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Surface Tension of Aqueous Solutions of Prostaglandins and Related Unsaturated Fatty Acids

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The surface properties of prostaglandins $F_{2\alpha}$, E_2, D_2, A_2 and B_2 at the air–water interface were studied under conditions where these compounds are stable. Our values of critical micelle concentrations (cmc) of prostaglandins $F_{2\alpha}$, E_2, A_2 and B_2 at pH 7.80 were 2.7×10^{-2}, 1.3×10^{-2}, 8.8×10^{-3} and 6.2×10^{-3} mol·dm^{-3}, respectively, whereas the formation of micelles at pH 4.96 was not detected for any of the prostaglandins tested. The cmc for prostaglandin D_2 could not be determined due to the instability of the compound at pH 7.80. However, the surface behavior of prostaglandin D_2 at pH 4.96 was very similar to that of prostaglandin E_2, indicating that prostaglandin D_2 may have a value of cmc at pH 7.80 close to that of prostaglandin E_2. These values of prostaglandins are comparable to those of sodium decyl sulfate, dodecanoic acid and tetradecyl phosphate. Possible structures of prostaglandins at the air–water interface and probable mechanisms of membrane penetration and permeation are discussed.

Keywords——prostaglandin; eicosapolyenoic acid; stability; surface tension; critical micelle concentration; adsorption; adsorptivity; area occupied; tilting angle; permeability

Prostaglandins are extremely potent, ubiquitous compounds with a variety of physiological and pharmacological actions.[2)] The chemistry of prostaglandins has been well studied, including their chemical synthesis and structural determination.[3)] Further, the conformational structures of PGA, PGE, and the Tris–*p*-bromobenzoate of $PGF_{1\beta}$ have been elucidated by X-ray crystallographic[4)] and nuclear magnetic resonance studies.[5)] In short, the α-chain (C_1—C_7) and the ω-chain (C_{13}—C_{20}) are aligned in parallel in close proximity, constituting a hair-pin structure. The five-membered ring of PGE or PGF is somewhat distorted, whereas that of PGA is nearly planar due to the enone structure causing greater separation of the two chains. Analysis of the proton magnetic resonance spectrum of $PGF_{2\alpha}$ indicated that the dihedral angle of the C_8 and the C_{12} protons is close to 180°, resulting in a half-chair conformation of the ring, which has pseudo-equatorial orientation at the C_{11} and C_{15} hydroxyl groups and pseudo-axial orientation at the C_9-hydroxyl group.

In spite of these chemical studies, the degree of association of prostaglandin molecules in water has not yet been determined for many biologically important prostaglandins. Johnson and Saunders[6)] first measured the surface tension of $PGF_{2\beta}$ and PGE_1 up to 4×10^{-3} and 4×10^{-4} mol·dm^{-3}, respectively. Roseman and Yalkowsky[7)] reported the solubility behavior and surface properties of the tromethamine salt of $PGF_{2\alpha}$, indicating that the properties are qualitatively similar to those of bile acids. No work has been reported on the surface properties of prostaglandins other than PGE_1, $PGF_{2\alpha}$ and $PGF_{2\beta}$, however. In view of the importance of prostaglandins in pharmaceutical applications, and their biological functions, it is important to know the physiochemical parameters for the association of prostaglandins

with water. In this study, we report the surface tension of aqueous solutions of $PGF_{2\alpha}$, PGE_2, PGD_2, PGA_2 and PGB_2 over a wide concentration range in comparison with related fatty acids and detergents.

Experimental

Materials——PGE_2, $PGF_{2\alpha}$ and PGD_2 were 99.7, 98—99 and 98% pure, respectively. PGA_2 and PGB_2 were prepared from a solution of PGE_2 by dehydration and isomerization reactions at 60 °C under an N_2 atmosphere as described by Monkhouse *et al.*[8] The sample of PGA_2 consisted of 92% PGA_2, 7% PGB_2 and 1% other products, and that of PGB_2 had a purity of 98%. 11,14-Eicosadienoic acid (11,14-EA) and 8,11,14-eicosatrienoic acid (8,11,14-EA) were purchased from Sigma. Their purities were 99 and 99%, respectively. 11,14,17-Eicosatrienoic acid (11,14,17-EA) and 5,8,11,14-eicosatetraenoic acid (5,8,11,14-EA) were obtained from Wako Pure Chemicals. Their purities were 99 and 99%, respectively. Purity was examined by both high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC).

Preparation of Solutions——Prostaglandins were dissolved at room temperature either in a solution containing 1/5 M NaH_2PO_4 (12.5 ml), 1/5 M NaOH (10.7 ml) and H_2O (26.8 ml) (pH 7.80) or a solution containing 1/11.4 M NaH_2PO_4 (33 ml) and 1/11.4 M Na_2HPO_4 (1.0 ml) (pH 4.96). Since PGD_2 is unstable at pH 7.80, the solution was made at pH 4.96 only. Solutions of unsaturated fatty acids were prepared as follows. A solution containing an equivalent amount of NaOH was heated to 90 °C. To this solution, a methanol solution containing the fatty acid was added dropwise with stirring. After complete saponification, the phosphate buffer solution (pH 7.80 or pH 4.96) was added. Eicosapolyenoic acids with no or one double bond did not dissolve at pH 7.80, and those with less than four double bonds did not dissolve in high concentration at pH 4.96. Accordingly, these fatty acids were not subjected to the measurement of surface tension.

Measurement of Surface Tension——The surface tension was measured with a Du Nöuy tensiometer (Shimadzu) with a platinum ring (d=23 mm) at 25±0.1 °C, and ionic strength 0.093 mol·dm^{-3}, at pH 7.80 or 4.96. This instrument was the same as that used by Nakagaki and Yokoyama[9] and its handling was described in that paper. For the calculation of the surface tension of aqueous solutions, the value of 71.96 mN·m^{-1} was used as the surface tension of pure water at 25 °C. The experimental determination of the surface tension was precise to ±0.1 mN·m^{-1}. Deionized and then twice-distilled water was used throughout this study.

Examination of Purity and Stability——For examination of the purity and stability of the prostaglandins used, HPLC was carried out at 24 °C using a column (Toyo Soda LS-410, 4 mm × 20 cm) with 0.02 M KH_2PO_4 : CH_3CN : isopropyl alcohol (35 : 13 : 11, v/v) as a solvent. The flow rate was 1.0 ml/min and the absorbance at 205 nm was recorded. TLC was carried out using a plate (Kieselgel 60 F_{254}, Merck) with $CHCl_3$: tetrahydrofuran : acetic acid (10 : 2 : 1, v/v) as a solvent. The spots were detected by using an ethanol solution of a phosphomolybdate reagent. The stability of prostaglandins was examined by determining the remaining quantity after measurement of surface tension. Volatility of eicosapolyenoic acids was found to be negligible under our conditions, since we obtained the same result using commercially available sodium salts.

Results

Stability of Prostaglandins

$PGF_{2\alpha}$ is known to be very stable (for 6 months at room temperature at pH between 5 and 11),[10] whereas PGE_2 is unstable due to the conversion of PGE_2 to PGA_2 and PGB_2 by dehydration and isomerization reactions.[8,11] In accordance with the reported observations,[8] our estimation of PGE_2 degradation to PGA_2 and unidentified product, possibly an 8-iso product, was approximately 30% after treatment at pH 10 and 70 °C for 2 min, whereas PGE_2 was stable for 2 h at pH 7.8 and 5.0 (it decomposed to the extents of approximately 2% and 0.3%, respectively, at 25 °C). It has been reported[8] that PGA_2 is very stable in the acidic region and that the rate constant for degradation of PGA_2 in the alkaline region is approximately 1/10 of that of PGE_2. Therefore, the degradation of PGA_2 at pH 7.8 and 4.96 and 25 °C is considered to be negligible. Furthermore, PGB_2 is considered to be very stable because it is the final decomposition product. Since the stability of PGD_2 has never been reported, we examined it under various conditions. At pH 4.5 and 20 °C, PGD_2 decomposed to the extent of approximately 2% after 6 h, whereas at pH 10 and 20 °C decomposition was approximately 12% after 3 h. The extents of degradation of PGD_2 were 11 and 28% at pH 4.5

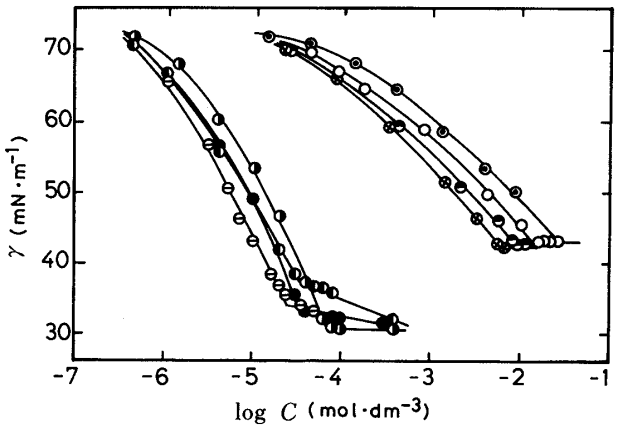

Fig. 1. Surface Tension of Prostaglandins $F_{2\alpha}$, E_2, A_2, B_2 and Related Unsaturated Fatty Acids at 25 °C and pH 7.80

◎, $PGF_{2\alpha}$; ○, PGE_2; ◓, PGA_2; ⊗, PGB_2; ◑, 5,8,11,14-EA; ●, 8,11,14-EA; ◐, 11,14,17-EA; ⊖, 11,14-EA.

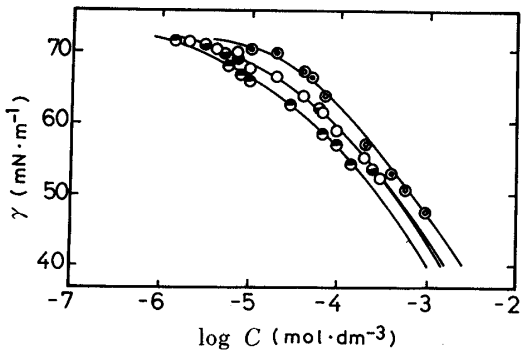

Fig. 2. Surface Tension of Prostaglandins $F_{2\alpha}$, E_2, D_2 and A_2 at 25 °C and pH 4.96

◎, $PGF_{2\alpha}$; ○, PGE_2; ◒, PGD_2; ◓, PGA_2.

and 10 after 3 h of treatment at 37 °C, respectively. The degradation of PGD_2 appears to proceed through dehydration and isomerization, being similar to that of PGE_2. Consequently, experimental conditions were selected for individual PG's: for PGD_2 the measurement of surface tension was performed only at pH 4.96 and 25 °C, and for PGE_2, PGA_2 and $PGF_{2\alpha}$ the measurement was done at pH 4.96 and 7.80 at 25 °C. Additionally, the measurement of surface tension of PGB_2 was done only at pH 7.80 because insufficient material was available. All measurements were finished within 120 min. Therefore, degradation of PG's should be less than 2%. The formation of small amounts of degradation products should not affect our measurements seriously. Nevertheless, our data at pH 4.96 are more reliable than those at pH 7.80 because of the greater stabilities, at the former pH.

Surface Tensions of the Solutions

Figures 1 and 2 show the changes of surface tension, γ, of PGE_2, $PGF_{2\alpha}$, PGD_2, PGA_2, PGB_2 and related unsaturated fatty acids as a function of the logarithm of concentration ($\log C$).

Adsorption at the Air–Water Interface

The adsorption amount, Γ, is defined by the Gibbs adsorption equation[12]:

$$\Gamma = -\frac{C}{iRT}\left(\frac{\partial \gamma}{\partial C}\right) \tag{1}$$

where R is the gas constant (8.314 J $mol^{-1} \cdot K^{-1}$), T is the absolute temperature (298.15 K at 25 °C), and C is the molar concentration of surface-active substances. In addition,

$$i = \nu_+ \left(\frac{\nu_+ C}{\nu_+ C + \nu_S C_S}\right) + \nu_- \tag{2}$$

where ν_+ and ν_- are the numbers of mol of cation and anion, respectively, formed from 1 mol of ionic surface-active molecule, ν_S is the number of mol of cations per mol of salt added, and C_S is the concentration of the salt. Under our experimental conditions, $i \fallingdotseq \nu_- = 1$. Therefore, Eq. 1 simply becomes:

$$\Gamma = -\frac{C}{RT} \cdot \frac{\partial \gamma}{\partial C} \tag{3}$$

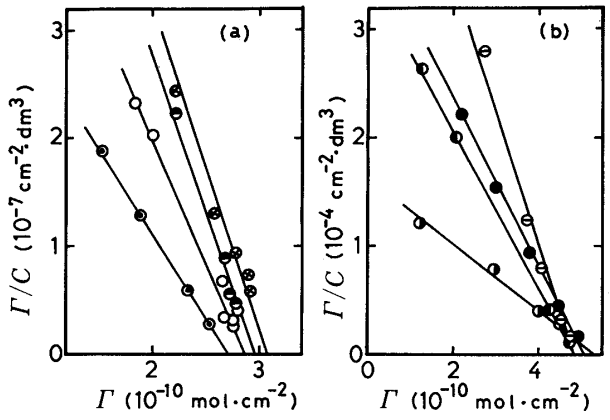

Fig. 3. Langmuir Plots for Prostaglandins (a) and Unsaturated Fatty Acids (b) at 25 °C and pH 7.80

Symbols are the same as in Fig. 1.

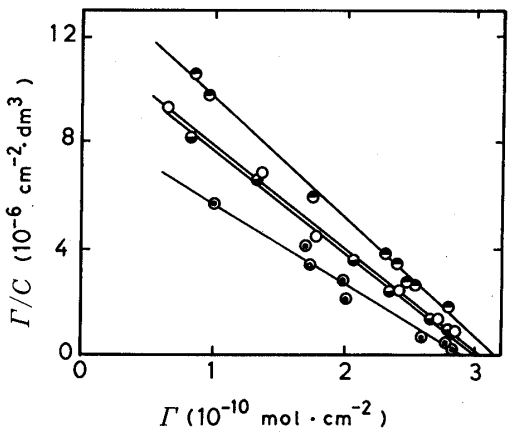

Fig. 4. Langmuir Plots for Prostaglandins at 25 °C and pH 4.96

Symbols are the same as in Fig. 2.

Using Eq. 3, the adsorption amount, Γ, was calculated from the slopes of the curves in Figs. 1 and 2.

Next, the Langmuir equation[12] was applied to these systems.

$$\frac{\Gamma}{C}=k\,(\Gamma_\infty-\Gamma) \tag{4}$$

where Γ_∞ is the saturated adsorption amount and k is a constant related to the strength of adsorption. The plots of Γ/C against Γ are presented for unsaturated fatty acids and prostaglandins in Figs. 3 and 4, at pH 7.80 and 4.96, respectively, and show good linear relationships. From the values of slope and intercept, k and Γ_∞ were calculated.

The area occupied by a molecule under saturated conditions for adsorption, A_∞, was obtained by the following equation:

$$A_\infty=\frac{1}{\Gamma_\infty\times N} \tag{5}$$

TABLE I. Surface Properties of Prostaglandins and Unsaturated Fatty Acids at 25 °C and pH 7.80

Unsaturated fatty acids C_{20}	cmc ($mol\cdot dm^{-3}$)	k ($mol^{-1}\cdot dm^3$)	Γ_∞ ($mol\cdot cm^{-2}$)	A_∞ ($Å^2\cdot molecule^{-1}$)
11,14-EA	2.6×10^{-5}	1.237×10^{6}	4.82×10^{-10}	34.5
11,14,17-EA	3.4×10^{-5}	7.990×10^{5}	4.84×10^{-10}	34.3
8,11,14-EA	3.6×10^{-5}	7.530×10^{5}	5.10×10^{-10}	32.6
5,8,11,14-EA	6.8×10^{-5}	3.080×10^{5}	5.30×10^{-10}	31.3
$PGF_{2\alpha}$	2.7×10^{-2} a)	1.676×10^{3}	2.70×10^{-10}	61.5
PGE_2	1.3×10^{-2}	2.275×10^{3}	2.87×10^{-10}	57.9
PGA_2	8.8×10^{-3}	2.888×10^{3}	2.96×10^{-10}	56.1
PGB_2	6.2×10^{-3}	3.025×10^{3}	3.07×10^{-10}	54.1

a) Extrapolated value.

where N is the Avogadro number. These results are summarized in Tables I and II.

Values of Critical Micelle Concentration (cmc) of Prostaglandins and Unsaturated Fatty Acids

The values of cmc at pH 7.80 were estimated from the break points, whereas at pH 4.96

TABLE II. Surface Properties of Prostaglandins at 25 °C and pH 4.96[a]

Prostaglandins	Molecular weight	k ($mol^{-1} \cdot dm^3$)	Γ_∞ ($mol \cdot cm^{-2}$)	A_∞ ($Å^2 \cdot molecule^{-1}$)	d (Å)	$\theta°$
$PGF_{2\alpha}$	354.5	3.013×10^4	2.89×10^{-10}	57.5	11.38	28.9
PGE_2	352.5	3.875×10^4	3.02×10^{-10}	55.1	11.80	24.8
PGD_2	352.5	3.875×10^4	2.97×10^{-10}	55.8	11.66	26.2
PGA_2	334.5	4.488×10^4	3.14×10^{-10}	52.9	11.67	—

a) Micelle formation could not be estimated because of the limited solubility at this pH.

no break point was detectable up to a concentration close to the solubility limit. It has been reported[7] that $PGF_{2\alpha}$ does not form any micelles in the acidic region.

Since the available amount of $PGF_{2\alpha}$ was limited, the estimation of cmc for $PGF_{2\alpha}$ at pH 7.80 was carried out as follows. The relation between surface pressure ($\gamma_0 - \gamma$) and concentration can be expressed by an empirical equation of Szyszkowski[12]:

$$\gamma_0 - \gamma = K \log(1 + kC) \tag{6}$$

where γ_0 is the surface tension of pure water ($\gamma_0 = 71.96\,mN \cdot m^{-1}$ at 25 °C), K is the Szyszkowski constant and k is the Langmuir constant. The value of K is obtained from the following equation:

$$K = 2.303\,RT\Gamma_\infty \tag{7}$$

Using Eq. 6, the curve of γ against $\log C$ was extrapolated (Fig. 1). When the γ value at the cmc, γ_{cmc}, for PGE_2 ($43\,mN \cdot m^{-1}$) is used, the cmc value for $PGF_{2\alpha}$ is obtained as 2.7×10^{-2} $mol \cdot dm^{-3}$ (Table I). This cmc value for $PGF_{2\alpha}$ is in good agreement with the value (2.6×10^{-2} $mol \cdot dm^{-3}$ in aqueous NaOH solution, pH 8.0) given by Roseman and Yalkowsky.[7] In addition, the value of γ_{cmc} ($43\,mN \cdot m^{-1}$) is also consistent with the value of Roseman and Yalkowsky.[7] Therefore, the results obtained in this paper seem to be reasonable.

Discussion

Values of cmc of Prostaglandins

The cmc's of prostaglandins were compared with those of eicosapolyenoic acids and higher alcohols (Tables I and III). Among eicosapolyenoic acids, the cmc increases as the number of double bonds increases. Among eicosatrienoic acids tested, the position of the double bonds does not affect the cmc. The cmc's of prostaglandins are very much higher than those of eicosapolyenoic acids, and are close to those of dodecanoic acid, decyl sulfate and tetradecyl phosphate (Table III). Since heptanoic acid and octanoic acid do not readily form micelles, a value of cmc for prostaglandins close to that for dodecanoic acid would indicate a weak ability of prostaglandins to form micelles. The comparison with higher alcohols reveals that the cmc of prostaglandin is approximately 10 times larger than that of 1,2-decanediol.

Area Occupied by a Prostaglandin Molecule

The values for the occupied area under maximum compression were 61.5 and 57.9 $Å^2$ at pH 7.80 for $PGF_{2\alpha}$ and PGE_2, respectively. Those at pH 4.96 were 55.8, 57.5 and 55.1 $Å^2$ for PGD_2, $PGF_{2\alpha}$ and PGE_2, respectively. The smaller values at pH 4.96 can be explained by the proximity of the pK_a of prostaglandin (5.44 for $PGF_{2\beta}$),[6] where the molecules are more strongly adsorbed and more compacted on the air–water interface. The occupied area for $PGF_{2\alpha}$ is larger than that for PGE_2, and that for PGE_2 is larger than that for PGA_2. The

TABLE III. Critical Micelle Concentrations of Various Compounds

Compounds	cmc ($mol \cdot dm^{-3}$)	Solvent	Reference
$C_nH_{2n+1}COONa$			
$n=6$	0.4	H_2O	13
$n=7$	0.36	H_2O	14
$n=9$	9.4×10^{-2}	H_2O	14
$n=11$	2.4×10^{-2}	H_2O	14
$n=13$	6.4×10^{-3}	H_2O	14
$C_nH_{2n+1}SO_4Na$			
$n=8$	0.13	H_2O	13
$n=10$	3.3×10^{-2}	H_2O	14
$n=12$	8.1×10^{-3}	H_2O	15
$n=14$	2.1×10^{-3}	H_2O	14
$n=16$	5.2×10^{-4}	H_2O	14
$C_nH_{2n+1}PO_4Na_2$			
$n=12$	4.0×10^{-2}	0.1 M NaOH	16
$n=14$	1.7×10^{-2}	0.1 M NaOH	16
$n=16$	6.0×10^{-3}	0.1 M NaOH	16
$C_nH_{2n}(OH)_2$			
1,2-$C_{10}(OH)_2$	2.3×10^{-3}	H_2O	17
1,3-$C_{11}(OH)_2$	2.3×10^{-3}	H_2O	17
1,2-$C_{12}(OH)_2$	1.8×10^{-4}	H_2O	17
1,3-$C_{13}(OH)_2$	1.4×10^{-4}	H_2O	17
1,3-$C_{15}(OH)_2$	1.3×10^{-5}	H_2O	17

numbers of hydroxyl groups in the prostaglandin molecules are 3, 2 and 1, for $PGF_{2\alpha}$, PGE_2 and PGA_2, respectively. The occupied area is, therefore, correlated with the number of hydroxyl groups in the molecule. PGD_2 is an isomer of PGE_2; the hydroxyl group in PGD_2 is at position 9, whereas that in PGE_2 is at position 11. The occupied area for PGD_2 is slightly larger than that of PGE_2. Based on the 3-dimensional model of $PGF_{2\beta}$,[6] the terminal carboxyl group and 3 hydroxyl groups in the molecule are aligned in approximately the same plane. Accordingly, the polar groups lie in the water surface and the hydrophobic portions orient toward the air phase. This model suggests that the prostaglandin molecule is tilted at the air–water interface. An analogous model may be relevant to the association of prostaglandin with phospholipid membrane: the polar groups orient toward the water phase and polar heads of phospholipids and the hydrophobic portions interact with fatty acyl groups of phospholipids. In addition, the occupied areas for eicosapolyenoic acids are approximately 1/2 of those for prostaglandins, supporting the tilting model of prostaglandins at the air–water interface.

Tilting Angles of Prostaglandins at the Air–Water Interface

The thickness of the monolayer membrane, d, can be calculated from the following equation:

$$d=\frac{M_r}{\rho\times A_\infty\times N} \tag{8}$$

where M_r is the molecular weight and ρ is the density. Next, the tilting angle is calculated from Eq. 9 by using the value of the length of a molecule, l:

$$\theta=\cos^{-1}\left(\frac{d}{l}\right) \tag{9}$$

In Table II, calculated tilting angles are shown for prostaglandins with comparable molecular size. Here, we have assumed that the density is 0.9 and the length of molecule is 13 Å. Since PGA_2 has a five-membered ring with a planar structure, the alignment of its α and ω chains is not the same as those of $PGF_{2\alpha}$, PGE_2 and PGD_2. Thus, the molecular length of PGA_2 may significantly deviate from 13 Å. Therefore, comparison among only $PGF_{2\alpha}$, PGE_2 and PGD_2 is more likely to be valid. The calculated tilting angle of $PGF_{2\alpha}$ is larger (approximately 3—4 °) than those of PGE_2 and PGD_2. The small difference between PGE_2 and PGD_2 (1.4 °) may suggest that the orientation of PGE_2 toward the air–water interface is similar but not identical with that of PGD_2. The relative locations of the ring hydroxyl group, the hydroxyl group at position 15, and the terminal carboxyl group in these prostaglandins may result in different orientations of these molecules on the interface.

Our tilting model suggests that the diameter (8.84 Å) of the prostaglandin-occupied area (60 $Å^2$) roughly corresponds to the length of five C–C bonds and one double bond. The length between C_1 and C_9 has been estimated to be 10.5 Å by X-ray crystallography.[4] Furthermore, the calculated thickness of a monolayer of PG's is 11—12 Å with a tilting angle of 26—29 ° (Table II). The hydrophobic portion of the prostaglandin molecule between positions C_2 and C_8 may be exposed to the air phase. Interestingly, it is known that chemical modifications at positions C_2, C_3 and C_4 result in loss of biological activity.[2]

Permeability of Membranes to Prostaglandins

In biological systems, the ability of prostaglandins to permeate across membranes is important. If prostaglandins can not permeate readily, receptor proteins may exist in membranes. In this connection, prostaglandin receptors have been partly characterized in the corpus luteum,[18] liver,[19] adipocytes,[20] adrenal,[21] myometrium,[22] thyroid,[23] erythrocytes[24] and kidney,[25] but little is known about their chemical nature. Based on our present study of the surface properties of prostaglandins, and their high solubilities in water, we predict the presence of a receptor for PG's on membranes.

In kidney, the $PGF_{2\alpha}$-receptor interactions are significantly enhanced in the presence of $MgCl_2$ or $CaCl_2$, which influence PGE_2 binding only minimally.[25] The metal interaction might be most important for the polar prostaglandin, $PGF_{2\alpha}$. Clearly the hydrophilic groups of a prostaglandin molecule rather than its hydrophobic domain should be responsible for the interaction with metal ions and water.

In many tissues, prostaglandin receptors are localized in the plasma membranes, but substantial binding capacity has also been found in mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes and nuclei.[20-22] The mechanisms for penetration and permeation of prostaglandins may not be the same for different membranes within a cell. Nonetheless, our present results seem to provide some basis for further studies of these biochemical problems.

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