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Possible Models of Adsorbed Deoxyadenosine-5′-monophosphate on a Gold Electrode derived from the Characteristic Specular Reflectance Data

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The adsorption behavior of deoxyadenosine-5′-monophosphate (dAMP) on a gold electrode has been studied by specular reflectivity measurement in 0.1 M $NaClO_4$ solutions ranging in concentration from 2.5×10^{-6} to 2.5×10^{-4} M. The reflectivity change due to the adsorption of dAMP was suggestive of the formation of two types of adsorbed layer depending on both the bulk concentration and the electrode potential; one of them, termed the type I layer, appeared at low concentrations at potentials around the point of zero charge (pzc) as well as over the whole concentration range at rather negative potentials, and the type II layer appeared at high concentrations around pzc. The results obtained by analysis of the isotherms corresponding to these layers suggest that the adsorbed molecule is oriented with its adenine moiety nearly flat on the surface in the type I layer, but perpendicular to it in the type II layer. Such a change in orientation was not observed for adenine, deoxyadenosine, or deoxyadenosine-5′-diphosphate or -triphosphate. The characteristics of the adsorption behavior of dAMP were reasonably explained on the basis of its structural effect, *i.e.*, the ease of orientational change of the adsorbed molecules and the resulting mutual interaction between adjacent adsorbates in the type II layer.

Keywords——deoxyadenosine-5′-monophosphate; adsorption; gold electrode; specular reflectivity; reorientation

Extensive investigations of the interaction and association of nucleic acid components such as nucleosides and nucleotides have been made in aqueous solutions using various experimental techniques[1–8] with a view to obtaining information about the state of nucleic acids in solution. In view of their roles in living organisms, their interactions with biological interfaces are also of particular interest in relation to the appearance of their biological effects both *in vivo* and *in vitro*.[9–11] However, difficulties in the detailed investigation of such interactions arise because of the structural complexity of biosurfaces.

An electrode surface may be regarded as a very simple model of a biosurface in terms of its being a charged surface in contact with an electrolyte solution.[12] A number of studies have been made on the adsorption of nucleic acids and their components at electrode surfaces from this viewpoint.[13–16] Detailed investigations on mercury electrodes over relatively wide concentration ranges were made for adenine, thymine, uracil, their nucleosides and nucleotides, and cytidine; in all cases, these compounds were found to form both so-called dilute and compact adsorption layers.[12,17–24] Association and/or rearrangement of adsorbed molecules take place in the latter layer, but the facility of such behavior seems to differ with the electrode material.[25–29]

Recently the adsorption behavior of adenine, deoxyadenosine and deoxynucleotides on a gold electrode was investigated by the present authors over a relatively low concentration range by means of specular reflectivity measurement.[30] All the compounds were found to be adsorbed in the potential region around the point of zero charge (pzc), the results were suggestive of flat orientation on the electrode surface in contact with their adenine moiety. Among the compounds, deoxyadenosine-5′-monophosphate(dAMP) exhibits a double step isotherm at potentials around pzc when its bulk concentration is relatively high. Such a trend was not be observed for adenine, deoxyadenosine, or deoxyadenosine-5′-diphosphate(dADP) or -triphosphate(dATP).

The purpose of this paper is to discuss how the adsorption states of dAMP change with surface excess of the compound on a gold electrode.

Experimental

Chemicals——Adenine obtained from Wako Pure Chemical Industries, Ltd., 2′-deoxyadenosine, 2′-deoxyadenosine-5′-phosphate (dAMP) and 2′-deoxyadenosine-5′-triphosphate (dATP) obtained from P-L Biochemicals, Inc., and 2′-deoxyadenosine-5′-diphosphate (dADP) obtained from Sigma Chemicals, were used without further purification. Stock solutions were prepared by dissolving known amounts of these compounds in 0.1 M $NaClO_4$. The pH of the deoxynucleotide solutions was adjusted to 6.5—7.0 by addition of NaOH solution.

0.1 M $NaClO_4$ solution was prepared by dissolving guaranteed grade material obtained from Kanto Chemical Co., Inc. in redistilled water, which served as a supporting electrolyte to avoid the effect of adsorabble buffer anions.

Apparatus and Procedure——A gold plate, 25 mm × 22 mm, was used as a working electrode. All the potentials were referred to the Ag/AgCl electrode. A gold plate was also used as a counter electrode. The working electrode was pretreated by the procedure described in a previous paper,[31] and fixed to a Teflon holder held at the center of the electrolytic cell.

Electrolysis was carried out by the linear potential sweep and potential step methods, the potential being applied with an HA-101 potentiostat (Hokuto Denko Ltd.) in conjunction with an HB-107A function generator (Hokuto Denko Ltd.). Cyclic voltammetry was done by a conventional method and current-potential (i-E) curves were recorded on an XY-recorder (Yokogawa Ltd., type 3077).

Specular reflectivity measurements were carried out using an apparatus purchased from Mikasa Co. Ltd. Perpendicularly polarized light of a fixed wavelength of 500 nm from a halogen lamp (Osram) was directed on the surface of the working electrode with an incident angle of 15°. The reflected light was detected as described in the previous paper.[31] Reflectivity measurements were made during a linear potential sweep and a potentiostatic electrolysis according to the procedure described previously.[31,32] Reflectivity-potential (R/R_0-E) and reflectivity-time (R/R_0-t) curves were recorded using an XY-recorder (Yokogawa Ltd., type 3077).

All the measurements were carried out at 26 ± 1°C. The pH of the test solutions was kept at 7.0 ± 0.5, which was checked before and after every measurement. Before each measurement a sufficient amount of pure nitrogen gas was bubbled through the electrolytic solution to remove dissolved oxygen and it was continuously passed above the solution during the measurement to prevent the re-entry of air into the solution.

Results and Discussion

i-E and R/R_0-E curves on gold were measured simultaneously in the solution containing dAMP while the potential scan was started from −1.1V to the positive potential side and reversed at +0.4 V with a sweep rate of 100 mVs^{-1}. Measurements were made on solutions ranging in concentration from 2.5×10^{-6} to 2.5×10^{-4} M. Some examples of the results are shown in Fig. 1. The i-E curve shows a flat double layer region without dAMP (curve a). Addition of dAMP (curve b) causes no appreciable change indicating that no redox reaction of dAMP occurs in this potential region. R/R_0-E curve for the supporting electrolyte solution (curve c) is characterized by two approximately linear portions intersecting at about −0.1 V, which corresponds to pzc of gold in neutral solution.[33–35] In the presence of dAMP, the R/R_0-E curves show no change compared with curve c in the potential region between −1.1 and −0.8 V, but show a marked decrease in reflectivity at more positive potential regions (curves d and e in Fig. 1, where the R/R_0-E curves are drawn separately to avoid overlap upon each other). The reflectivity further decreases with increase of dAMP concentration but tends to a limiting value. These results indicate that the dAMP molecules are adsorbed on the gold electrode surface. The hysteresis of the R/R_0-E curves may be associated with a slow rate of adsorption-desorption of the dAMP.

The reflectivity changes corresponding to the adsorption equilibrium at various potentials between −0.8 and +0.3 V was obtained as follows. The potential was first set at −1.0 V, where no specific adsorption of dAMP is expected, and then stepped to a potential more positive than −1.0 V, and the R/R_0-t curve was recorded for a sufficient adsorption time to attain the equilibrium value of R/R_0. The same procedure was also applied to the supporting elec-

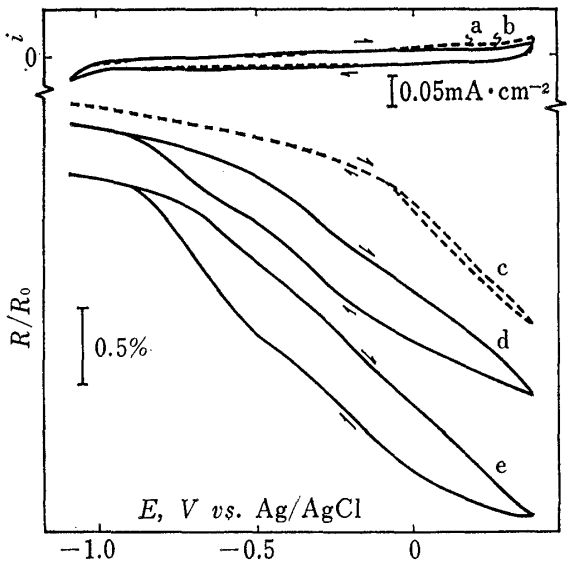

Fig. 1. i-E (a and b) and R/R_0-E (c-e) Curves on Gold in the Absence and Presence of dAMP

Concentrations: a) and c) 0, d) 7.4×10^{-6}, b) and e) 7.7×10^{-5}M. Sweep rate: 100 mVs^{-1}. λ: 500 nm.

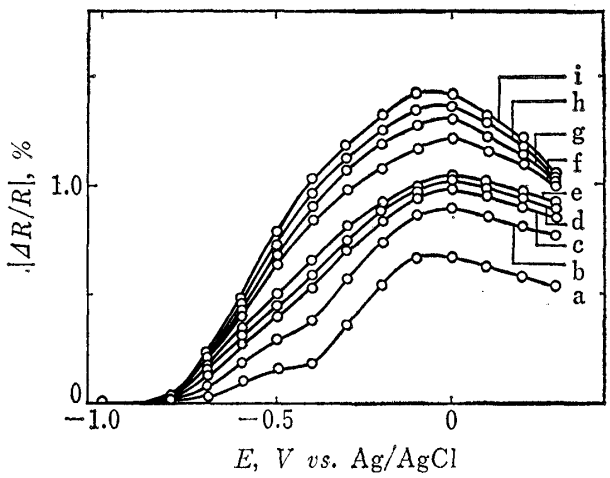

Fig. 2. Potential Dependence of the Reflectivity Change due to the Adsorption of dAMP

Concentrations: a) 2.8×10^{-6}, b) 1.5×10^{-5}, c) 2.6×10^{-5}, d) 3.3×10^{-5}, e) 4.1×10^{-5}, f) 7.7×10^{-5}, g) 9.9×10^{-5}, h) 1.7×10^{-4}, i) 2.4×10^{-4}M.

trolyte solution as the reference. The net change in reflectivity due to the adsorption, denoted as $\Delta R/R_0$, can then be obtained by subtracting the latter values from the former values at the same potential.

In Fig. 2, the $|\Delta R/R_0|$[36] thus obtained for dAMP is plotted against the applied potential. The $|\Delta R/R_0|$ value at each potential increases with increase of the concentration. As shown in Figs. 3A and 3B, the shape of the $|\Delta R/R_0|$ versus concentration curves differs depending on the applied potential. In the potential region between −0.8 and −0.4 V, $|\Delta R/R_0|$ increases monotonously with increase of concentration and tends to saturate (Fig. 3A). On the other hand, at potentials around pzc, $|\Delta R/R_0|$ increases stepwise with the concentration (Fig. 3B). Such a shape of the $|\Delta R/R_0|$ *vs.* c curves suggests that two types of adsorbed layer of dAMP may be formed on the electrode surface depending on both potential and concentration. One of them would be formed with low bulk concentrations (less than 5×10^{-5}M) around pzc as well as over the whole concentration range investigated at potential more negative than *ca.* −0.3 V, and the other with high bulk concentrations (more than 5×10^{-5}M) around pzc. In this paper, they are termed type I layer and type II layer for convenience, respectively.

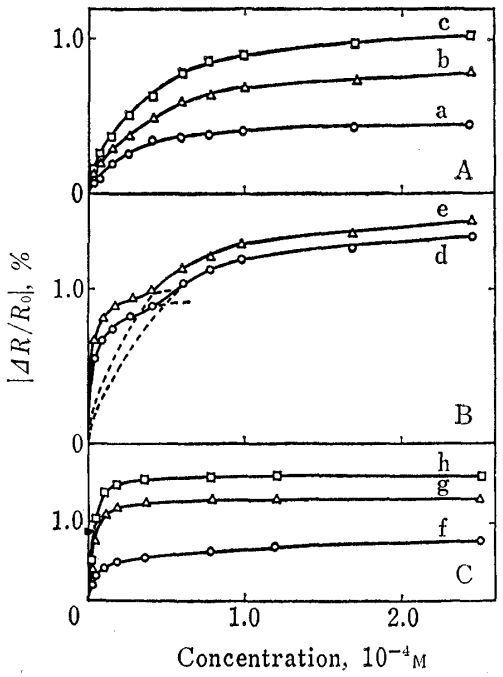

Fig. 3. Concentration Dependence of the Reflectivity Change for dAMP (A and B) at a) −0.6, b) −0.5, c) −0.4, d) −0.2, e) −0.1V, and for Adenine (C) at f) −0.6, g) −0.4, h) −0.1V

Adenine, deoxyadenosine, dADP and dATP are also adsorbed on a gold electrode as described in the previous paper,[30] but such anomalous $|\Delta R/R_0|$ *vs.* c relations were not observed.

Some typical results for adenine are shown in Fig. 3C. Next, the adsorption states of dAMP in both the layers will be discussed.

Structural Model for the Type I Layer

The reflectivity change, $\Delta R/R_0$, due to the presence of an adsorbed layer formed on the electrode surface is given by equation (1) according to McIntyre and Aspnes, based on a three-phase model,[37]

$$\left(\frac{\Delta R}{R_0}\right)_{\perp,\,\theta=1} = \frac{8\pi\, d n_s \cos\phi}{\lambda} \mathrm{Im}\left(\frac{\hat{\varepsilon}_{ad}-\hat{\varepsilon}_M}{n_s^2-\hat{\varepsilon}_M}\right) \quad (1)$$

where d is the thickness of the monolayer, ϕ the incidental angle, λ the wavelength, and n_s the refractive index of the bulk solution. $\bar{\varepsilon}_{ad}$ and $\bar{\varepsilon}_M$ are the complex dielectric constants of the adsorbed layer and the metal substrate, respectively.

A change in the amount of adsorption has been found to cause a change in the reflectivity of the electrode surface.[31] When the surface coverage is less than unity, the reflectivity change at coverage θ, $(\Delta R/R_0)_{\perp,\theta}$, is expressed as

$$\left(\frac{\Delta R}{R_0}\right)_{\perp,\,\theta} = \left(\frac{\Delta R}{R_0}\right)_{\perp,\,\theta=1} \cdot \theta \quad (2)$$

provided that d, $-_{ad}$ and $-_M$ remain unaltered during adsorption.[37-39] A change in the orientation of the adsorbed molecule to the electrode surface will cause a change in d and/or $-_{ad}$, and the reflectivity will consequently be changed. Thus, the equation (2) holds in the region where reorientation is not expected to occur. As can be seen in Figs. 3A and 3B, $|\Delta R/R_0|$ *vs.* c curves of dAMP give a Langmuir-type relation for concentrations lower than 5×10^{-5} M at the potential around pzc and also over the whole concentration range investigated at more negative potentials, suggesting that the orientation dose not change, at least under these conditions.

In our previous paper,[30] we calculated the θ value from equation (2) assuming the limiting value of $|\Delta R/R_0|$ at pzc to correspond to the reflectivity change at $\theta=1$, and the data obtained in the negative potential region were analyzed based on the isotherm

$$\theta/(1-\theta)^p = cK \quad (3)$$

where p is the number of water molecules replaced by one molecule of the adsorbed species, and c is the concentration, used in place of activity in a dilute solution. The constant K is the adsorption coefficient expressed as

$$K = \exp(-\Delta G^0_{ad}/RT + \alpha F\bar{E}/RT) \quad (4)$$

where ΔG^0_{ad} is the standard free energy change of adsorption at pzc, α the constant, and $\bar{E}$ the potential difference between the pzc and the potential at which the observation is made. The use of this isotherm is convenient to calculate the area occupied by the adsorbed molecule in terms of p. The adsorption parameters thus obtained are listed in Table I (in the lower part) for comparison with the present data. In the calculation of the area occupied by one adsorbed molecule from p, that occupied by one water molecule was assumed to be 0.09 nm^2.

The present results were analyzed in the same way. The limiting value in the $|\Delta R/R_0|$ *vs.* c relation corresponding to the type I layer, which is about 1%, was assumed to correspond to the reflectivity change at $\theta=1$. The adsorption parameters are given in the first row of Table I. The area of adsorbed dAMP in the type I layer is somewhat smaller than that of adenine of 0.42 nm^2, which agrees with its planar area estimated from the crystal structure reported by Donohue,[40] but is larger than the value of 0.22 nm^2 estimated for the perpendicular orientation of adenine with its amino group and N(7) in contact with the surface. These results suggest a nearly flat orientation of the adenine moiety of dAMP. The deoxyribose-phosphate group may be in the *syn* conformation, since adsorbed dAMP should occupy a

Table I. Adsorption Parameters of Adenine and Its Derivatives on Gold from 0.1 M $NaClO_4$

Compound	ΔG^0_{ad} (kj mol^{-1})	p	Area occupied by one adsorbed molecule (nm^2)[a]
dAMP			
Type I layer	−41.5±2.0	3.6±0.4	0.32±0.04
Type II layer	−29.8±1.8	1.9±0.3	0.17±0.03
Adenine[b]	−58.6±1.6	4.7±0.2	0.42±0.02
dAMP[b]	−41.4±1.2	3.7±0.3	0.33±0.03
dADP[b]	−42.3±2.4	3.8±0.3	0.34±0.03
dATP[b]	−43.1±2.0	3.6±0.4	0.32±0.04

a) Calculated from the p value by assuming the area occupied by one adsorbed water molecule to be 0.09 nm^2.
b) Data taken from our previous paper.[30]

larger area than adenine in the *anti* conformation. A possible model of the orientation is illustrated in Fig. 4, in which the adenine moiety comes in contact with the surface and the deoxyribose-phosphate group is directed to the solution side away from the surface. Fig. 4 also shows a flat orientation of adenine. dADP and dATP occupy about the same area as dAMP, implying similar orientations of all these molecules. The ΔG^0_{ad} value of dAMP is about 17 kj mol^{-1} smaller than that of adenine (Table I). This lower adsorptivity seems to result from the hydrophilic nature of the phosphate group and the repulsive interaction between the negative charge of the phosphate group and the electrode. Such effects are also observed for dADP and dATP.

Structural Model for the Type II Layer

As noted above, the $|\Delta R/R_0|$ *vs.* c curves of dAMP obtained at around pzc show a double-stepped shape (Fig. 3B). In spite of the higher concentration, a much longer time was needed to attain the equilibrium value of $|\Delta R/R_0|$ in the second step region compared with that in the first one. This result suggests a somewhat compact arrangement of adsorbed dAMP molecules in the layer corresponding to the second step region, *i.e.*, the type II layer. Two possibilities can be considered in this regard; one is an alteration of orientation of adsorbed

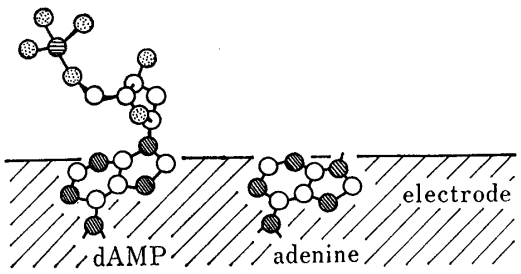

Fig. 4. Possible Orientation of dAMP in the Type I Layer and Adsorbed Adenine at the negatively Charged Surface of the Gold Electrode

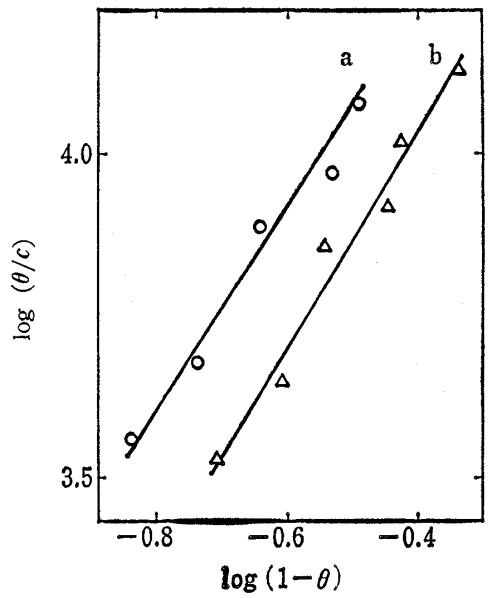

Fig. 5. Plots of log (θ/c) *vs.* log (1−θ) for dAMP at a) −0.2, b) −0.3V

dAMP (for example, from the flat to the perpendicular orientation), and the other is multilayer formation.

Recently, Krznarič *et al.* obtained double step adsorption isotherms of adenosine and its nucleotides and concluded that these compounds first form a dilute adsorption layer over a wide potential range and then form a compact film within a limited potential range around maximum adsorption at elevated bulk concentration.[21] In their study, the isotherms were successively treated by regarding them as being composed of two isotherms of the Frumkin type involving different adsorption parameters. Similarly, we considered the obtained $|\Delta R/R_0|$ *vs.* c curves as an overlap of two isotherms (see Fig. 3B, the second one being extrapolated by the dashed line) and attempted a quantitative analysis of the second by using equation (3) as follows. Combination of equations (3) and (4) leads to the equation

$$2.30 \log(\theta/c) = 2.30\, p \log(1-\theta) - \Delta G^0_{ad}/RT + \alpha F\bar{E}/RT \qquad (5)$$

Assuming that the limiting value of $|\Delta R/R_0|$ corresponds to the reflectivity at $\theta=1$, θ was calculated according to equation (2) in the concentration range higher than 5×10^{-5} M. $\log(\theta/c)$ is plotted against $\log(1-\theta)$ at -0.1 and -0.2 V in Fig. 5. The plots give straight lines, and the values of p and ΔG^0_{ad} can be evaluated from the slopes and the intercepts of the lines. The results are listed in Table I. The area occupied one adsorbed dAMP molecule is about a half of that in the type I layer, implying that adsorbed dAMP is no longer lying with the adenine moiety flat on the surface. The following results were evaluated from the structural data[40] as the projected areas of the adenine molecule in the perpendicular orientation; 0.12 nm^2 with the exocyclic amino group of the adenine moiety in contact with the surface, 0.19 nm^2 with the exocyclic amino group and N(1), 0.22 nm^2 with the exocyclic amino group and N(7). The obtained area is closest to the second value, but such orientation seems inappropriate since the phosphate group of the adsorbed molecule would exist close to the surface in the *syn* conformation of the deoxyribose-phosphate group. Thus, the third value may be most reasonable, where the deoxyribose -phosphate group is oriented to the solution side. In any event, the result indicates perpendicular orientation of the adenine moiety of dAMP in the type II layer.

On the other hand, it is difficult to consider multilayer formation for the type II layer since the increment of $|\Delta R/R_0|$ for the second step region of the $|\Delta R/R_0|$ *vs.* c curves is relatively small compared to that in the first one. In addition, the hydrophilic nature of the deoxyribose-phosphate group, which is oriented to the solution side in the type I layer of adsorbed dAMP, would prevent the second layer formation.

As described above, in the type I layer dAMP is adsorbed with its adenine moiety and the deoxyribose-phosphate group directed to the solution. The hydrophylicity of the phosphate group would serve as the driving force for the orientational change of the adenine moiety from flat to perpendicular to the surface (N(9) of the adenine moiety may be directed to the solution side). Such reorientation would be possible when the surface excess of dAMP increases.

After the reorientation, adenine moieties in adjacent dAMP molecules can exist closely in parallel in the type II layer, where intermolecular interaction would occur. Mutual interaction of adenine derivatives is known to occur in solution but at rather high concentrations, of millimolar order or above.[3-5,41] Thus, the self-association in the present supporting electrolyte solution was actually examined in the cases of dAMP and deoxyadenosine by proton magnetic resonance(PMR). The concentration dependence of the proton chemical shift is shown in Fig. 6. The base protons (H-2 and H-8) and also the H-1′ proton are shielded upfield progressively with increase of the concentration. The result is in good agreement with the findings for various purines, their nucleosides and nucleotides in aqueous solution obtained by PMR,[2,3,6-8,42] in which association was found to occur by means of vertical stacking of their adenine moieties. Such association was also found by vapor pressure osmometric[1,3,42,43] and sedimentation equilibrium[4,5] measurements. The ultraviolet spectra of adenine, deoxy-

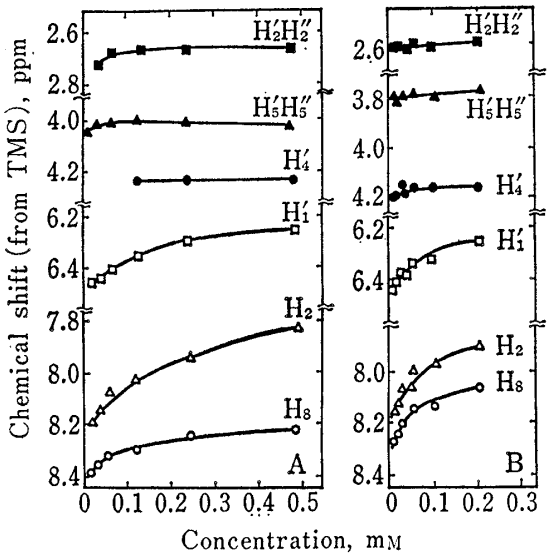

Fig. 6. Concentration Dependence of Proton Cemical Shifts for dAMP (A) and Deoxyadenosine (B) in 0.1 M $NaClO_4$ D_2O Solution

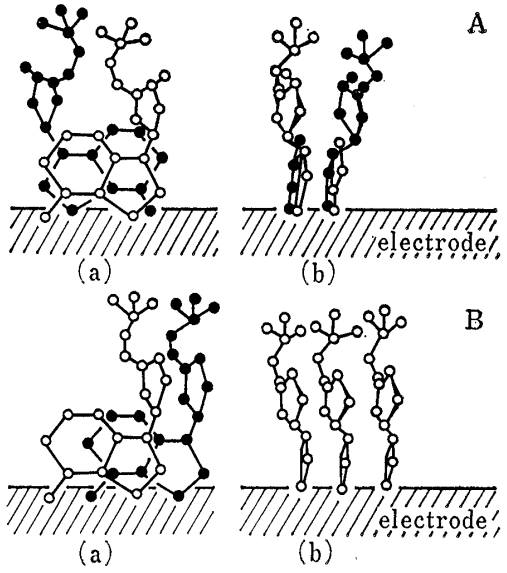

Fig. 7. Possible Orientation of Adsorbed dAMP Molecules on the Electrode Surface at Potentials around pzc and with High Surface Concentration. Adjacent Adenine Moieties are stacked with the Deoxyribosephosphate Groups lying at Opposite Sides (A) and at the Same Side (B). (a) and (b); Side Views from the Directions Perpendicular and Parallel to the Adenine Planes, respectively

adenosine and dAMP were also measured in 0.1 M $NaClO_4$. The concentration dependence of their absorbance at 260 nm showed a hypochromic effect in the concentration range above 1 mM. This result is in accord with that reported for deoxyadenosine by Solie and Chellman,[5] who considered that the effect is attributable to base stacking.

These findings indicate that in all cases, adenine, deoxyadenosine and dAMP, association would occur in solution when the concentration exceeds about 1 mM. Contrary to this, the association of dAMP in the adsorbed layer would probably occur even when the bulk concentration is as low as 5×10^{-5} M because of the increasing proximity of the molecules.[44] On the other hand, this would not be expected for adenine, deoxyadenosine, dADP or dATP from the $|\Delta R/R_0|$ *vs.* c relations.

Thus, there seem to be some factors facilitating the association, which is presumably due to the stacking interaction between adsorbed dAMP molecules. Adenine is adsorbed in a flat orientation in its adsorption layer, so it is difficult for the stacking interaction between neighboring molecules to occur. On the contrary, in the case of dAMP molecules, in which the adenine moieties lie perpendicular in contact with the electrode surface in the type II layer, a lateral interaction such as stacking may take place easily.[45] dADP and dATP do not show such a reorientation as dAMP even though their adsorption orientation is similar to that for dAMP in the type I layer. This is presumably attributable to the significant electrostatic repul sion among negatively charged diphosphate or triphosphate groups. Such a repulsive force should interfere with the closer approach and consequently the interaction between neighboring molecules. Thus, adsorbed dAMP is the most likely among the three to show association on the electrode surface.

Based on the above discussion, possible models of the orientation of dAMP in the type II layer are schematically shown in Fig. 7. Two modes of stacking may be possible; in the model shown in Fig. 7A, the deoxyribose-phosphate groups of adjacent molecules lie at opposite

sites, while in Fig. 7B, they lie on the same side but the molecules are mutually displaced to avoid steric hindrance.

The type I and the type II layers discussed here can readily be identified with the dilute and the compact layers reported by Krznarič *et al.*,[21] and Dryhurst and coworkers.[16,23] They observed the formation of both layers for some adenine derivatives on mercury electrodes. Such characteristic adsorption behavior, however, was observed only for dAMP in the present study. The differences presumably also arise from differences in the electrode materials and the experimental conditions. In any event, the association of adsorbed dAMP takes place on a gold electrode under conditions in which the association dose not occur in bulk solution. This indicates that dAMP has the most favorable structure for reorientation and stacking among adenine derivatives.

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44) The surface excess of dAMP for the monolayer can be estimated to be about 10^{-11} mol/real cm^2 from the area occupied by one adsorbed molecule (listed in Table I).
45) The interaction seems to be rather weak, since the free energy change for the association of such purine derivatives in solution is reported to be about 1.5—7.7 kj mol^{-1}.[3)]