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Micellarization of Chlorpromazine

Implications in the Binding of the Drug to Brain Tubulin

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SUMMARY

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The micellar characteristics of chlorpromazine in 0.025 M sodium pyrophosphate-0.00125 M magnesium chloride-0.125 M sodium chloride-1.0 M sucrose, pH 6.8, 37°, have been investigated by frontal gel chromatography on Sephadex G-25. Results indicate a critical micelle concentration of 0.2 mM, a value that substantiates the necessity to consider the coexistence of monomeric and micellar forms of the drug in its interaction with tubulin under these conditions. Taken in conjunction with published results of such binding studies, this investigation provides experimental support for the concept that the micellar state of chlorpromazine interacts preferentially with one site on brain tubulin.

In a previous publication (1), binding curves for the interaction of chlorpromazine with brain tubulin were considered in terms of binding to a site of relatively high affinity plus weaker, positively cooperative, binding of an additional eight or nine chlorpromazine molecules. This interpretation has since been queried (2) as the result of theoretical studies of the effects of ligand self-association on the nature of Scatchard plots for systems in which the acceptor exhibits a preference for one ligand form. It was shown (2) that preferential binding of chlorpromazine micelles, comprising nine to eleven monomers, to a single brain tubulin site would provide an adequate quantitative description of the experimental results in terms of a simpler model than that suggested initially (1). However, this alternative quantitative interpretation of the binding curves is reliant upon the critical micelle concentration being in the vicinity of 0.2 mM rather than 4–5 mM, the range previously reported (1, 3) from investigations of micelle formation by chlorpromazine in salt-free and dilute salt solutions. The purposes of the present gel chromatographic study of chlorpromazine were (a) to provide evidence favoring the lower value for the critical micelle concentration of chlorpromazine in 0.025 M sodium pyrophosphate-0.00125 M magnesium chloride-0.125 M NaCl-1.0 M sucrose, pH 6.8, 37°, the environment used for the binding studies (1); and (b) thus to provide support for the revised interpretation (2) of those studies in terms of preferential binding of micellar chlorpromazine to a single brain tubulin site.

Chlorpromazine hydrochloride, a commercial sample obtained from Sigma Chemical Company (St. Louis, Mo.), was dissolved directly in 0.025 M sodium pyrosphosphate-0.00125 M magnesium chloride-0.125 M sodium chloride-1.0 M sucrose, pH 6.8 (PMSS buffer of ref. 1), the concentrations of these solutions being determined spectrophotometrically on the basis of a molar extinction coefficient of 3162 at 300 nm (4). Solutions were then incubated at 37° for 15 min prior to frontal gel chromatography (5) on a column (1.2 × 6.5 cm) of Sephadex G-25, pre-equilibrated at 37° with the same buffer. The column effluent, maintained at a flow rate of 30 ml/hr, was monitored spectrophotometrically at 300 nm. To minimize chlorpromazine adsorption (1), Teflon tubing was used to connect the outlet from the glass chromatographic column to the flow cell, which preceded a peristaltic pump in the continuous-flow assay system. A routine check on the flow rate was made by determining the weight of column effluent collected in a given time interval during each experiment. The weight-average elution volume, V_w, was determined from the centroid (6) of the advancing elution profile, in which the chlorpromazine concentration increased from zero to that of the applied solution, $\bar{c}$, in each experiment. This elution volume was then converted to the corresponding weight-average partition coefficient, σ_w, by the expression $\sigma_w = (V_w - V_0)/(V_t - V_0)$, the void ($V_0$) and total ($V_t$)

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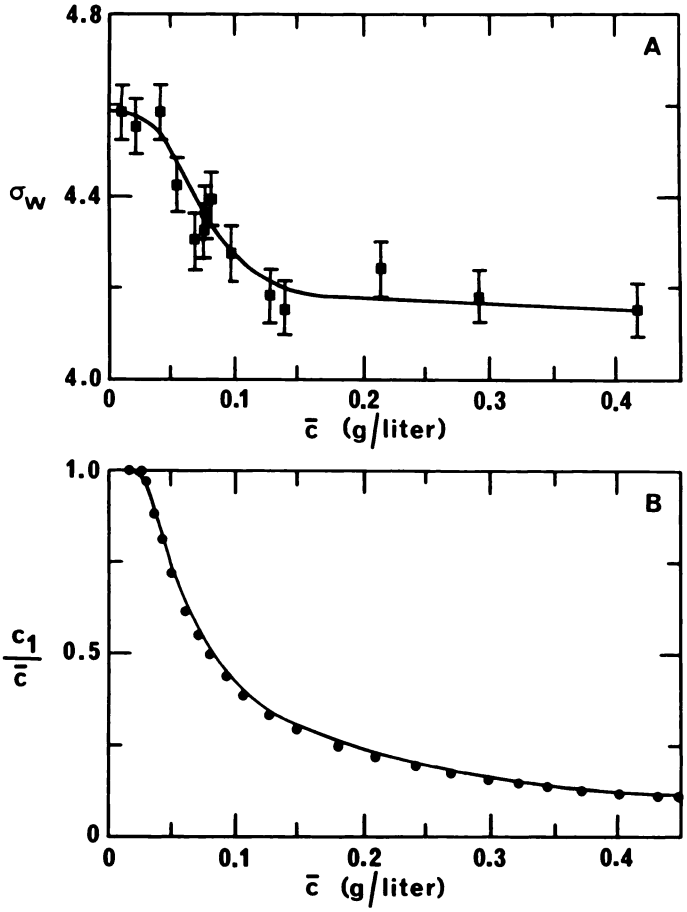

FIG. 1. *Gel chromatography of chlorpromazine on Sephadex G-25*

A. Dependence of the apparent weight-average partition coefficient, σ_w, upon total concentration, $\bar{c}$.

B. Corresponding dependence of the weight-fraction of monomer, $c_1/\bar{c}$, for two proposed (2) modes of chlorpromazine micellarization: ——, $m = 9$ and $Y = 3.8 \times 10^{30}$ M^{-8}; ●, $m = 11$ and $Y = 3.6 \times 10^{38}$ M^{-10}.

volumes of the column having been taken as the elution volumes of bovine serum albumin and potassium chromate, respectively.

Results of the frontal gel chromatographic experiments on chlorpromazine are summarized in Fig. 1A, about which the following points require comment. (a) Throughout the range of chlorpromazine concentration studied (0.01–0.42 g/liter) the magnitude of the apparent weight-average partition coefficient, σ_w, is well in excess of unity, the maximal value if gel permeation were the sole chromatographic process. It must therefore be concluded, in agreement with the previous finding (1), that reversible adsorption of the drug to the polysaccharide matrix has a pronounced influence on the gel chromatographic behavior of chlorpromazine on Sephadex. (b) Despite its occurrence, this reversible adsorption does not obscure the sigmoidal dependence of σ_w upon total chlorpromazine concentration, $\bar{c}$. The simplest interpretation of this phenomenon is self-association of the drug—in particular, an equilibrium between monomeric and micellar forms. (c) From the position of the sigmoid it is evident that the proportions of presumed micellar and monomeric forms exhibit greatest sensitivity to total drug concentration at $\bar{c} \simeq 0.07$ g/liter, a result that is in keeping with a critical micelle concentration of approximately 0.2 mM (0.071 g/liter) under these conditions, which duplicate the environment used to study the binding of chlorpromazine to brain tubulin (1).

The observed dependence of σ_w upon $\bar{c}$ is in accord with that expected for the chlorpromazine association proposed (2) to account for the interaction of the drug with brain tubulin under these conditions. In that study (2) it was established that the binding results could be described quantitatively by an envelope of theoretical curves calculated with the following sets of association parameters for chlorpromazine micelle formation ($mS \rightleftarrows T$, $Y = [T]/[S]^m$): $m = 9$, $Y = 3.8 \times 10^{30}$ M^{-8} and $m = 11$, $Y = 3.6 \times 10^{38}$ M^{-10}. Figure 1B shows the variation of the weight-fraction of monomer as a function of total chlorpromazine concentration that is calculated for these sets of association parameters: the close correspondence between the two curves shows that a virtually identical critical micelle concentration is relevant to the entire envelope. This predicted critical micelle concentration (0.07 g/liter) is in striking agreement with that inferred from the point of inflection observed in the concentration dependence of the weight-average partition coefficient (Fig. 1A). In this connection it is noted that the chlorpromazine concentrations reported in the binding studies (1) were calculated on the premise that one-third of the drug was in the form of a 1:1 complex with sucrose that did not bind to brain tubulin. Since the critical micelle concentration predicted from those binding data coincides with the present experimental value, the chlorpromazine-sucrose complex and the pure drug presumably exhibit similar micellarization behavior.

In summary, this investigation provides experimental support for the basic tenets of the model proposed (2) to account for the chlorpromazine-tubulin binding results (1) and hence for the conclusion (2) that the binding curves reflect preferential binding of the micellar form of chlorpromazine to a single site on brain tubulin. It is conceivable that this inference may be of pharmacological significance in relation to drug dosage when chlorpromazine is used as a tranquilizer (7).

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