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MICROCALORIMETRIC INVESTIGATIONS OF PHARMACEUTICAL COMPLEXES II. DRUG-ALBUMIN INTERACTIONS

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The binding parameters for the non-cooperative binding assumed to occur between drugs and human serum albumin (HSA) have been investigated by a variety of separation and spectroscopic techniques (1,2). Most of these techniques lend themselves to investigations at a single temperature and very few reports contain estimates of the entropies and enthalpies of the reaction after use of the van't Hoff equation (3-7).

In this preliminary report, we show that microcalorimetry can be used to estimate the thermodynamic parameters after drug-albumin interactions in certain cases. An L.K.B. flow microcalorimeter was used to measure the heat flux generated by the binding of salicylate, sulfaethidole, fenoprofen, indomethacin and flufenamic acid to HSA. The experiments were performed in a phosphate buffer at 25°. In the experiments, the solutions of drug and albumin are pumped at a total flow rate of approximately 30 ml hr^{-1} into the reaction cell of volume approximately 0.5 ml; the residence time within the cell is approximately one minute. The mixed solutions then pass through the flow cell. By measuring heat flux differences between the two cells, heat effects due to friction and turbulence are essentially eliminated.

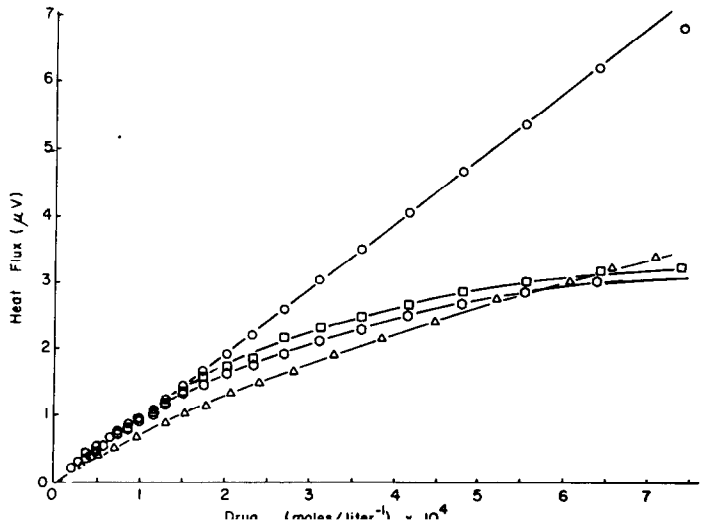

Fig. 1. Heat flux as a function of drug concentration at 25°. Conditions: [HSA] = 1.45 x 10^{-4} M; pH 7.4.
Key: (Δ-Δ) fenoprofen, (○-○) salicylate, (□-□) sulfaethidole, and (o-o) flufenamic acid.

The experimental details are similar to those reported for the investigation of β-cyclodextrin - drug interactions (8). The time of residency within the reaction cell is many orders of magnitude larger than the time necessary for attainment of equilibrium between small molecules and proteins (1). Figure 1 shows the heat flux as a function of drug concentration with the HSA concentration fixed. The heat flux generated is proportional to the amount of drug bound to the albumin. If only one binding site contributes to the heat flux, then the data are readily interpreted in terms of the binding constant, ΔG ΔH and ΔS for binding to that site. If many sites are involved having different enthalpies of binding, then unambiguous interpretation of the data may be impossible. Taking the data of Fig. 1 at only very low drug to albumin ratios, a 1:1 binding constant can be calculated for salicylic acid (9), sulfaethidole (10) and fenoprofen (11), these drugs having been reported to have secondary sites of significantly lower affinity than that of their single primary site. The binding constants and enthalpies for the first binding sites shown in Table 1 are

Table 1. Binding constants and derived thermodynamic parameters for 1:1 complexes between the drugs and HSA

Drugs	K (moles liter^{-1})	ΔG (cal. mole^{-1})	ΔH (cal. mole^{-1})	ΔS (cal. mole^{-1}K^{-1})	K_{Lit}	Ref.
Salicylic acid	2.1×10^5	-7260	-5669	+5.3	1.2×10^5	9
Sulfaethidole	1.1×10^5	-6877	-5537	+4.5	1.2×10^5	10
Fenoprofen	3.4×10^5	-7545	-6280	+4.2	1.9×10^5	11
Indomethacin	7.5×10^5	-8014	-5309	+9.1	3.0×10^5	12

calculated from the experimental data by an iterative least squares technique (8), and are compared to the literature values. The values of the binding constants for salicylate and sulfaethidole are in excellent agreement with those obtained by Perrin et al. (9,10). A value of 2.5×10^5 for fenoprofen supports the suggestion of Vallner et al. (11) that fenoprofen has a binding affinity for albumin greater than 10^5. The affinity constant for indomethacin is in good agreement with that found by Mason and McQueen (12). These values are for the first binding site, and in the above cases good agreement with the literature is obtained because either little heat is evolved on binding to the second site or the second site has a much lower affinity for the drug than does the first. From Fig. 1 it can be seen that more than one site is contributing to the heat evolved for the binding of flufenamic acid to albumin. No binding constants have been estimated from this data; flufenamic acid has been reported to have three very high affinity sites for HSA and eight sites of lower affinity (13).

From Table 1 it can be seen that the large negative enthalpy makes a much larger contribution to the free energy term than does the positive entropy for the four drugs investigated. A

positive entropy is frequently taken as evidence for hydrophobic interactions, but it has been pointed out that the positive entropy may also be a manifestation of electrostatic forces. However, the enthalpy change is expected to be near zero for purely electrostatic interactions (14). Clearly the large enthalpy observed indicates considerable contributions of van der Waals forces and hydrogen bonds to the binding of these anionic drugs to albumin. It is possible that the changes in behavior of the solvent, water, after the binding process account for a large fraction of the thermodynamic parameters reported in Table 1 (14-16). More insight into the mechanism of binding can be gained by measuring enthalpy changes as a function of temperature; such measurements can be made with the flow microcalorimeter.

The thermodynamic parameters for salicylate are different from those reported by Zaroslinski et al. (3). The difference of binding constant (55×10^3) reflects the variation of enthalpy and entropy values. The ΔH value (-9.811 k cal. $mole^{-1}$) in the study of Zaroslinski et al. (3) is large and negative compared with that in this study. Also the ΔS value (-11 cal. $K^{-1}mole^{-1}$) for their study is relatively large and negative, while it is positive in the present investigations. The literature values may be somewhat suspect because insufficient data were collected at low drug to protein ratios. This is in comparison to the large number of data points obtainable by microcalorimetry and used to estimate the thermodynamic parameters in Table 1. Moreover, the microcalorimeter method can be expected to be more reliable than any techniques using the van't Hoff method where binding constants are determined over a temperature range.

Recent reports (17,18) have suggested that the N↔B transition may be involved in drug-albumin interactions (19,20), and Figure 2 suggests that microcalorimetry may be a useful tool for studying this phenomenon. In the figure the change of heat flux for the dilution of albumin by phosphate

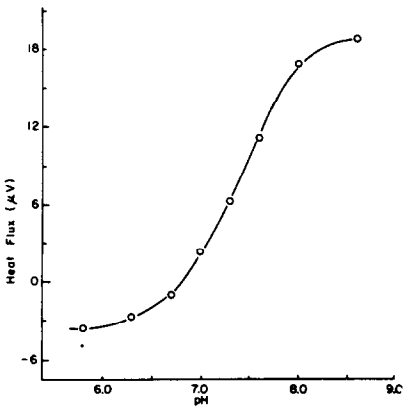

Fig. 2. Heat of dilution of HSA as a function of pH in the presence of sodium phosphate buffer (0.1 M).

buffers as a function of pH is shown. The heat flux is corrected for the heat of dilution of albumin and of the phosphate buffer, but has not been corrected for the changes in the state of ionization of the albumin. The change in heat flux shown in Fig. 2 occurs in the pH region associated with the N↔B transition (18).

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