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Binding Data Analysis for Interaction of n- Alkyl Sulfates with Insulin

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ABSTRACT

The binding data for interaction of a homologous series of n-alkyl sulfates with alkyl chain lengths from C_8 to C_{12} with insulin were analyzed on basis of Hill equation for two classes of binding sites .The intrinsic Gibbs free energies were calculated and resolute on basis of electrostatic and hydrophobic contributions The estimation of these contributions reveals the major role of electrostatic interactions on first binding set and the minor one in the second.

Keywords: Insulin, n–Alkyl sulfates, Surfactant, Hill equation, Electrostatic interaction, Hydrophobic interaction.

INTRODUCTION

The interaction between ionic surfactants and globular proteins has been studied extensively [1-3]. Ionic surfactants can binds to proteins and destruct its native structure at mM concentration scale. It is well known that a combination of electrostatic and hydrophobic forces contributes in the formation of ionic surfactant-protein complexes [4]. However there is not any quantitative method for resolution of these forces. One of the logic approaches for this purpose is analysis of binding data for interaction of a homologous series of n-alkyl surfactants with a special protein. These data can provide sufficient information for an extrapolation procedure that provides the contribution of electrostatic and hydrophobic forces. This extrapolation procedure can be on basis of hydrophobic interaction dependency to hydrophobic tail length of surfactant.

In the present study a novel extrapolation procedure has been proposed and used for resolution of electrostatic and hydrophobic interaction in the binding of n-alkyl sulfates with insulin.

The interaction between a range of n-alkyl sulfates with alkyl chain lengths from C_8 to C_{12} with insulin have been measured previously using equilibrium dialysis and micro calorimetric techniques [5, 6] The experimental binding data have been taken directly from these references and analyzed on basis of our model in spite of resolution for electrostatic and hydrophobic contributions in intrinsic Gibbs free energy of binding.

BINDING DATA ANALYSIS AND RESULTS

The binding isotherms for interaction of n-alkyl sulfates with insulin plotted as the number of bound surfactants (υ) vs. $\log[s]_f$, where $[s]_f$ is the free surfactant concentration, are shown in figures 1a and b(These figures directly taken free reference 5)

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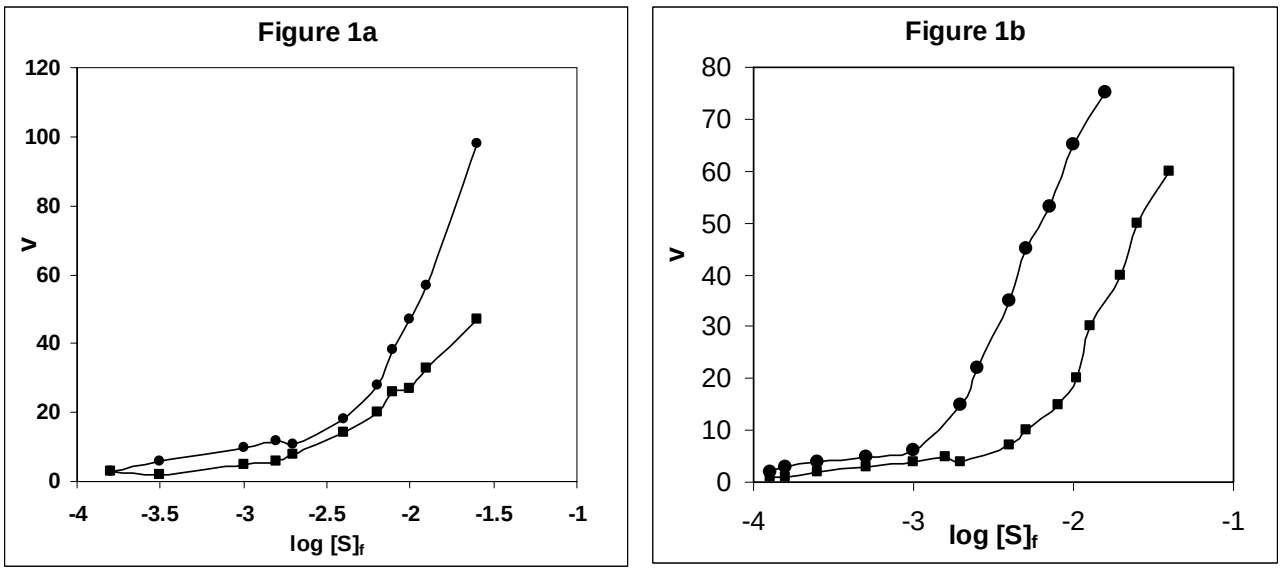

Fig. 1. Binding isotherms for interaction of n-alkyl sulfate with insulin a) C_n=8 (●),C_n=9 (■) and b) C_n=10 (●),C_n=12 (■).

These data should be fitted to the Hill equation for two binding sets system (equation (1))

$$v = \frac{g_1 (k_1 [s]_f)^{n_{H1}}}{1 + (k_1 [s]_f)^{n_{H1}}} + \frac{g_2 (k_2 [s]_f)^{n_{H2}}}{1 + (k_2 [s]_f)^{n_{H2}}} \quad (1)$$

Where g_1 , k_1 and n_{H1} are the number of binding sites ,Hill binding constant and Hill coefficient for first binding set, respectively, and g_2 , K_2 and n_{H2} are the corresponding parameters for second binding set. The binding data were fitted to this equation using computer program for non-liner least-square fatting.The fitting parameters were listed in Table1.

The intrinsic Gibbs free energy of binding per mole of surfactant for the first, $\Delta G_{b,\upsilon}^{(1)}$ and second, $\Delta G_{b,\upsilon}^{(2)}$, binding set can be calculated by the following equations [7].

Figures 2 and 3 show the variation of $\Delta G_{b,\upsilon}^{(1)}$ and $\Delta G_{b,\upsilon}^{(2)}$ for interaction of n-alkyl sulfates with insulin respectively.

$$\Delta G_{b,v}^{(1)} = -RTn_{H1} \ln k_1 + RT(1-n_{H1}) \ln [s]_f \; if \quad o < v < q_1 \quad (2)$$

$$\Delta G_{bv}^{(2)} = -RTn_{H2} \ln k_2 + RT(1-n_{H2}) \ln [s]_f \; if \quad g_1 < v < g_1 + g_2$$

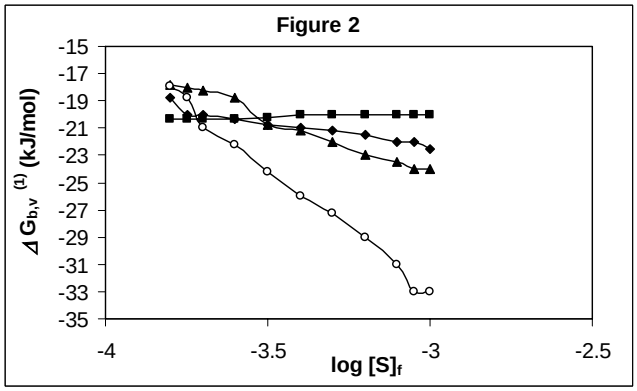

Fig. 2. The variation of $\Delta G_{b,\upsilon}^{(1)}$ versus log $[S]_f$ for interaction of n-alkyl sulfates with insulin. (♦) C_n=8, (■) C_n=9, (▲) C_n= 10 and (○) C_n=12.

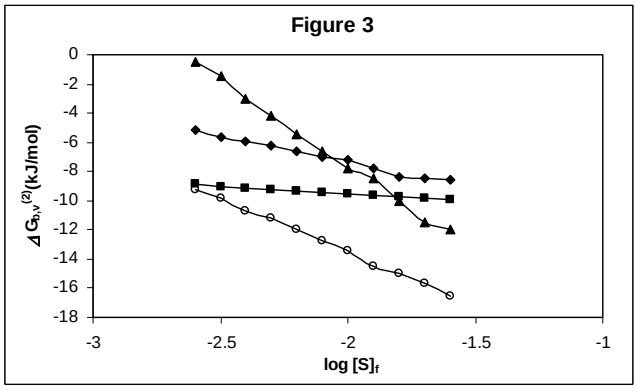

Fig. 3. The variation of $\Delta G_{b,\upsilon}^{(2)}$ versus log $[S]_f$ for interaction of n-alkyl sulfates with insulin. (♦) C_n=8, (■) C_n=9, (▲) C_n= 10 and (○) C_n=12.

It is reasonable that $\Delta G_{b,v}^{(i)}$ is considered as a summation of two parts as follows;

$$\Delta G_{b,v}^{(i)} = \Delta G_{b,v}^{(i)}(ele) + \Delta G_{b,v}^{(i)}(hyd) \qquad (3)$$

Where $\Delta G_{b,v}^{(i)}(ele)$ and $\Delta G_{b,v}^{(i)}(hyd)$ are the electrostatic and hydrophobic contributions in overall intrinsic Gibbs free energy of binding, respectively .For a series of n- alkyl sulfates with various alkyl chain lengths, C_n, $\Delta G_{b,v}^{(i)}(hyd)$ should be function of C_n while $\Delta G_{b,v}^{(i)}(ele)$ not. In the other word

$$\Delta G_{b,v}^{(i)}(hyd) = f(C_n) \qquad (4)$$

The limiting value of $\Delta G_{b,v}^{(i)}$ when C_n goes to zero should be equal to $\Delta G_{b,v}^{(i)}(ele)$:

$$\lim_{C_N \to 0} \Delta G_{b,v}^{(i)} = \Delta G_{b,v}^{(i)}(ele) \qquad (5)$$

This strategy provides an extrapolation procedure for resolution of these forces in the binding of n-alkyl sulfates to insulin.

Figure (4) and (5) show the variation of $\Delta G_{b,v}^{(i)}$ at any specified free surfactant concentration of surfactant versus C_n. By fitting of these curves to any arbitrary function and calculating the intercept values , $\Delta G_{b,v}^{(i)}(ele)$ at any specific surfactant concentration was estimated .The values of $\Delta G_{b,v}^{(i)}(hyd)$ can be calculated by subtracting of $\Delta G_{b,v}^{(i)}(ele)$ from $\Delta G_{b,v}^{(i)}$. Figures (6) and (7) show the variation of $\Delta G_{b,v}^{(i)}(ele)$ and $\Delta G_{b,v}^{(i)}(hyd)$ versus $\log[S]_f$ for first binding set and figures (8) and (9) show the corresponding curves for second binding set.

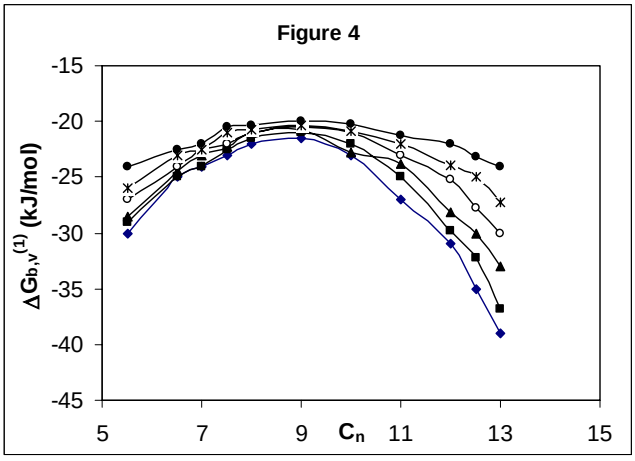

Fig. 4. The variation of $\Delta G_{b,\upsilon}^{(1)}$ versus C_n for interaction of n-alkyl sulfates with insulin. (♦) $\log[S]_f$ = -3.1, (■) $\log[S]_f$ = -3.2, (▲) $\log[S]_f$ = -3.3, (○) $\log[S]_f$ = -3.4, (×) $\log[S]_f$= -3.5 and (●) $\log[S]_f$= -3.6.

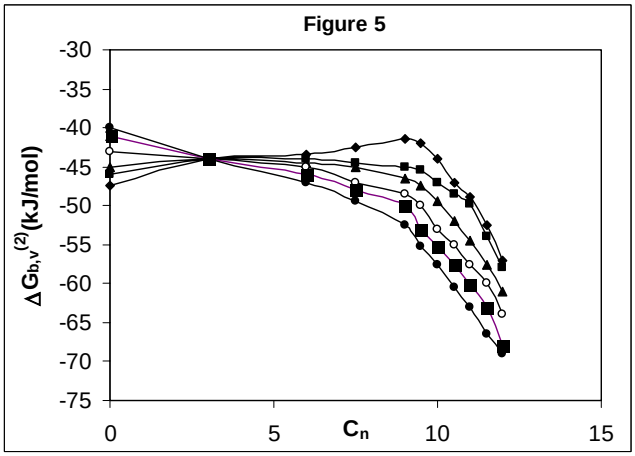

Fig. 5. The variation of $\Delta G_{b,\upsilon}^{(2)}$ versus C_n for interaction of n-alkyl sulfates with insulin. (♦) $\log[S]_f$= -2.1, (■) $\log[S]_f$= -2.0, (▲) $\log[S]_f$= -1.9, (○) $\log[S]_f$= -1.8, (×) $\log[S]_f$= -1.7 and (●) $\log[S]_f$= -1.6.

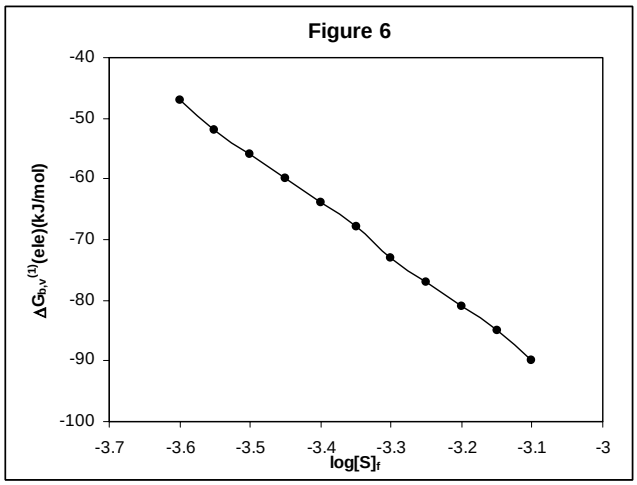

Fig. 6. The variation of $\Delta G_{b,v}^{(1)}(ele)$ versus $\log[S]_f$ for interaction of n-alkyl sulfates with insulin.

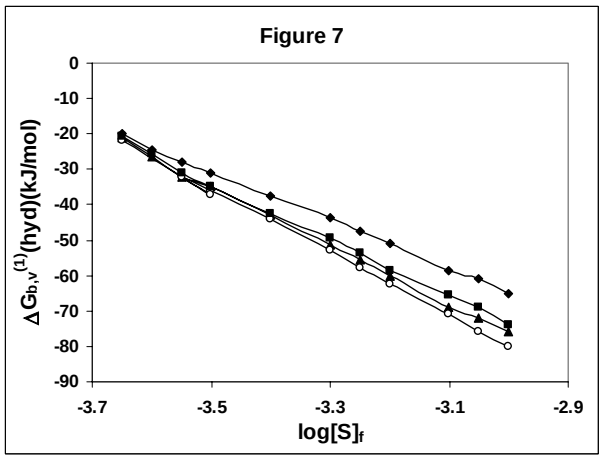

Fig. 7. The variation of $\Delta G_{b,\upsilon}^{(1)}(hyd)$ versus $\log[S]_f$ for interaction of n-alkyl sulfates with insulin. (♦) C_n=8, (■) C_n=9, (▲) C_n= 10 and (○) C_n=12.

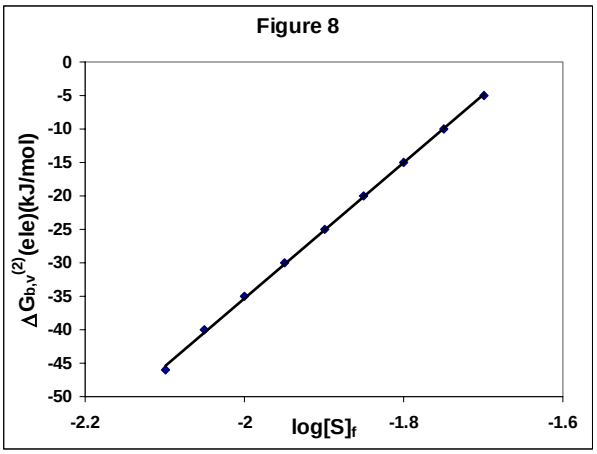

Fig. 8. The variation of $\Delta G_{b,v}^{(2)}(ele)$ versus $\log[S]_f$ for interaction of n-alkyl sulfates with insulin.

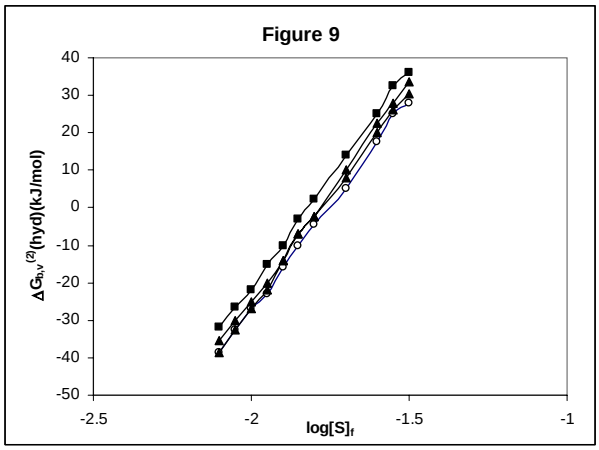

Fig. 9. The variation of $\Delta G_{b,v}^{(2)}(hyd)$ versus $\log[S]_f$ for interaction of n-alkyl sulfates with insulin. (♦) C_n=8, (■) C_n=9, (▲) C_n= 10 and (○) C_n=12.

DISCUSSION AND CONCLUSION

The negative slopes of lines in figures 2 and 3 represent the positive cooperatively in binding process of both sets. The positive cooperatively in first binding set represents that both electrostatic and hydrophobic interactions contributes in this set. This is in good agreement with previous results for other surfactant –protein system [8, 9] .The intrinsic binding affinity for C_8 and C_9 shows less difference with respect to C_{10} and C_{12}.

In the mean time, the affinity increased by increasing of C_n which represents the special role of hydrophobic interaction (hydrocarbon tail length) in first binding set. However the result represents anon-linear relation between the extent of hydrophobic interactions and C_n. Comparison of figures (6) and (7) reveals the higher role of electrostatic interactions in the first binding set. In spite of our expectation, the electrostatic forces increase by progress of binding. This may be due to special feature of insulin structure, as a very small globular protein.

The curves in figure (5) shows an isoaffinity point at C_n= 7.8. This point can be taken as inflection point for cooperatively. It seems that the alkyl sulfates with chain length less than 7.8 cannot interact with insulin in a cooperative manner. Comparison of figures (8) and (9) represents the predominate role of hydrophobic force in the second binding set. This is expected due to this fact that unfolding and exposure of many hydrophobic regions of protein is usually occurred in the second binding set. The electrostatic contributions to intrinsic Gibbs free energy goes to more positive values which represents the increasing of repulsive electrostatic interaction in the formation of insulin-surfactant complexes.

Table. 1 The values of Hill parameters for interaction of n-alkyl sulfates with insulin.

surfactant	g_1	n_{H1}	$k_1(M^{-1})$	g_2	n_{H2}	$k_2(M^{-1})$
C_8	12.0	1.55	3737.44	192	1.66	37.82
C_9	5.0	0.93	3354.76	170	1.30	58.49
C_{10}	10.0	2.33	3581.87	160	3.20	64.58
C_{12}	6.0	4.04	6133.60	149	2.26	144.34

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