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SOLVOPHOBIC FORCES AND MOLECULAR SURFACE AREA CHANGES IN DRUG-BIOMOLECULE ASSOCIATIONS AS WITH ACTINOMYCIN-DEOXYGUANOSINE IN A WIDE RANGE OF METHANOL/WATER MIXTURES

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A method is given to predict the unitary free energies of complexation between drug-like and nucleoside-like biomolecules in a range of mixed solvent compositions. A stability maximum for the actinomycin (A)-deoxyguanosine (D) complex at 8% MeOH (v/v) in methanol/water mixtures is correctly predicted by the theory in agreement with existing experimental data. The molecular surface areas of A and D exposed to the solvent are found to diminish by 36.4 Å^2 upon association. The 'microthermodynamic differential surface tension' of the solvophobic theory obtained for nucleoside-like and organic molecules in contact with MeOH/H_2O can be used to predict the solvent effect free energies in other such molecular or biopolymeric associations in solution.

1. Introduction

Confirmation of the prediction that the 'cavity term' of the solvent effects in the standard unitary free energy of association between molecular groups or between molecules forming a complex in solution [1] is the one that provides the solvent driving force that stabilizes the proteins was given by Lee and Timasheff [2]. In the present paper, the variations of free enthalpy of denaturation of a complex (AD) between actinomycin (A) and deoxyguanosine (D) in different methanol/water mixtures will be studied: again it will be shown that the 'cavity term' provides the driving force for the association. The thermodynamic data were obtained by Crothers and Ratner [3].

The microthermodynamic interfacial tension, introduced in ref. 4, in an alternative treatment of solvophobic theory [1] between the hydrocarbonaceous parts of the solute and different methanol/water mixed solvent systems of varying composition will be evaluated. These data will be used to calculate the free enthalpy of formation of actinomycin-deoxyguanosine complexes formed in methanol/water mixtures. This version [4] of the solvophobic theory [1,4] used eliminates the necessity of evaluating the 'interaction term' [1] in the free energy of denaturation.

2. The stoichiometry of the actinomycin-deoxyguanosine (AD) complex, the model

Good linear correlation is found as anticipated from the first method [1] of the solvophobic theory when $\Delta G^0_{\text{dissoc.}}$ of AD in different methanol/water mixtures is plotted vs. the solvent microthermodynamic surface tension against vacuo [1], i.e., $k_1^g(1)\gamma_1$ (γ_1, bulk macroscopic surface tension of the mixture (vs. vacuum); $k_1^g(1)$ is the term converting to the microscopic surface in the free energy cavity term: $G_c = k^g(i)\gamma_1\sigma$; for further details see refs. 1 and 4); G_c, free energy of creation of a cavity of inner surface area σ of molecular dimen-

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sions [1,4]). The deviations from linearity appear over the small range of concentrations 0–8% (v/v) MeOH in H_2O. They can be explained in terms of the solvophobic theory [1,4]. The corrective factors $k_1^g(1)$ were obtained from experimental data from high-pressure liquid chromatography (HPLC) obtained by Horvath et al. [5] on the capacity factor of *o*-toluic acid on an octadecylsilica column with water/methanol mixtures as eluents at 25°C.

The equilibrium $A + D \rightleftharpoons AD$ has been previously studied by Crothers and Ratner [3] using optical titration methods. Based on evidence shown below, the following general scheme for the associations of A and D in solution is proposed: This scheme uses only the species detected by Crothers and Ratner [3] (A_U, unfolded conformer; A_F, folded conformer; A_U^2, dimer of A_U).

$$\begin{array}{ccccc} AF \rightleftharpoons A_U & \overset{+D}{\rightleftharpoons} & A_UD & \overset{+D}{\rightleftharpoons} & A_UD_2 \\ \Updownarrow + A_U & & & & \\ A_U^2 & & & & \end{array}$$

The conformational change $A_U \rightleftharpoons A_F$ was detected by Crothers et al. [6] using optical titration methods. It is not clear a priori that one can assign a stoichiometry of 1 : 1 to the complex AD, since a large excess of nucleotide was used in the experiments. (The existence of a 1 : 2 stoichiometry was indicated by NMR studies of Krugh and Neely [7] (large excess of nucleotide was used) and by direct inspection of the crystal [8].) The correct stoichiometry may be decided as follows: The NMR studies show that d(pA) (deoxyadenosine phosphate) binds to A with a comparable strength to that with d(pG) (deoxyguanosine phosphate), while optical titration methods show a big difference. The two experiments were done at very different concentrations of A. This can be explained by assigning a 1 : 2 stoichiometry in the experiment of Krugh and Neely [7] and a 1 : 1 stoichiometry in the optical titration case. In the d(pA) case, a first residue might produce a conformational change in A when it attaches to A such that the affinity of A for a second residue is now increased.

In the d(pG) case, both affinities remain approximately the same.

3. The solvophobic effect [1]

The preceding discussion indicates that the stoichiometry in the measurements of Crothers and Ratner [3] corresponds to $A_U + D \rightleftharpoons A_UD$. The standard unitary free energy is given [1] by:

$$\begin{aligned} \Delta G^0_{\text{assoc.}} &= \Delta G^{0,\text{in vacuo}}_{\text{vdw,assoc.}} + \Delta G_{\text{red}} + \Delta G_{\text{int}} \\ &\quad + \Delta G_c - kT \ln\left(\frac{RT}{P_0 V}\right) \\ &= \Delta G^{0,\text{in vacuo}}_{\text{vdw,assoc.}} + \Delta G^0_{\text{vdw,AD}} - \frac{N}{2}\mathscr{D}\mathscr{P}\frac{\mu^2_{\text{AD}}}{v_{\text{AD}}} \\ &\quad + \Delta G_{\text{red}} + Nk^g_{\text{AD}}(1 - W_{\text{AD}})\gamma\sigma_{\text{AD}} \\ &\quad - \Delta G^0_{\text{vdw,A}} + \frac{N}{2}\frac{\mu^2_{\text{A}}}{v_{\text{A}}}\mathscr{D}\mathscr{P} \\ &\quad - Nk^g_{\text{A}}(1 - W_{\text{A}})\gamma\sigma_{\text{A}} - \Delta G^0_{\text{vdw,D}} \\ &\quad + \frac{N}{2}\frac{\mu^2_{\text{D}}}{v_{\text{D}}}\mathscr{D}\mathscr{P} - Nk^g_{\text{D}}\gamma(1 - W_{\text{D}})\sigma_{\text{D}} \\ &\quad - RT \ln(RT/P_0V) \end{aligned} \tag{1}$$

(The notation is consistent with ref. 1.) The following assumptions will be made:

$W_{\text{AD}} = W_{\text{A}} = W_{\text{D}} = 0$

$k^g_{\text{M}} = 1 + (k^g_{\text{M}} - 1)(v/v_{\text{M}})^{2/3}$. For M = A, D or AD

$k^g_{\text{M}} \approx k^c_{\text{M}}$

$\mathscr{D} \approx 1$

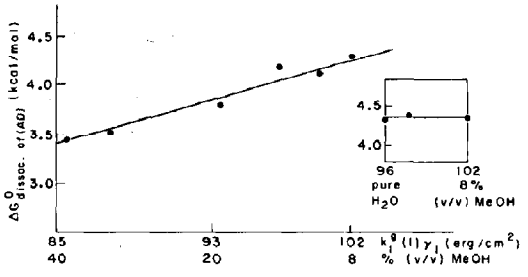

Fig. 1. $\Delta G^0_{\text{dissoc.}}$ of AD vs. the microscopic surface tension $k^g_1(1)\gamma_1$ ($\approx k^c_1(1)\gamma_1$) for the range of compositions 8–40% (v/v) of MeOH in water. (The $\Delta G^0_{\text{dissoc.}}$ values were taken from ref. 3; the $k^g_1(1)$ values were obtained by Horvath et al. [5] according to ref. 1).

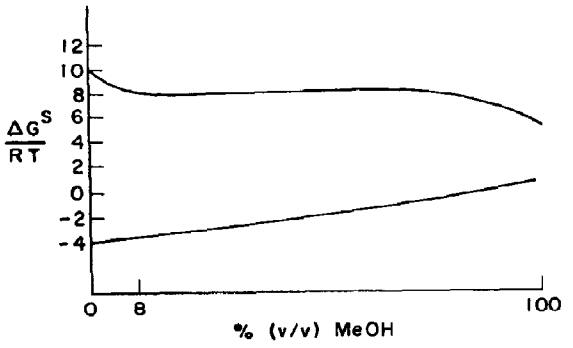

Fig. 2. The entropic contribution $RT\ln(RT/P_0V_1) \equiv \Delta G^s$ ($V_1 \equiv$ mole volume of the solvent) and $12 - \Delta G_{vdw}$ in reduced (RT) units plotted vs. MeOH concentration (cf. also ref. 5).

We can then write approximately:

$$\Delta G^0_{assoc.} = \Delta G^{0,\,in\,vacuo}_{vdw,assoc.} + \Delta(\Delta G_{vdw}) - \frac{N}{2}\mathscr{P}\Delta\left(\frac{\mu^2}{v}\right) + Nk_1^e(i)\gamma_1\Delta\sigma - RT\ln(RT/P_0V) + \Delta G_{red}$$

(Δ indicates the difference AD − A − D.)
The term corresponding to the electrostatic interaction: $(N/2)\mathscr{D}\mathscr{P}\Delta(\mu^2/v)$ is insensitive to solvent effects (cf. ref. [9].)

The variations in the terms $-RT\ln(RT/P_0V)$ and $\Delta(\Delta G_{vdw})$ are partially compensated in a wide range of concentrations: 8–100% (v/v) MeOH in water as displayed in fig. 2. The sum $(\Delta G^{0,\,in\,vacuo}_{v.dw} + \Delta G_{red})$ is fairly insensitive to solvent effects in the range 0–40% MeOH, as shown in ref. 10.

Therefore, a linear correlation between the unitary free energy of dissociation and the microthermodynamic surface tension [1] is expected. This is confirmed by fig. 1. It is also important to observe that the maximum in the free energy of dissociation is obtained at 8% (v/v) MeOH in H_2O which also corresponds to the maximum in the quantity $k_1^g(1)\gamma_1$ ($\simeq 102$ erg/cm^2). According to section 2, the slope can be attributed to the molecular surface area change $\Delta\sigma$ for the process AD $\rightleftharpoons$ A + D as predicted in ref. 1.

4. Second type derivation of the solvophobic theory [4] eliminating the explicit solute-solvent interaction terms

The sum of the cavity and the interaction terms in eq. 2 has recently been demonstrated to be proportional to $\Delta\sigma$ by Sinanoğlu [4]: $\Delta G_c + \Delta G_{int} = \delta\Delta\sigma$. The proportionality constant is called the 'microthermodynamic differential surface tension', and has been calculated from HPLC data [5] in ref. 10 for methanol/water mixtures. In the notation of ref. 4 we obtain:

$$\Delta G^0_{assoc.} = \left[\Delta G^{0,\,in\,vacuo}_{assoc.} + \Delta G_{red}\right] + (k^g_{1HC}\gamma_{1HC} - k^g_{HC}\gamma_{HC})\Delta\sigma - RT\ln(RT/P_0V_1) \quad (3)$$

$\delta = k^g_{1HC}\gamma_{1HC} - k^g_{HC}\gamma_{HC}$ is the difference between the interfacial microthermodynamic tension between a polar solvent (1) and a hydrocarbonaceous (HC) liquid phase equivalent to that of the solutes [10] and the microscopic surface tension of that hydrocarbonaceous phase.

Comparing eqs. 2 and 3 we obtain:

$$(k_{1HC}\gamma_{1HC} - k_{HC}\gamma_{HC})\Delta\sigma = k_1\gamma_1\Delta\sigma + \Delta G_{es} + \Delta(\Delta G_{vdw}) \quad (4)$$

The terms on the right-hand side of eq. 4 are obtained in ref. 10 directly from HPLC data. It was demonstrated in ref. 10 that the quantity $(\Delta G^{0,\,in\,vacuo}_{assoc.} + \Delta G_{red})$ can be taken to be at its average value in the range where $(k_{1HC}\gamma_{1HC} - k_{HC}\gamma_{HC} - k_1\gamma_1)$ remains insensitive to solvent composition.

Since, for pure water $\Delta G^0_{dissoc.} = 4.3$ kcal/mol and

$$\Delta G^0_{dissoc.} = -\left(\Delta G^{in\,vacuo}_{assoc.} + \Delta G_{red}\right) + RT\ln(RT/P_0V_1) + \delta\Delta\sigma_{dissoc.} \quad (5)$$

and we already had $\Delta\sigma_{dissoc.} = 36.4$ Å^2 (obtained from fig. 1) using the first ($k\gamma$) version of the theory [1], we can give the approximate formula (in reduced (RT) units) for predicting $\Delta G^0_{dissoc.}$ in the range 0–40%.

$$\Delta G^0_{dissoc.} = 7.0(RT\text{ units}) + \delta(36.4\text{ Å}^2) + \ln(RT/P_0V_1) \quad (6)$$

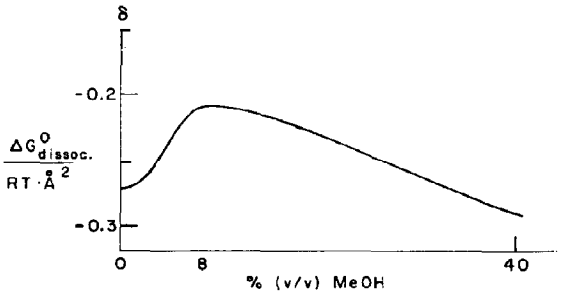

Fig. 3. ($\Delta G^0_{dissoc.}/RT \cdot Å^2$) plotted vs. % (v/v) MeOH.

This formula reproduces the $\Delta G^0_{dissoc.}$ experimental values closely in the range 8–40% MeOH (v/v).

In the range 8–40% (but not below 8%), the $k_1\gamma_1$ of version I of the theory [1] is a monotonically decreasing function of the methanol concentration which allows one to plot $\Delta G^0_{dissoc.}$ vs. $k_1\gamma_1$ as well as vs. % (v/v) MeOH as shown in fig. 1 as compared to fig. 3.

5. Deviations in the range 0–8% (v/v) MeOH in water

Within the range 0–8% (v/v) MeOH, the standard unitary free energies of dissociation at 0 and 8% (v/v) MeOH are the only data available [3].

These values indicate a deviation from the line obtained by plotting standard unitary free energies of dissociation vs. microscopic surface tension of the solvent for the range of concentrations 8–40% as shown in fig. 1.

Since the data available in the region 0–8% (v/v) are so few, one cannot tell for sure which of the two explanations given below is the correct one to account for these deviations. We therefore suggest that experiments be made at a number of solvent compositions in the range 0–8% to examine the following explanations:

(a) The entropic contribution term: $-RT \times \ln(RT/P_0V)$ increases considerably as the concentration of MeOH diminishes from 8% (v/v) to 0% while $\Delta(\Delta G_{vdw})$ remains almost constant.

This view is supported by the fact that for conformational changes (denaturation of lysozyme in $MeOH/H_2O$ mixtures [11]), where the entropic contribution $-RT \ln RT/P_0V$ does not appear in eq. 2, the deviations from the linear correlation in the range 0–8% (v/v) are smaller [12].

(b) The decrease of the microthermodynamic surface tension $k^g_f\gamma_1$ as we move from 8% MeOH (v/v) (maximum of $k^g_f\gamma_1$) to pure water implies that the maximum stability of the species A^2_U and A_F (the processes $A_U \rightleftharpoons A_F$, $A_U \overset{+A_U}{\rightleftharpoons} A^2_U$ are coupled to $A_U + D \rightleftharpoons A_UD$) is reached at 8% MeOH rather than at pure water. The increases in the equilibrium constants for the processes $A^2_U \rightleftharpoons 2A_U$ and $A_F \rightleftharpoons A_U$ as we move to pure water are reflected in an increase in the concentration of A_U, thus partially inhibiting the denaturation of the complex A_UD.

6. Conclusion

We have demonstrated above that drug-biomolecule type associations in as complex a mixed solvent system as methanol/water follow closely the predictions of the solvophobic theory [1,4]. The analysis presented allowed the extraction from solution data [3,6] of the molecular surface area change [1] of the present example, the actinomycin-deoxyguanosine complex upon complex formation yielding $\Delta\sigma(AD - A - D) = -36.4$ Å^2.

The theory correctly predicted the interesting maximum observed at 8% (v/v) MeOH in the complex formation stabilization free energy vs. MeOH/water composition curve.

The differential surface free energy (δ) version of the theory [4] we also used and from the AD data in the solutions we obtained the δ for methanol/water in contact with DNA-type bases and drug molecules, in particular, actinomycin. This δ may now be used in the equations above (and in ref. 4) to predict the solvent effect free energies of new drug-nucleoside, drug-DNA, etc., associations in methanol/water mixtures.

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