solid lines represent the computer-generated best-fit curves, assuming a one- or two-class binding model. The estimated values of binding and thermodynamic parameters for a one-class binding model are listed in Table 1.

The parameter, ΔH_m in Table 1, is the molar enthalpy change for complete binding to all sites of protein, and the values were calculated from the equation:

$$\Delta Q_{max} = \Delta H_m\ F_r\ P_t \quad (9)$$

where ΔQ_{max} is the heat effect estimated from the plateau value of the titration curve. In the comparison of ΔH_m with the total amount of binding enthalpy change of all binding

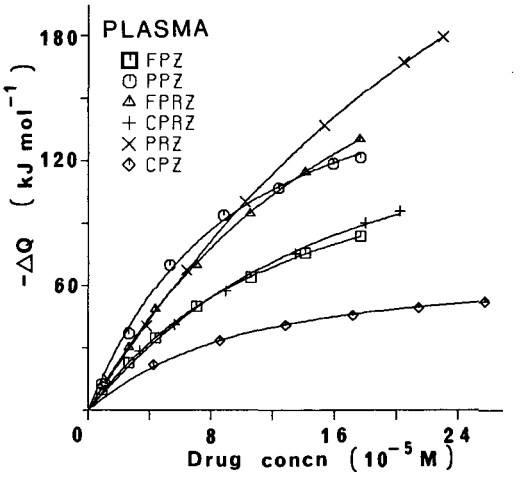


FIG. 1. Calorimetric titration curves for the binding of phenothiazines to human plasma at 37°C and pH 7·4 in 10 mM sodium phosphate buffer. The final concentration of plasma was in the range of $3{\cdot}87$–$5{\cdot}13 \times 10^{-5}$ M by the Lowry method (Lowry et al 1951) with HSA as a standard. Points show experimental values calculated from the single calorimetric titration. Solid lines represent computer-generated best-fit curves assuming a one-class binding model with binding parameters given in Table 1.

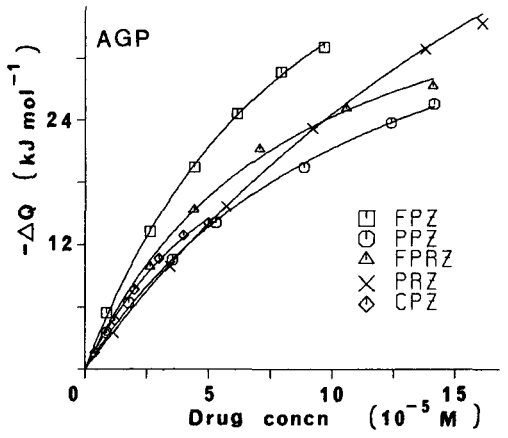


FIG. 2. Calorimetric titration curves for the binding of phenothiazines to AGP at 37°C and pH 7·4 in 10 mM sodium phosphate buffer. The final concentration of AGP was in the range of $1{\cdot}12$–$1{\cdot}24 \times 10^{-5}$ M. Each point shows the mean value of three measurements. Solid lines represent computer-generated best-fit curves assuming a one-class binding model with binding parameters given in Table 1.

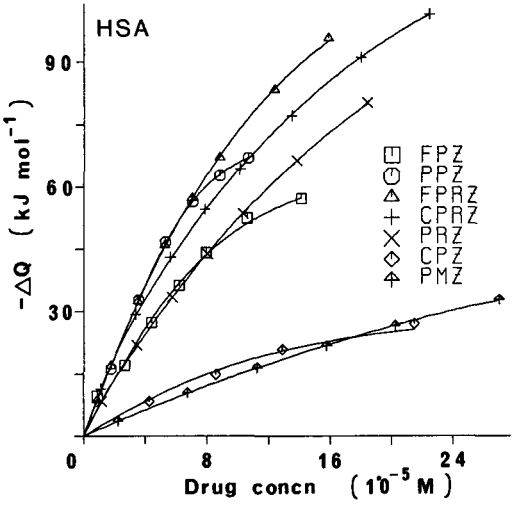


FIG. 3. Calorimetric titration curves for the binding of phenothiazines to HSA at 37°C and pH 7·4 in 10 mM sodium phosphate buffer. The concentration of HSA was 0·25% for FPZ, PPZ, FPRZ, CPRZ and PRZ, and 0·5% for CPZ and PMZ, and final concentration was in the range from $1{\cdot}85$ to $2{\cdot}22 \times 10^{-5}$ M and from $3{\cdot}21$ to $3{\cdot}84 \times 10^{-5}$ M, respectively. Each point shows the mean value of three measurements. Solid lines represent computer-generated best-fit curves assuming a two-class binding model with binding parameters given in Table 2.

sites, $n\Delta H$, an agreement between the two values validates the mathematical model, indicating that n is the molar ratio of the drug-protein complexation and ΔH_m the extent of the binding area or binding capacity on the protein molecule.

The interaction of phenothiazines with plasma proceeded exothermically with increasing concentrations of drug (Fig. 1). The values of ΔH and ΔS varied widely from $-47{\cdot}5$ to -209 kJ mol^{-1} and from -75 to -698 J K^{-1} mol^{-1}, respectively. The values of $-\Delta H$ and $-\Delta S$ fell with decrease in the length of aliphatic side chains at the position N-10 of the phenothiazine nucleus (Table 1). On the other hand, ΔG values calculated from the K values remained steady, amounting from $-22{\cdot}8$ to $-24{\cdot}7$ kJ mol^{-1}. Therefore, the

Table 1. Binding and thermodynamic parameters of phenothiazines binding to human plasma proteins considering one-class binding model at pH 7·4 and 37°C.

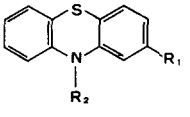
	K (10^4 M^{-1})	n	$-\Delta H$*	$-\Delta H_m$*	$-\Delta G$*	$-\Delta S$**	MSE***
Plasma							
FPZ	0·92	0·7	209	141	23·5	−598	0·007
PPZ	1·36	1·0	184	181	24·5	−513	0·006
FPRZ	1·07	2·0	116	225	23·9	−297	0·008
CPRZ	1·15	1·6	97·2	151	24·1	−236	0·009
PRZ	0·70	3·1	69·5	245	22·8	−150	0·010
CPZ	1·46	1·3	52·3	66·5	24·7	−89	0·006
PMZ	1·22	1·3	47·5	60·6	24·3	−75	0·004
AGP							
FPZ	1·48	1·8	31·1	54·5	24·8	−20	0·003
PPZ	1·01	1·4	31·0	42·6	23·8	−23	0·003
FPRZ	1·62	1·4	29·1	39·6	25·0	−13	0·006
CPRZ	1·80	1·6	28·7	46·8	25·3	−11	0·004
PRZ	1·09	2·4	29·9	70·9	24·0	−19	0·005
CPZ	2·92	1·0	27·2	27·7	26·5	−2	0·004
PMZ	0·99	1·0	28·5	28·0	23·7	−16	0·004
HSA							
FPZ	6·19	3·5	20·0	68·9	28·4	27	0·022
PPZ	15·8	3·9	19·9	77·2	30·8	35	0·010
FPRZ	4·87	6·1	20·0	120	27·8	25	0·024
CPRZ	4·91	6·7	17·5	116	27·8	33	0·043
PRZ	1·76	7·2	18·0	127	25·2	23	0·014
CPZ	1·72	3·1	12·4	37·8	25·1	41	0·007
PMZ	0·74	8·2	7·9	61·8	23·0	49	0·004

* kJ mol^{-1}, ** J K^{-1} mol^{-1}, *** mean of squared errors.

interactions were assumed to be driven by the enthalpic contribution to the free energy change.

Fig. 2 shows the calorimetric titration curves of phenothiazines binding to AGP. Calorimetric data fitted well to the one-class binding model with small values of the mean of squared errors (MSE) as shown in Table 1. Stoichiometrically, no significant changes were recognized among the drugs; all were bound to AGP at only one binding site (n = 1–2) with binding constants of the order of 10^4 M^{-1} and a ΔG for the binding of around -25 kJ mol^{-1}. The values of $-\Delta H$ and $-\Delta S$ were within the limits of 27·2–31·1 kJ mol^{-1} and 2–23 J K^{-1} mol^{-1}, respectively. Therefore, the binding was assumed to be less influenced by the C-2 substituent R_1 and the N-10 substituted side chain R_2 on the phenothiazine nucleus.

FPZ	R_1=CF$_3$	R_2=C$_3$H$_6$N(piperazine)C$_2$H$_4$OH
PPZ	R_1=Cl	R_2=C$_3$H$_6$N(piperazine)C$_2$H$_4$OH
FPRZ	R_1=CF$_3$	R_2=C$_3$H$_6$N(piperazine)CH$_3$
CPRZ	R_1=Cl	R_2=C$_3$H$_6$N(piperazine)CH$_3$
PRZ	R_1=H	R_2=C$_3$H$_6$N(piperazine)CH$_3$
CPZ	R_1=Cl	R_2=C$_3$H$_6$N(CH$_3$)$_2$
PMZ	R_1=H	R_2=C$_3$H$_6$N(CH$_3$)$_2$



Phenothiazine binding to HSA was characterized by a small ΔH and a large positive ΔS. The values of $-\Delta H_m$ were larger than those for AGP; the drugs had a high capacity for binding sites on the HSA molecule (n > 3). Although the calorimetric data for binding of phenothiazines to HSA fitted the one-class binding model, with large values of MSE (Table 1), a two-class binding model with independent binding sites of different affinities gave better agreement between the data and the curves (Fig. 3). The best-fit parameters of ΔH, K_1, K_2, n_1 and n_2 for two classes of binding sites and MSE are shown in Table 2. These results indicated that the drugs were bound to HSA on at least two classes of independent binding sites containing one strong binding site of $n_1 = 1$ with the binding constant of $K_1 = 10^5$–10^6 M^{-1} and

Table 2. Parameters of phenothiazines binding to HSA for two classes of binding sites at pH 7·4 and 37°C.

Drug	K_1 (10^5 M^{-1})	n_1	K_2 (10^4 M^{-1})	n_2	$-\Delta H^*$	MSE**
FPZ	1·63	1·0	6·30	3·0	20·0	0·006
PPZ	2·06	1·5	14·6	2·4	19·9	0·006
FPRZ	4·65	1·2	2·59	5·5	20·0	0·005
CPRZ	10·7	0·9	1·16	6·0	22·4	0·008
PRZ	8·41	1·0	1·23	8·2	18·0	0·003
CPZ	0·74	1·0	2·44	2·7	12·4	0·003
PMZ	0·75	2·6	0·45	8·0	6·0	0·002

* kJ mol^{-1}, ** mean of squared errors.

some weak binding sites of $K_2 = 10^3$–10^4 M^{-1}. The value of ΔH for each drug was independent of the binding model, and the drugs with an *N*-methylpiperazinyl group in R_2 showed a more negative ΔH than those with a dimethylamino group.

Thermodynamics for the binding sites of plasma proteins

To characterize the binding site of each plasma protein, the molar enthalpy change of protein for the ith class of binding sites (ΔH_{mi}) was calculated. The reaction enthalpy change obtained by the calorimetric measurement is a composite quantity resulting from the algebraic summation of the enthalpy change of the ith class of binding sites for drug-protein complexation.

$$\Delta H = f_1 \Delta H_1 + f_2 \Delta H_2 + \ldots + f_i \Delta H_i + \ldots$$
$$= n_1/n \, \Delta H_1 + n_2/n \, \Delta H_2 + \ldots + n_i/n \, \Delta H_i + \ldots (10)$$

where, f_i is the fraction of drug binding contributing to the ith class, ΔH_i the intrinsic enthalpy change of binding of the drug for the ith class of binding sites and n the total number of binding sites of all classes as follows:

$$n = \Sigma n_i \quad (11)$$

And then, the molar enthalpy change of protein (ΔH_m) is shown by

$$\Delta H_m = \Sigma \, n_i \, \Delta H_i \quad (12)$$

The combination of equations 10–12 gives ΔH_{mi} as follows:

$$\Delta H_{mi} = n_i \, \Delta H \quad (13)$$

On the other hand, the free energy and the entropy changes in the ith class of binding sites are calculated by the following equations.

$$\Delta G_{mi} = -RT \ln n_i K_i \quad (14)$$

$$\Delta S_{mi} = (\Delta H_{mi} - \Delta G_{mi})/T \quad (15)$$

In Fig. 4, the $\Delta H_m - \Delta S_m$ relationships are shown for phenothiazines binding to HSA, AGP, and plasma, respectively. HSA clearly has two binding sites; the first class being characterized by a small negative enthalpy change, $-29 < \Delta H_{m1} < -12$ kJ mol^{-1} and a positive entropy change, $11 < \Delta S_{m1} < 63$ J K^{-1} mol^{-1}, and the second class having a low affinity was characterized by large negative ΔH_{m2} and ΔS_{m2} values. The linear relationships between enthalpy and entropy changes for plasma, AGP and each class of the binding sites on HSA indicate that all the drugs are bound to a common binding site on the individual plasma protein. Since the value ΔH_m is the extent of the binding area for the drug binding to protein as described above, plasma has a large capacity where ΔH_m for the drug binding to plasma is resultant of the sum of ΔH_{m1} and ΔH_{m2} for HSA and ΔH_m for AGP.

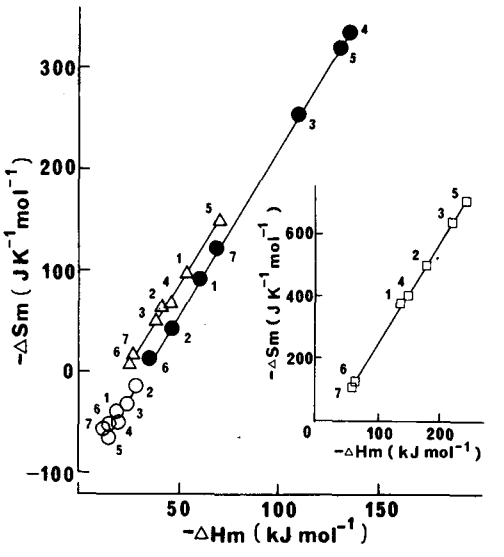


FIG. 4. Relationships between the enthalpy and the entropy changes in phenothiazines binding to plasma proteins. ○: the first class of binding sites in HSA, ●: the second class of binding sites in HSA, △: AGP binding sites, □: plasma binding sites. 1 FPZ, 2 PPZ, 3 FPRZ, 4 CPRZ, 5 PRZ, 6 CPZ, 7 PMZ.

Discussion

Enthalpic titration measurements of the interactions of the phenothiazines with human plasma and plasma proteins have been analysed thermodynamically to determine their stoichiometric binding and thermodynamic characteristics. The thermodynamic parameters can be interpreted in terms of the forces that stabilize the protein-ligand complex. In general, the contribution to positive enthalpy and entropy changes arises from the occurrence of a large degree of hydrophobic interaction; while the sources of negative enthalpy and entropy changes arise from van der Waals' interaction and hydrogen bonding formation in low dielectric media. Although the electrostatic forces contribute to the positive entropy change, the enthalpy change is expected to be very small or almost zero for purely electrostatic interactions (Ross & Subramanian 1981). From a purely pragmatic point of view, ΔG determines the direction of any spontaneous change in the protein-ligand equilibrium under specific conditions. In each case described in this study, however, the ΔG changed by no more than 3 kJ mol^{-1} for the binding of the drugs to plasma or the individual plasma proteins (Table 1). Thus, ΔG values are relatively insensitive to variation in the molecular details of the binding reaction.

Under our experimental conditions, the overall binding of the drugs was exothermic as indicated by the negative sign of the enthalpy change, which was compensated for with a variation of the entropy change (Fig. 4). The values of ΔH, ΔS and ΔH_m for phenothiazines binding to plasma, were more negative than those for AGP and HSA binding. These thermodynamic parameters result from contributions from

the overall binding to individual plasma proteins in plasma. In the present study, the binding capacity of the plasma was the sum of that for AGP and for HSA, indicating that the drugs were bound mainly to AGP and HSA in plasma. Furthermore, the large negative values of ΔS suggest that the drugs are stabilized in plasma by a binding equilibrium.

Recent reports have indicated that AGP is an important binding protein in plasma for basic drugs such as phenothiazines, the presence of one high-affinity binding site alone therein has been proved by the high association constants obtained (Kornguth et al 1981; Verbeeck et al 1983; Schley & Müller-Oerlinghausen 1986). Müller & Stillbauer (1983) indicated that a single common binding site with high affinity on AGP mediates the binding of various basic drugs such as CPZ, propranolol and imipramine with AGP. A few observations suggest that this binding site represents a remote hydrophobic area within the protein but not the carbohydrate part of the glycoprotein molecule (El-Gamal et al 1982; Brunner & Müller 1985). By investigating the interaction of several phenothiazines with AGP using circular dichroism, El-Gamal et al (1983) suggest that the structural parameters, except lipophilicity, are more important and that for the intensity of the extrinsic Cotton effects the substituent R_1 is much more important than the aliphatic side chain R_2.

From the thermodynamic analysis of the titration curves, it was found that the phenothiazines were bound at one class of binding site on AGP (Table 1). Also the linear relationship between ΔH_m and ΔS_m for AGP (Fig. 4) supported that the drugs binding to AGP was mediated mainly by one common high-affinity binding site on the protein. The binding of phenothiazines to AGP was characterized by the negative values of ΔH and ΔS not being so influenced by the variation of R_1 and R_2 on the phenothiazine nucleus. Therefore, it is suggested that the interactions between the two molecules are dominated by van der Waals' interaction with approximately one mole of drug per mole of AGP.

The nature of the binding site for the phenothiazines on serum albumin has been widely investigated and discussed. Several authors have demonstrated a correlation between the hydrophobic characteristics and the binding affinities of phenothiazines, and the binding to albumin is considered to be the result of hydrophobic interaction (Krieglstein et al 1972; Huang & Gabay 1974; Hulsoff & Perrin 1977). On the other hand, Sharples (1976) suggests that the major factor in binding of the tricyclic tranquillizers and antidepressants to HSA is electrostatic, with only a minor contribution from hydrophobic parameters.

Phenothiazine binding to HSA was characterized by more than one class of binding site, containing a strong binding site with a high affinity constant K_1 in the range of 10^5 to 10^6 M^{-1} (Table 2). In the HSA molecule, the first class of binding site with a high affinity was characterized by small negative ΔH_m and positive ΔS_m values (Fig. 4), which reflect the hydrophobic interaction. The forces of the hydrophobic interaction appeared to exert a certain influence in the R_2 moieties. For instance, FPRZ, CPRZ and PRZ with a shorter methylpiperazinylpropyl side chain were bound to HSA more strongly than other drugs with either the dimethylpropylamino or 2-hydroxyethylpiperazinylpropyl side chain for the first class of binding sites. With FPZ and PPZ, however, the degree of binding was decreased further by the introduction of a hydrophilic group (—OH) at the end of R_2: steric effects must have played an important role here. The K_2 values of FPZ and PPZ were much higher than those of other phenothiazines (Table 2). The binding of those having a substituted R_2 side chain must be governed by other factors, e.g. ionic binding, in addition to the hydrophobic interaction. Therefore, only hydrophobic interaction might contribute to the first class of binding sites, while other binding mechanisms such as ionic binding, hydrogen bonding, and steric effects, must be involved in the second class of binding sites for the drugs binding to HSA.

In conclusion, plasma was found to have a large binding capacity to the drugs, which was resultant of the AGP and HSA binding. AGP possessed a common single binding site mainly with van der Waals' interaction. HSA possessed at least two classes of binding sites, those of high-affinity and low-capacity with hydrophobic interaction, and sites of low-affinity and high-capacity contributing to the large negative ΔH and ΔS. The substituted R_2 side chains have more effect on the variation of the binding and thermodynamic parameters than the R_1 substituents. A similar tendency is shown in the interactions between plasma and phenothiazines. The HSA binding, therefore, is a factor in determining the binding process of drugs such as phenothiazines, as well as acidic drugs, in plasma.

References

Bickel, M. H. (1975) Binding of chlorpromazine and imipramine to red cells, albumin, lipoproteins and other blood components. J. Pharm. Pharmacol. 27: 733–738

Brunner, F., Müller, W. E. (1985) The stereoselectivity of the 'single drug binding site' of human α_1-acid glycoprotein (orosomucoid). Ibid. 37: 305–309

Cohn, E. J., Hughes, W. L., Weare, F. H. (1947) Preparation and properties of serum and plasma proteins. XIII. Crystallization of serum albumins from ethanol-water mixtures. J. Am. Chem. Soc. 69: 1753–1761

El-Gamal, S., Wollert, U., Müller, W. E. (1982) Optical studies on the specific interaction of dipyridamole with α_1-acid glycoprotein (orosomucoid). J. Pharm. Pharmacol. 34: 152–157

El-Gamal, S., Wollert, U., Müller, W. E. (1983) Binding of several phenothiazine neuroleptics to a common binding site of α_1-acid glycoprotein, orosomucoid. J. Pharm. Sci. 72: 202–205

Freedberg, K. A., Innis, R. B., Creese, I., Snyder, S. I. (1979) Antischizophrenic drug: differential plasma protein binding and therapeutic activity. Life Sci. 24: 2467–2474

Gucker, F. T., Pickard, H. B., Planck, R. W. (1939) A new microcalorimeter: the heats of dilution of aqueous solutions of sucrose at 20 and 30°C and their heat capacities. J. Am. Chem. Soc. 61: 459–470

Huang, P. C., Gabay, S. (1974) Examination of phenothiazine albumin interaction by ultraviolet difference spectrophotometry. Biochem. Pharmacol. 23: 957–972

Hulshoff, A., Perrin, J. H. (1977) Quantitative correlations between albumin binding constants and chromatographic R_M values of phenothiazine derivatives. J. Med. Chem. 20: 430–439

Kornguth, M. L., Hutchins, L. G., Eichelman, B. S. (1981) Binding of psychotropic drugs to isolated α_1-acid glycoprotein. Biochem. Pharmacol. 30: 2411–2435

Krieglstein, J., Meiler, W., Staab, J. (1972) Hydrophobic and ionic interactions of phenothiazine derivatives with bovine serum albumin. Ibid. 21: 985–997

Lowry, O. H., Rosebrough, N. F., Farrand, A. L., Radall, R. J. (1951) Protein measurement with folin phenol reagent. J. Biol. Chem. 193: 265–275

Lund, A. (1980) The distribution of chlorpromazine between plasma and erythrocytes. Acta Pharmacol. Toxicol. 47: 300–304

Müller, W. E., Stillbauer, A. E. (1983) Characterization of a common binding site for basic drugs on human α_1-acid glycoprotein (orosomucoid). Naunyn-Schmiedeberg's Arch. Pharmacol. 322: 170–173

Piafsky, K. M., Bôrga, O. (1977) Plasma protein binding of basic drugs. II Importance of α_1-acid glycoprotein for interindividual variation. Clin. Pharmacol. Ther. 22: 545–549

Piafsky, K. M. (1980) Disease-induced changes in the plasma binding of basic drugs. Clin. Pharmacokinet. 5: 246–262

Ross, P. D., Subramanian, S. (1981) Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry 20: 3096–3102

Schley, J., Müller-Oerlinghausen, B. (1986) Investigation of the binding of various tricyclic neuroleptics and antidepressants to α_1-acid glycoprotein. J. Pharm. Pharmacol. 38: 102–106

Schmidt, K. (1953) Preparation and properties of serum and plasma proteins. XXIX. Separation from human plasma of polysaccharides, peptides and proteins of low molecular weight. Crystallization of an acid glycoprotein. J. Am. Chem. Soc. 75: 60–65

Sharples, D. (1976) Factors affecting the binding of tricyclic tranquillizers and antidepressants to human serum albumin. J. Pharm. Pharmacol. 28: 100–105

Sugiyama, Y., Suzuki, Y., Sawada, Y., Kawasaki, S., Beppu, T., Iga, T., Hanano, M. (1985) Auramine O as a fluorescent probe for the binding of basic drugs to human α_1-acid glycoprotein (α_1-AG). Biochem. Pharmacol. 34: 821–829

Suurkuusk, J., Wadsö, J. (1982) A multichannel microcalorimetry system. Chemica Scripta 20: 155–163

Vallner, J. J. (1977) Binding of drugs by albumin and plasma protein. J. Pharm. Sci. 66: 447–465

Verbeeck, R. K., Cardinal, J. A., Hill, A. G., Midha, K. K. (1983) Binding of phenothiazine neuroleptics to plasma proteins. Biochem. Pharmacol. 32: 2565–2570

Yamamoto, M., Aki, H. (1988) Application of differential flow microcalorimetry for study of drug interactions in the blood system. J. Biochem. Biophys. Methods 16: 271–282