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SURFACE ACTIVITIES OF TERTIARY AMINE LOCAL ANESTHETICS AT AIR/WATER INTERFACE IN THE PRESENCE AND ABSENCE OF PHOSPHOLIPID MONOLAYERS

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Summary

Adsorption of procaine and tetracaine to the dipalmitoyl phosphatidylcholine monolayers at the air/water interface is analyzed in terms of two types of interaction: (1) between the phospholipid molecules and the ligand molecules, and (2) among the ligand molecules themselves.

The presence of the phospholipid monolayer increases the surface concentration of the anesthetics. The interaction energy, ω_{AB}, between the phospholipid molecules and the anesthetic molecules at the interface accounts for this excess adsorption. The values were $-2.95\,kT$ for procaine and $-2.99\,kT$ for tetracaine where k is the Boltzmann constant and $T = 298$ K.

The adsorption of the local anesthetics to the interface was cooperative. The interaction energy, ω_{AA}, between the anesthetics molecules on the surface determines the cooperativity. The values were $-0.056\,kT$ for procaine and $-0.397\,kT$ for tetracaine, where $T = 298$ K. This parameter determines the slope of the curve plotted relating the surface concentration (Γ) and the logarithm of the bulk concentration (log C). When $|\omega_{AA}/kT| \geqslant 1$, the adsorption follows the phase-transition.

A parameter K_A, which is related to the difference of the free energy of anesthetics between the surface and the bulk molecules, locates the take-off point of the adsorption curve at the log C axis. The values were $2.15 \cdot 10^3$ for procaine and $7.00 \cdot 10^3$ for tetracaine.

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In spite of the general assumption that the difference in the clinical potency among local anesthetics are attributable to their lipid solubility, the present results showed that the phospholipid-anesthetic interaction energies for procaine and tetracaine were similar. The larger surface concentration of tetracaine than procaine at the same bulk concentration was due to the combined effect of K_A and ω_{AA}. K_A represents the tendency of the anesthetic molecules to escape from the hydrogen-bonded water phase, and ω_{AA} determines the cooperativity factor causing these molecules to aggregate at the interface.

It was also observed that the charged forms of the anesthetics have nonzero surface activities.

Introduction

Phospholipid or lipid surface monolayers have been used for the study of anesthetic interaction with model membranes [1—12]. The penetration into surface monolayers by ligand molecules has usually been analyzed by the Gibbs surface excess or the adsorption isotherm to the empty space available at the surface unoccupied by the monolayer molecules. The interaction between the phospholipid molecules and the ligand molecules is generally not considered.

The present study compares the equilibrium surface adsorption of the local anesthetics, procaine and tetracaine, at the air/water interface in the presence and absence of the dipalmitoyl phosphatidylcholine monolayer. The presence of the phospholipid monolayer increased the surface concentration of the local anesthetics, which indicated that the penetration into the monolayer is not a simple adsorption on the vacant area of the water surface.

A statistical mechanical method was used to separate the interaction energy between the phospholipid molecules and the anesthetic molecules from that among the anesthetic molecules. The adsorption is analyzed according to these two interaction energies and a parameter, K_A, which is related to the free energy difference between the local anesthetics in the bulk phase and those in the surface phase.

Materials and Methods

Synthetic 1,2-dipalmitoyl-*sn*-glycerophosphorylcholine was purchased from Sigma and its purity was checked by thin-layer chromatography which was found to show a single spot. Crystalline tetracaine and procaine were generous gifts from The Sterling Winthrop Institute. All other chemicals were the highest grade available.

Water was purified by distillation followed by passing through columns of activated charcoal and mixed-bed ion-exchange resins and then by ultrafiltration in a Millipore water purifying system. The specific resistance was maintained above 18 $M\Omega \cdot cm^{-1}$ and the absence of surface active impurities was checked by dynamic surface tension measurements as previously described [10].

Surface tension was measured by the platinum hanging plate method with

a Cahn Electrobalance with a surface tension accessory and recorded with a Hewlett-Packard X-Y recorder. The surface of the platinum plate was roughened by scrubbing with scouring powder to ensure wettability. The sample trough was Kel-F lined and had dimensions of 11.5 × 6.0 × 1.2 cm. The platinum plate and the trough were washed with running tap water, rinsed with distilled water followed by ethanol and acetone. The platinum plate was further brought to white heat with a Bunsen burner before use.

The temperature of the sample was monitored by a thermistor immersed in the solution and was maintained at 25 ± 0.1°C by circulating water from a constant-temperature water bath.

The phospholipid was dissolved in a 9 : 1 (v/v) mixture of hexane and ethanol in appropriate concentrations. An aliquot of 20 μl was spread on the surface of subphase water with a Hamilton microsyringe.

The local anesthetics were dissolved in water. Both local anesthetics were supplied as HCl salt and dissociate into charged (LH^+) and uncharged (L) species according to the pK_a of the compound and the pH of the solution.

$$pH - pK_a = \log([L]/[LH^+]) \quad (1)$$

The uncharged forms were prepared by adding 0.1 M NaOH. The local anesthetic solutions were prepared just prior to use to avoid alkaline hydrolysis. For the study of the pH dependency of the local anesthetic adsorption, 0.1 M phosphate buffer was used. The presence of phosphate buffer did not show any measurable effect on the surface pressure. A Beckman model 3500 Digital pH meter with a glass electrode was used for the pH measurements.

The obtained data were interpretated according to the theory presented below. The best fit for the experimental data to the theory was obtained by the non-linear, least-square method with a Hewlett-Packard 9810-A calculator and an X-Y plotter.

Theory

One-component system

Thermodynamically, surface free energy, F, is related to temperature, T, surface area, a, and the number of surface molecules of composition i, N_i, by the Eqn. [13]:

$$dF = -SdT + \gamma da + \sum_i \mu_i dN_i \quad (2)$$

where S is surface entropy, γ is surface tension and μ_i is the chemical potential of component i. The decrease of the surface energy is defined at constant temperature and constant N_i as

$$-dF = -\gamma da - (-\gamma_0 da) = (\gamma_0 - \gamma)da \quad (3)$$

or

$$-dF = \pi da \quad (4)$$

where the area covered by the film molecules increases by da and the clean or

uncovered area decreases by da. π is the surface pressure and

$$\pi = \gamma_0 - \gamma \tag{5}$$

where γ_0 is the surface tension of the clean solution. Eqn. 2 can be rearranged as

$$dF = -SdT - \pi da + \sum_i \mu_i dN_i \tag{6}$$

Hence

$$\mu_i = (\partial F/\partial N_i)_{T,a} \tag{7}$$

and

$$\pi = -(\partial F/\partial a)_{T,Ni} \tag{8}$$

For a one-component system and by the Bragg-Williams approximation, Fowler and Guggenheim [13] gave the relation

$$F = kT\left\{-N_A \ln q(T) + \frac{\omega_{AA}}{kT}\frac{N_A^2}{N_s} + N_A \ln \frac{N_A}{N_s} + (N_s - N_A)\ln\frac{N_s - N_A}{N_s}\right\} \tag{9}$$

and from Eqn. 7,

$$\mu_A = kT\left\{\ln\left(\frac{N_A}{N_s - N_A}\right) - \ln q(T) + \frac{2\omega_{AA}}{kT}\frac{N_A}{N_s}\right\} \tag{10}$$

where N_s is the total number of sites on the surface, N_A is the number of sites occupied by the A molecules, and $(N_s - N_A)$ is the number of unoccupied sites. The interaction energy between pairs of nearest neighbors of A molecules is denoted by ω and $\omega_{AA} = (z/2)\omega$ with z being the coordination number of an adsorbed molecule on the surface and 1/2 being the factor included to prevent counting bonds twice. The parameter $q(T)$ is the partition function for the internal degrees of freedom of an adsorbed molecule, and it is temperature dependent. We define

$$a = a_0 N_s \tag{11}$$

where a_0 is the area per unit site, assuming every site occupies the same area. Thus, from Eqn. 8:

$$\pi = -\frac{1}{a_0}\left(\frac{\partial F}{\partial N_s}\right)_{T,N_A} \tag{12}$$

or

$$\pi = -\frac{kT}{a_0}\left\{-\frac{\omega_{AA}}{kT}\frac{N_A^2}{N_B^2} + \ln\left(\frac{N_s - N_A}{N_s}\right)\right\} \tag{13}$$

Now let

$$X_i = N_i/N_s \tag{14}$$

so that Eqns. 10 and 13 become

$$\mu_A = kT\left\{\ln\left(\frac{X_A}{1 - X_A}\right) - \ln q(T) + \frac{2\omega_{AA}}{kT}X_A\right\} \tag{15}$$

and

$$\pi = -\frac{kT}{a_0}\left(-\frac{\omega_{AA}}{kT}X_A^2 + \ln(1-X_A)\right) \tag{16}$$

Since both μ_A and π are functions of X_A, we have

$$\left(\frac{\partial \mu_A}{\partial X_A}\right)_{T,q(T)} = kT\left(\frac{1}{X_A} + \frac{1}{1-X_A} + \frac{2\omega_{AA}}{kT}\right) \tag{17}$$

and

$$\left(\frac{\partial \pi}{\partial X_A}\right)_{T,q(T)} = \frac{kT}{a_0}\left(\frac{2\omega_{AA}}{kT} + \frac{1}{(1-X_A)X_A}\right)X_A \tag{18}$$

This leads to

$$\left(\frac{\partial \pi}{\partial \mu_A}\right)_{T,q(T)} = \frac{(\partial \pi/\partial X_A)_{T,q}}{(\partial \mu_A/\partial X_A)_{T,q}} = \frac{X_A}{a_0}$$

$$= \frac{N_A/N_s}{a_0} = \frac{N_A}{a} = \Gamma_A \tag{19}$$

where Γ_A is the surface density of A, and has the dimension of molecules per Å^2. At equilibrium,

$$\mu_A^{surface} = \mu_A^{bulk} = \mu_A^0 + kT \ln \gamma C \tag{20}$$

where γ is the activity coefficient based on concentration, and as $C \to 0$, then $\gamma \to 1$ [14]; μ_A^0 is the chemical potential of the corresponding standard state, which is a hypothetical state of the solute A in the solution at a concentration of 1 mol/l. We have, at constant temperature,

$$d\mu_A^{surface} = kT \, d \ln \gamma + kT \, d \ln C \tag{21}$$

In low concentration range, 10^{-2} M or less, the change of lnγ is smaller than that of ln$C$ so that dlnγ is neglected.

Eqn. 19 becomes

$$\left(\frac{\partial \pi}{kT \, \partial \ln C}\right)_{T,a} = \Gamma_A \tag{22}$$

which is the Gibbs equation of surface excess. Eqns. 15 and 16 can be further rearranged as follows.

$$\ln C = -\ln K + \ln\left(\frac{\Gamma_A}{1/a_0 - \Gamma_A}\right) + \frac{2\omega_{AA}}{kT}a_0\Gamma_A \tag{23}$$

where

$$K = \exp(\mu_A^0/kT)\gamma q(T)$$

and

$$\pi = \frac{kT}{a_0}\left(\frac{\omega_{AA}}{kT}\Gamma_A^2 a_0^2 - \ln(1-a_0\Gamma_A)\right) \tag{24}$$

Experimentally, one obtains Γ_A from the slope of the curve relating π vs.

log C for each concentration by using Eqn. 22. When there are enough points of (log C, Γ_A) and (π, Γ_A), the parameters K, a_0 and ω_{AA} can be obtained from Eqn. 23 and checked with Eqn. 24.

When ω_{AA} is very small as in the ideal case, Eqn. 16 reduces to

$$\pi = -\frac{kT}{a_0} \ln(1 - X_A) \tag{25}$$

In a dilute system, $X_A << 1$ and

$$-\ln(1 - X_A) = X_A = N_A/N_s \tag{26}$$

thus

$$\pi a_0 N_s = N_A kT = \pi a \tag{27}$$

which is the ideal gas equation of state for the surface.

Two-component system

The surface free energy is related to the surface canonical partition function, $Q(N_A, N_B, T)$, by

$$F = -kT \ln Q(N_A, N_B, T) \tag{28}$$

and

$$Q(N_A, N_B, T) = [q(A, T)]^{N_A}[q(B, T)]^{N_B} Q_c(N_A, N_B, T) \tag{29}$$

where $Q_c(N_A, N_B, T)$ is the configurational canonical partition function which is defined as

$$Q_c(N_A, N_B, T) = \sum_{N_{AA}} \sum_{N_{AB}} \sum_{N_{BB}} g(N_A, N_B, N_{AA}, N_{AB}, N_{BB}) \exp(-E_{int}/kT) \tag{30}$$

with $g(N_A, N_B, N_{AA}, N_{AB}, N_{BB})$ being the degeneracy and E_{int} being the interaction energy. The quantity E_{int} is defined as

$$E_{int} = N_{AA}\epsilon_{AA} + N_{BB}\epsilon_{BB} + N_{AB}\epsilon_{AB} + N_{AV}\epsilon_{AV} + N_{BV}\epsilon_{BV} + N_{VV}\epsilon_{VV} \tag{31}$$

where N_{ij} denotes the number of nearest neighbor pairs having the interaction energy ϵ_{ij}. The subscript V denotes the quantity of vacant sites. Only interaction between the nearest neighbors is considered in Eqn. 31. Notice that [13]

$$N_{AV} = zN_A - 2N_{AA} - N_{AB} \tag{32}$$

$$N_{BV} = zN_B - 2N_{BB} - N_{AB} \tag{33}$$

and

$$2N_{VV} = zN_V - N_{AV} - N_{BV} \tag{34}$$

where z is the coordination number of an adsorbed molecule. Eqn. 31 can be rearranged as:

$$E_{int} = N_{AA}(\epsilon_{AA} - 2\epsilon_{AV} + \epsilon_{VV})$$

$$+ N_{BB}(\epsilon_{BB} - 2\epsilon_{BV} + \epsilon_{VV})$$

$$+ N_{AB}(\epsilon_{AB} - \epsilon_{AV} - \epsilon_{BV} + \epsilon_{VV})$$

$$+ N_A(z\epsilon_{AV} - z\epsilon_{VV}/2) + N_B(z\epsilon_{BV} - z\epsilon_{VV}/2)$$

$$+ N_V(z\epsilon_{VV}/2) \tag{35}$$

Thus the canonical ensemble partition function, $Q(N_A, N_B, T)$ of Eqn. 29 becomes

$$Q(N_A, N_B, T) = [q(A, T)]^{N_A} \exp(-N_A(z\epsilon_{AV} - z\epsilon_{VV}/2)/kT)$$

$$\times [q(B, T)]^{N_B} \exp(-N_B(z\epsilon_{BV} - z\epsilon_{VV}/2)/kT)$$

$$\times \exp(-N_V z\epsilon_{VV}/2kT)$$

$$\times \sum_{N_{AA}} \sum_{N_{AB}} \sum_{N_{BB}} \{g(N_{AA}, N_{AB}, N_{BB}, N_A, N_B) \exp(-E(N_{AA}, N_{AB}, N_{BB})/kT)\} \tag{36}$$

where

$$E(N_{AA}, N_{AB}, N_{BB}) = N_{AA}(\epsilon_{AA} - 2\epsilon_{AV} + \epsilon_{VV})$$

$$+ N_{BB}(\epsilon_{BB} - 2\epsilon_{BV} + \epsilon_{VV}) + N_{AB}(\epsilon_{AB} - \epsilon_{AV} - \epsilon_{BV} + \epsilon_{VV}) \tag{37}$$

Since $\Sigma_{N_{AA}} \Sigma_{N_{AB}} \Sigma_{N_{BB}} g \exp(-E/kT)$ is difficult to evaluate, we shall use the averaged values of N_{AA}, N_{AB} and N_{BB} according to the Bragg-Williams approximation. The 0-th-order random approximation then gives [13]

$$\overline{N_{AA}} = \tfrac{1}{2} z \frac{N_A^2}{N_s} \tag{38}$$

$$\overline{N_{BB}} = \tfrac{1}{2} z \frac{N_B^2}{N_s} \tag{39}$$

and

$$\overline{N_{AB}} = z \frac{N_A N_B}{N_s} \tag{40}$$

Using Eqns. 37—40, Eqn. 36 can be rearranged to give

$$Q(N_A, N_B, T) = \overline{q_A}^{N_A} \overline{q_B}^{N_B} \cdot \exp\left\{-\left(\frac{\omega_{AA}}{kT}\frac{N_A^2}{N_s} + \frac{\omega_{BB}}{kT}\frac{N_B^2}{N_s} + \frac{\omega_{AB}}{kT}\frac{N_A N_B}{N_s}\right)\right\}$$

$$\times \sum_{N_{AA}} \sum_{N_{AB}} \sum_{N_{BB}} g(N_A, N_B, N_{AA}, N_{AB}, N_{BB}) \tag{41}$$

where for g we have used the relation

$$\sum_{N_{AA}} \sum_{N_{AB}} \sum_{N_{BB}} g(N_A, N_B, N_{AA}, N_{AB}, N_{BB}) = \frac{N_s!}{N_A! N_B! (N_s - N_A - N_B)!} \tag{42}$$

$$\overline{q_A} = q(A, T) \exp(-z(\epsilon_{AV} - \epsilon_{VV})/kT) \tag{43}$$

and

$$q_B = q(B, T) \exp(-z(\epsilon_{BV} - \epsilon_{VV})/kT) \tag{44}$$

The energy origin of $Q(N_A, N_B, T)$ in Eqn. 41 has been shifted. In Eqn. 41, ω_{AA}, ω_{BB} and ω_{AB} are defined by

$$\omega_{AA} = z(\epsilon_{AA} - 2\epsilon_{AV} + \epsilon_{VV})/2 \tag{45}$$

$$\omega_{BB} = z(\epsilon_{BB} - 2\epsilon_{BV} + \epsilon_{VV})/2 \tag{46}$$

and

$$\omega_{AB} = z(\epsilon_{AB} - \epsilon_{AV} - \epsilon_{BV} + \epsilon_{VV}) \tag{47}$$

respectively.

From Eqn. 28, we now have

$$F = -kT\left\{N_A \ln \overline{q_A} + N_B \ln \overline{q_B} - \left(\omega_{AA}\frac{N_A^2}{N_s} + \omega_{AB}\frac{N_A N_B}{N_s} + \omega_{BB}\frac{N_B^2}{N_s}\right)\Big/kT - \left[N_A \ln(N_A/N_s) + N_B \ln(N_B/N_s) + (N_s - N_A - N_B)\ln\left(\frac{N_s - N_A - N_B}{N_s}\right)\right]\right\} \tag{48}$$

Thus, from Eqn. 12,

$$\pi_{AB} = \frac{kT}{a_0}\left\{\frac{\omega_{AA}}{kT}X_A^2 + \frac{\omega_{AB}}{kT}X_A X_B + \frac{\omega_{BB}}{kT}X_B^2 - \ln(1 - X_A - X_B)\right\} \tag{49}$$

where X_i is the fraction of sites occupied by composition i. From Eqn. 7, we have the chemical potential for

$$\mu_A = -kT \ln \overline{q_A} + 2\omega_{AA}\frac{N_A}{N_s} + \omega_{AB}\frac{N_B}{N_s} - kT \ln\left(\frac{N_s - N_A - N_B}{N_A}\right) \tag{50}$$

From Eqn. 20, we have

$$\mu_A = \mu_A^0 + kT \ln(\gamma_A C_A) \tag{51}$$

Eqns. 50 and 51 are combined as

$$\ln C_A = -\ln K_A + \frac{2\omega_{AA}}{kT}X_A + \frac{\omega_{AB}}{kT}X_B - \ln\left(\frac{1 - X_A - X_B}{X_A}\right) \tag{52}$$

where

$$K_i = \exp(\mu_i^0/kT)\gamma_i \overline{q_i} \tag{53}$$

A similar expression for $\ln C_B$ can be obtained. Here it is found that π_{AB}, $\ln C_A$ and $\ln C_B$ are all functions of X_A and X_B.

If X_B is kept fixed, π_{AB}, $\ln C_A$ and $\ln C_B$ will depend only on X_A, and we have

$$\left(\frac{\partial \ln C_B}{\partial X_A}\right)_{X_B,T} = \frac{\omega_{AB}}{kT} + \frac{1}{1 - X_A - X_B} - \left(\frac{\partial \ln \gamma_B}{\partial X_A}\right)_{X_B,T} \tag{54}$$

Assuming component B forms an insoluble monolayer such that C_B does not depend on X_A, we find

$$(\partial \ln C_B/\partial X_A)_{X_B,T} = 0\,, \tag{55}$$

$$\left(\frac{\partial \ln C_A}{\partial X_A}\right)_{X_B,T} = \frac{2\omega_{AA}}{kT} + \frac{1}{1-X_A-X_B} + \frac{1}{X_A} - \left(\frac{\partial \ln \gamma_A}{\partial X_A}\right)_{X_B,T} \quad (56)$$

and

$$\left(\frac{\partial \pi_{AB}}{\partial X_A}\right)_{X_B,T} = \frac{kT}{a_0}\left(\frac{\omega_{AB}}{kT}X_B + \frac{2\omega_{AA}}{kT}X_A + \frac{1}{1-X_A-X_B}\right) \quad (57)$$

Substituting Eqn. 54 into Eqn. 57, we obtain

$$\frac{(\partial \pi_{AB}/\partial X_A)}{(\partial \ln C_A/\partial X_A)} = \left(\frac{\partial \pi_{AB}}{\partial \ln C_A}\right)_{X_B,T} = \frac{kT}{a_0}X_A = kT\,\Gamma_A \quad (58)$$

or

$$\left(\frac{\partial \pi_{AB}}{kT\,\partial \ln C_A}\right)_{X_B,T} = \Gamma_A \quad (59)$$

where Γ_A is the surface density of composition A, and the dependence of γ_i on X_A has been ignored. Hence the slope of the curve of π_{AB} vs. $\ln C_A$ in the two-component system relates to the surface density of composition A. Eqn. 52 can be rewritten as

$$\ln C_A = -\ln K_A + \frac{2\omega_{AA}}{kT}a_0\Gamma_A + \frac{\omega_{AB}}{kT}a_0\Gamma_B + \ln\left(\frac{\Gamma_A}{1/a_0-\Gamma_A-\Gamma_B}\right) \quad (60)$$

From Eqn. 49, when the second component, say A, is absent, π reduces to a one-component system, or,

$$\pi_B = \frac{kT}{a_0}\left\{\frac{\omega_{BB}}{kT}X_B^2 - \ln(1-X_B)\right\} \quad (61)$$

which is the same as Eqn. 16.

Comparing Eqn. 49 for π_{AB} for a two-component system and Eqn. 61 for π_B for a one-component system, one may eliminate ω_{BB} by using $\Delta\pi = \pi_{AB} - \pi_B$,

$$\Delta\pi = \frac{kT}{a_0}\left\{\frac{\omega_{AB}}{kT}a_0^2\Gamma_A\Gamma_B + \frac{\omega_{AA}}{kT}a_0^2\Gamma_A^2 + \ln\left(1+\frac{\Gamma_A}{1/a_0-\Gamma_A-\Gamma_B}\right)\right\} \quad (62)$$

Eqns. 59, 60 and 62 are used to obtain the interaction energy terms ω_{ij} between molecules of different components.

Results

Fig. 1 shows the pH-dependent depression of the surface tension of the tetracaine solution at varying concentrations in the absence of phospholipid monolayer. Tetracaine lowered the surface tension of the solution at all pH values studied. According to Eqn. 22, tetracaine has non-zero surface density even at the low pH region where most of the molecules are protonated. In order to estimate the contribution of charged [LH^+] and uncharged [L] forms toward the surface excess, the concentrations of each form were estimated according to Eqn. 1. Fig. 2 shows the surface tension of the tetracaine solution

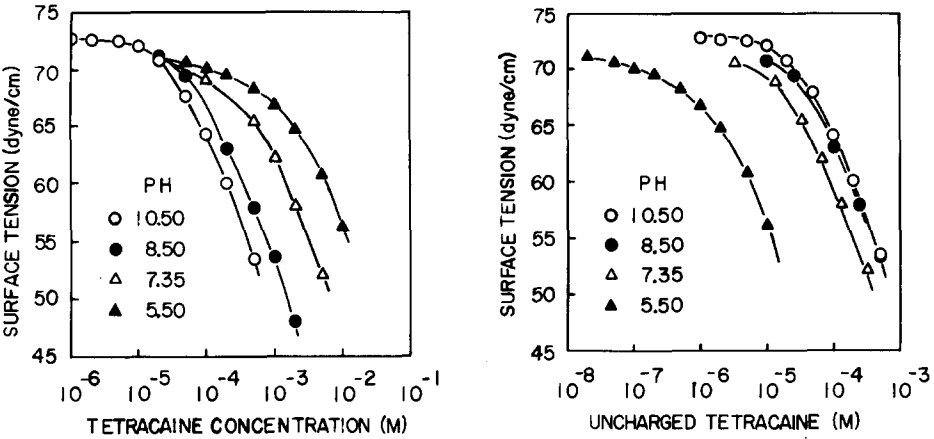


Fig. 1. The effects of subphase pH to the surface tension of the tetracaine solution in the absence of the monolayer. Abscissa is the bulk tetracaine concentrations and the pH values are shown in the figure.

Fig. 2. The values of the surface tension of the tetracaine solution of Fig. 1 are replotted against the concentrations of uncharged molecules of tetracaine. If only uncharged molecules were surface active, all lines should fall in a single curve.

plotted against the calculated concentration of the uncharged forms. If the surface-adsorbed molecules were solely composed of the uncharged species, all curves at different pH should fall on the same line. The deviation from this assumption shown in Fig. 2 indicates that the surface adsorbed molecules include the charged species as well as uncharged species.

Because uncharged species showed much larger surface activities, further

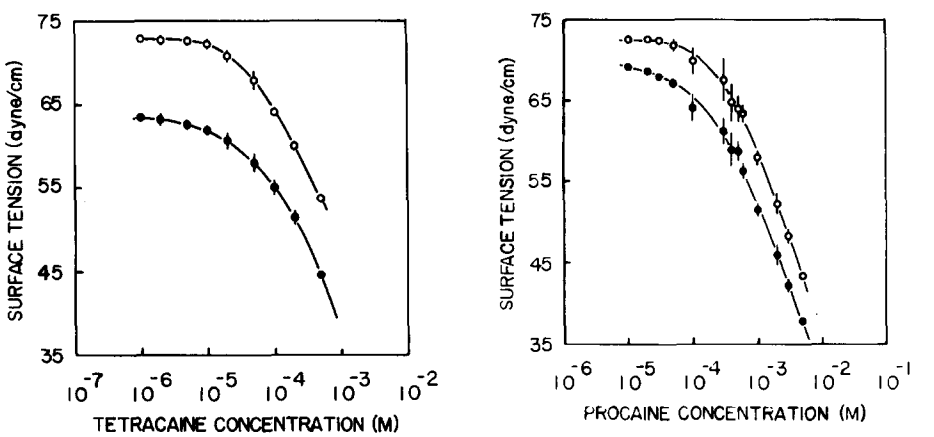
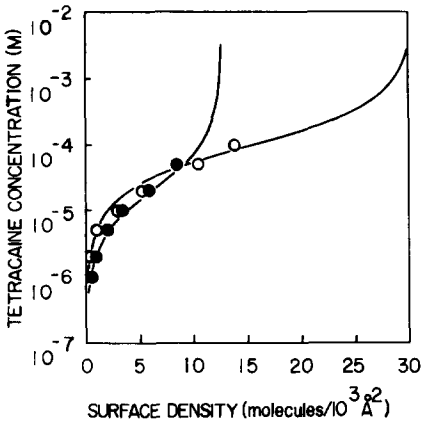


Fig. 3. Surface tensions of tetracaine solutions at pH 10.5 with (●) and without (○) dipalmitoyl phosphatidylcholine monolayer. The surface density of the phospholipid was $1.796 \cdot 10^{-2}$ molecules/Å^2. Each points are the mean of at least four separate studies and the standard error is indicated by the vertical bar.

Fig. 4. Surface tensions of procaine solutions at pH 11.0 with (●) and without (○) dipalmitoyl phosphatidylcholine monolayer. The surface density of the phospholipid was $1.183 \cdot 10^{-2}$ molecules/Å^2. Each points are the mean of at least four separate studies and the standard error is indicated by the vertical bar.



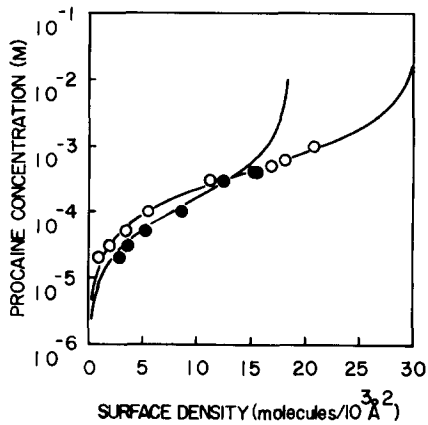
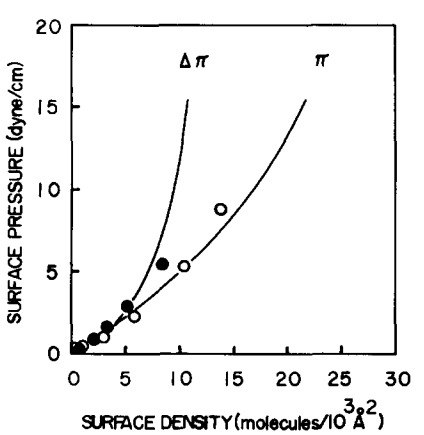


Fig. 5. Relations of the bulk concentration of tetracaine and its surface density at the air/water interface at pH 10.5, in the presence (●) and absence (○) of the monolayer. The curves are drawn according to Enq. 23 and 60.

Fig. 6. Relations of the bulk concentration of procaine and its surface density at the air/water interface at pH 11.0, in the presence (●) and absence (○) of the monolayer. The curves are drawn according to Eqns. 23 and 60.

studies were performed with uncharged species of the local anesthetics.

The surface tensions at various concentrations of tetracaine and procaine solutions at pH values two units above their pK_a values (8.5 and 8.95, respectively) with and without dipalmitoyl phosphatidylcholine monolayer are shown in Figs. 3 and 4, respectively. The surface concentrations of the phospholipid were $1.796 \cdot 10^{-2}$ molecules/Å^2 for the tetracaine study and



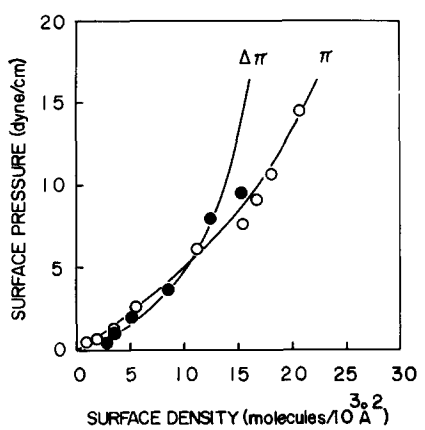


Fig. 7. Surface pressures of the tetracaine solution without the monolayer (○) and their difference from those with the monolayer (●) are plotted against the surface densities of tetracaine. The curves are drawn according to Eqns. 24 and 62.

Fig. 8. Surface pressures of the procaine solution without the monolayer (○) and their difference from those with the monolayer (●) are plotted against the surface densities of procaine. The curves are drawn according to Eqns. 24 and 62.

TABLE I

THE INTERFACIAL PARAMETERS OF PROCAINE OR TETRACAINE SOLUTION WITH OR WITHOUT DIPALMITOYL PHOSPHATIDYLCHOLINE MONOLAYER

	Procaine	Tetracaine
pK_a	8.95	8.50
a_0 in Å^2/site	32.65	32.65
K_A	2150	7000
ω_{AA}	$-0.056kT$ *	$-0.379kT$ *
ω_{AB}	$-2.95kT$ *	$-2.99kT$ *

* T = 298K

$1.183 \cdot 10^{-2}$ molecules/Å^2 for the procaine study. The values of the surface density of the local anesthetics, Γ_A, were obtained according to Eqns. 22 and 59 for the absence and presence of the monolayer, respectively, and are shown in Figs. 5 and 6.

Figs. 7 and 8 show the relation between the surface pressures and the surface densities of these anesthetics. The solid lines in these figures are the theoretical curves obtained according to Eqns. 24 and 62 for the surface pressures in the absence of the monolayer and for the difference of the surface pressures between the presence and absence of the monolayer, respectively.

The parameters, a_0, ω_{AA}, K_A and ω_{AB} are listed in Table I.

The calculated and experimental values of surface tension of the local anesthetics at varying bulk concentrations are shown in Figs. 9 and 10.

The agreements between the experimental values and the theoretical values are excellent except for the high concentration range of tetracaine in the

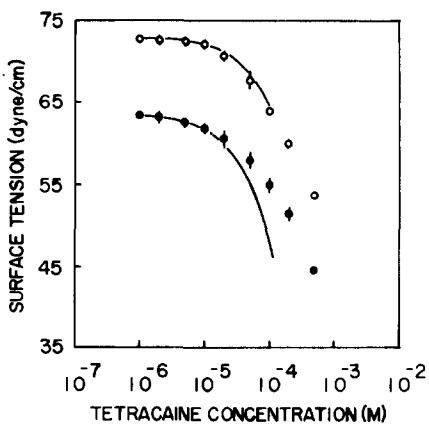


Fig. 9. Comparison of the experimental and calculated results of the surface tension of the tetracaine solution in the presence (●) and absence (○) of the monolayer. The curves are drawn through the theoretical values.

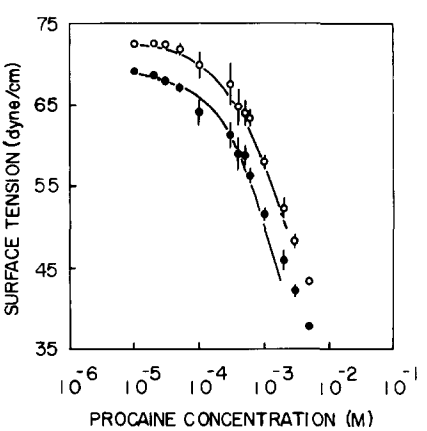


Fig. 10. Comparison of the experimental and calculated results of the surface tension of the procaine solution in the presence (●) and absence (○) of the monolayer. The curves are drawn through the theoretical values.

presence of the phospholipid monolayer. The monolayer was in the solid-condensed state in the tetracaine system with a molecular area of 56 Å^2 while in the procaine system it was 85 Å^2 and was in the expanded state. The better fit in the procaine system indicates that the random distribution approach to the surface adsorption in this analysis is more applicable to the liquid-expanded state of the phospholipid monolayer than to the solid-condensed state.

Discussion

For the evaluation of the numerical values obtained in the present study, the value of 32.65 Å^2 per site for a_0 is now examined. Eqn. 13 is rearranged as

$$\pi = \omega_{AA} a_0 \bar{a}^{-2} + \frac{kT}{a_0} \ln\left(\frac{1/a_0}{1/a_0 - 1/\bar{a}}\right) \tag{63}$$

where $\bar{a} = a/N_A$.

From this equation, the critical point of the phase-transition of the phospholipid monolayer can be found by taking

$$(\partial\pi/\partial\bar{a})_T = 0 \quad \text{and} \quad (\partial^2\pi/\partial\bar{a}^2)_T = 0 \tag{64}$$

This leads to $\bar{a} = 2a_0$ at the critical point. In the present study with the dipalmitoyl phosphatidylcholine, the calculated critical area per lipid molecule therefore becomes about 65 Å^2, which is in reasonable agreement with the experimental result of about 60 Å^2 reported by Phillips and Chapman [15].

Since the surface concentration of the anesthetics was higher when the phospholipid monolayers were present, the adsorption was not a simple penetration of anesthetics into the vacant space at the monolayer. The lipid monolayer pulls up more local anesthetic molecules to the interface from the bulk.

A parameter ω_{AB} represents the energy of this interaction. In this study, we found the ω_{AB} values for tetracaine and procaine were very close; $-2.99\,kT$ for tetracaine and $-2.95\,kT$ for procaine (T = 298 K).

In a one-component system, the parameter K_A, which locates the sigmoidal curve of log C_A versus Γ_A along the log C_A axis, has the value

$$K_A = \exp(\mu^0_{bulk}/kT) \cdot \gamma q_{surface}(T) \tag{65}$$

It is known that the equilibrium constant, K_{eq}, for the system A $\rightleftharpoons$ B is expressed as

$$K_{eq} = \frac{q_B}{q_A} \exp(-\Delta\mu/kT) \tag{66}$$

where $\Delta\mu = \mu^0_B - \mu^0_A$ is the standard molar free energy difference between the two states. It is obvious that the parameter K_A relates to K_{eq} with a specific activity coefficient γ. Hence besides this dependence on the activity coefficient, K_A is proportional to the equilibrium constant of local anesthetics between the bulk and surface phase which, in turn, is proportional to the free energy difference. Thus, this parameter represents the tendency of the anesthetic molecules to escape from the highly structured water phase. The larger the value of K_A the more the molecules tend go to the surface.

For a two-component system, Eqn. 60 can be rearranged to give

$$\ln C_A = -\ln\left\{K_A \exp\left(-\frac{\omega_{AB}}{kT} a_0 \Gamma_B\right)\right\} + \frac{2\omega_{AA}}{kT} a_0 \Gamma_A + \ln\left(\frac{\Gamma_A}{1/a_0 - \Gamma_A - \Gamma_B}\right) \quad (67)$$

Comparing this to a one-component system, the apparent K_{app} which is defined as

$$K_{app} = K_A \exp\left(-\frac{\omega_{AB}}{kT} a_0 \Gamma_B\right) \quad (68)$$

is responsible for the location of the sigmoidal curve of log C_A vs Γ_A along the log C_A axis. K_{app} increases with the absolute value of ω_{AB}, because the phospholipid molecules attract the anesthetic molecules. The higher this value, the smaller the concentration of local anesthetics in the bulk needed to reach a point where there is a sharp increase in its surface density. In cases where the ω_{AB} values are very close, such as for tetracaine and procaine, K_A determines the point where the condensation of anesthetic molecules at the interface starts.

The cooperative phenomenon seen in Figs. 5 and 6 depends upon the value of ω_{AA}, the interaction energy among surface molecules. The increase of surface density with bulk concentration is sharper with larger absolute values of ω_{AA}. When $|\omega_{AA}/kT| \geqslant 1$ the curves relating the surface and bulk concentrations behave as the phase-transition.

The teracaine solution has a higher absolute value of ω_{AA}, 0.379 kT, than the value of 0.056 kT of the procaine solution where $T = 298$ K. Therefore, the curves with tetracaine are more nearly parallel to the abscissa, showing a higher degree of cooperativity. The physical meaning of this cooperative effect is that the anesthetic molecules will not condense at the interface until there are enough molecules at the interface close enough together to attract each other. Strong condensation then sets in.

The phenomenon familiar to anesthesiologists that the sensation of pain returns abruptly in spinal anesthetized patients may be explained by this high cooperativity of the interfacial density of local anesthetics.

The clinical potency of tetracaine exceeds that of procaine by one order of magnitude [16]. This difference is often attributed to the higher lipophilicity of tetracaine dissolving into the lipid region of the membrane. The present findings, however, indicate that the lipid-anesthetic interaction is only slightly responsible for this difference, although the presence of the monolayer increased the surface concentration of the local anesthetics.

The difference in the surface concentration is mainly caused by the differences in K_A and ω_{AA}. The tendency of the tetracaine molecules to escape from the water phase is higher than that for procaine molecules. The take-off point of the surface concentration at the axis of the logarithm of the bulk concentration is lower with tetracaine than with procaine. After the take-off, the surface concentration of tetracaine increases more rapidly than that of procaine because ω_{AA} determines the slope of the curve.

Because the pK_a of tetracaine is closer to the physiological pH than is the pK_a of procaine, procaine needs to increase the bulk concentration about one order of magnitude higher than tetracaine to reach the same surface concentration at the clinical concentration range and at the pH 7.4.

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