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Evidence for the preferential interaction of micellar chlorpromazine with human serum albumin

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Although the interaction of chlorpromazine with human serum albumin has been the subject of several quantitative studies, there is considerable conflict about the stoichiometry and strength of the interaction [1–5], and also an indication [6] that the binding curve is sigmoidal. In an attempt to determine whether micelle formation by chlorpromazine might be a contributing factor to the seemingly diverse binding behaviour, an equilibrium dialysis study of the interaction between human serum albumin and the drug has been performed in acetate–chloride buffer, pH 5.5, I 0.154, conditions operative for the only quantitative characterization of chlorpromazine in terms of micelle size and association equilibrium constant [7]. It is concluded that albumin, like tubulin [8, 9], binds the micellar form of chlorpromazine preferentially to a single site; but that the existence of additional protein sites with weaker affinity for the drug precludes precise quantitative characterization of the system.

Materials and methods

Chlorpromazine hydrochloride was obtained from Sigma Chemical Co. and the human serum albumin (25 mg/ml solution) was kindly donated by the Red Cross Blood Transfusion Service in Brisbane. In order to remove the slight colouration resulting from residual haemoglobin and erythrocytic fragments, the albumin concentrate was further purified by ion-exchange chromatography on carboxymethyl-cellulose (Whatman CM32) pre-equilibrated with 10 mM sodium phosphate buffer, pH 5.5. Prior to binding studies the purified albumin solution, which passed unretarded through the ion-exchange column, was dialysed for 24 hr at 4° against acetate–chloride buffer, pH 5.5 (0.01 M sodium acetate/0.144 M sodium chloride, pH adjusted with acetic acid).

Dialysis sacs (Visking 18/32) containing human serum albumin (5 ml, 10 mg/ml) and chlorpromazine (20 μM–5 mM) in the acetate–chloride buffer were placed in the same buffer (450 ml) containing an identical concentration of chlorpromazine. Dialysis was allowed to proceed at 25° for 36 hr, after which the concentration of chlorpromazine in the diffusate, $[S]_0$, was determined spectrophotometrically on the basis of a molar absorptivity of 4400 $M^{-1}cm^{-1}$ at 300 nm [10]; and the total concentrations of albumin and drug in the inner solution ($[\bar{A}]_i$, $[\bar{S}]_i$) obtained by combining absorbance measurements at 300 nm and 280 nm in the expressions

$$A_{280} = 36{,}300[\bar{A}]_i + 1995[\bar{S}]_i \quad (1a)$$

$$A_{300} = 3940[\bar{A}]_i + 4400[\bar{S}]_i. \quad (2a)$$

The molar absorptivity of human serum albumin at 280 nm (36,300 $M^{-1}\,cm^{-1}$) is based on a molecular weight of 66,000 [11] and an absorption coefficient ($A^{1\%}_{1cm}$) of 5.50 [12]: magnitudes of the molar absorptivities of chlorpromazine at 280 nm and of albumin at 300 nm were deduced from spectral measurements designed to test the additivity of absorbances in albumin–chlorpromazine mixtures at these wavelengths. A value of the Klotz [13] binding function, r, was then determined from the expression $r = ([\bar{S}]_i - [S]_i)/[\bar{A}]_i$, where $[S]_i$, the concentration of free chlorpromazine in the albumin–drug mixture, was obtained from that of the diffusate ($[S]_0$) by allowance [14] for the Donnan redistribution of ions.

Solutions of chlorpromazine (0.05–4.85 mg/ml) in acetate–chloride buffer, pH 5.5, I 0.154, were subjected to frontal gel chromatography [15] on a column (0.9 × 12.5 cm) of Sephadex G-25, pre-equilibrated at 25° with the same buffer. The column effluent, maintained at a flow-rate of 0.75 ml/min, was divided into 1.5 ml fractions which were then assayed spectrophotometrically at 300 nm after appropriate dilution. The weight-average elution volume, V_w, was determined from the centroid [16] of the advancing profile, and converted to the corresponding partition coefficient, σ_w, by the expression $\sigma_w = (V_w - V_0)/(V_t - V_0)$, the void ($V_0$) and total ($V_t$) volumes of the column having been taken as the elution volumes of serum albumin and potassium chromate, respectively.

Results and discussion

Results of equilibrium dialysis experiments with free chlorpromazine concentrations in the range 20 μM–5 mM are presented in Scatchard format in Fig. 1, the most characteristic feature of which is the existence of a minimum in the vicinity of $r = 1$, and presumably, therefore, of a maximum, since the limiting value of $r/[S]_i$ as $[S]_i \rightarrow \infty$ is zero: although not established unequivocally, the present results suggest that this maximum occurs at $r = 12$–15. The general form of the plot resembles that for the interaction of chlorpromazine with brain tubulin [17], a system for which the unusual binding curve reflects the preferential interaction of micellar drug with a single site on the protein acceptor [8, 9]. A similar interpretation of Fig. 1 would be conditional upon chlorpromazine undergoing pronounced micellization at concentrations in the vicinity of 5 mM, a phenomenon for which there is certainly evidence for the drug in 0.154 M NaCl [7, 18]. The micellar characteristics of chlorpromazine under the present conditions (acetate–chloride, pH 5.5, I 0.154) have therefore been examined by gel chromatography on Sephadex G-25 [9].

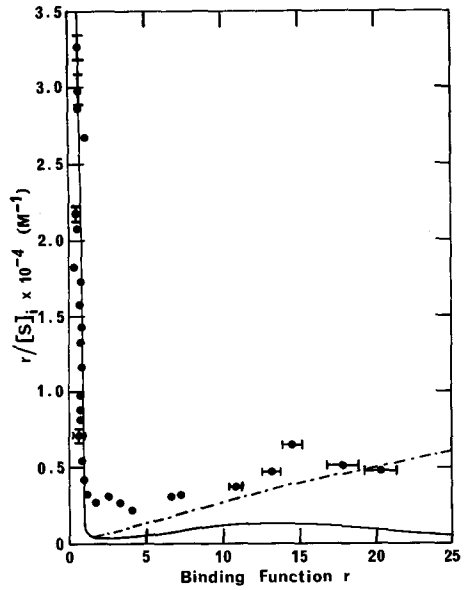

Fig. 1. Scatchard plot of equilibrium dialysis measurements of the interaction between chlorpromazine and human serum albumin in acetate–chloride buffer, pH 5.5, I 0.154, the curves being theoretical plots based on equation (2) with $p = 1$, $m = 35$, $Y = 7.9 \times 10^{78}\,M^{-34}$, $L_A = 10^5\,M^{-1}$ and $L_A/K_A = 10$ (——) or 1000 (–·–·–). Error bars denote extents of uncertainty associated with representative experimental points.

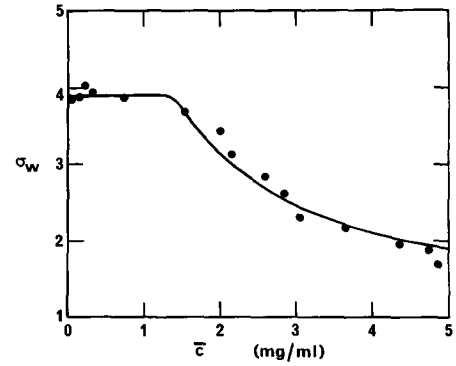

Fig. 2. Concentration-dependence of the weight-average partition coefficient, σ_w, for chlorpromazine on Sephadex G-25 equilibrated with acetate–chloride buffer, pH 5.5, I 0.154, the curve being the theoretical plot for a monomer–micelle system ($mS \rightleftarrows T$) with $m = 35$ and an association constant (Y') of 5.4×10^{-7} litre^{34}g^{-34} [7], $\sigma_S = 3.90$ and $\sigma_T = 1.95$.

From the results of the gel chromatography study of chlorpromazine (Fig. 2) it is evident that the effects of micelle formation on the weight-average partition coefficient (σ_w) are first detected at a drug concentration of approximately 1.5 mg/ml, a finding that compares favourably with the value of 1.3 mg/ml inferred from sedimentation equilibrium studies of the drug in 0.154 M NaCl (Fig. 1 of Ref. [7]), and which therefore suggests the likelihood that a comparable quantitative description of micelle formation pertains under the present conditions. Indeed, the earlier description [7] of chlorpromazine in terms of a two-state monomer–micelle equilibrium ($mS \rightleftarrows T$) with $m = 35$ and an association constant (Y') of 5.4×10^{-7} litre^{34}g^{-34} referred to drug in 0.154 M NaCl, the pH of which had decreased to 5.5 at chlorpromazine concentrations conducive to micelle formation (4–6 mM). In view of the agreement observed between the experimental points in Fig. 2 and the theoretical dependence (——) for such a system with values of 3.90 and 1.95 for the respective partition coefficients (σ_S, σ_T) of monomeric and micellar drug, it seems reasonable to proceed with analysis of the binding data in Fig. 1 in terms of the micellar model inferred from the sedimentation equilibrium studies [7].

Attempts have been made to curve-fit the experimental binding data in terms of the expression [8]

$$r = p(K_A[S]_i + mL_AY[S]_i^m)/(1 + K_A[S]_i + L_AY[S]_i^m) \quad (2)$$

where K_A and L_A denote the respective intrinsic association constants for the interaction of monomeric and micellar ligand with p equivalent and independent protein acceptor sites; and Y is the molar association constant describing micelle formation ($7.9 \times 10^{78}\,M^{-34}$). Theoretical considerations of the forms of binding curves for micellar ligand systems [8] have shown that the presence of a minimum in a Scatchard plot signifies that the micellar state of the ligand is bound preferentially by the acceptor ($L_A > K_A$). Furthermore, the existence of the minimum in the vicinity of $r = 1$ indicates a value of unity for p, the number of binding sites on albumin. Figure 1 displays two theoretical curves, which have been calculated on the basis of $K_A = 10^5\,M^{-1}$ and values of 10 (——) and 1000 (–·–·–) for the ratio L_A/K_A, the latter providing a reasonable description of the experimental binding data in the region of highest r values measured. However, this model consistently underestimates the experimental binding curve in the range $1 < r < 5$, a factor which is taken to signify the existence on albumin of additional binding sites with weak affinity for chlorpromazine. The system described by the solid line in Fig. 1 ($L_A/K_A = 10$) was selected for illustration to indicate the extent of preference for micellar ligand required to predict a Scatchard plot with a maximum in the vicinity of $r = 15$, which seems to be more in keeping with experimental findings. Clearly, a large number of possible L_A/K_A values coupled with weaker binding of chlorpromazine to additional protein sites could be invoked to provide reasonable descriptions of the experimental binding results; and hence precise quantitative characterization of the albumin–chlorpromazine system from the present results is precluded.

In summary, the aim of this investigation has been to demonstrate an effect of micelle formation on the interaction of chlorpromazine with serum albumin. Neglect of this phenomenon could well provide at least a partial explanation of the diverse binding behaviour reported [1–6] for the albumin–chlorpromazine system; but clearly a definitive answer to this dilemma must await quantitative characterization of the micellar characteristics of the drug under the conditions used in the various binding studies. Irrespective of that outcome, the present investigation serves a valuable role by emphasizing the need to consider possible effects of micelle formation in ligand-binding studies with all essentially hydrophobic molecules possessing a polar group for greater aqueous solubility. Micelle formation by the ligand is a potentially real but rarely considered phenomenon in studies of drug interactions.

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