Multi-site Recognition of Flavin Adenine Dinucleotide by Mixed Monolayers on Water

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(Received May 10, 1995)

Flavin adenine dinucleotide (FAD) formed a stoichiometric complex at the air-water interface with four molecules of a three-component monolayer that contain guanidinium, diaminotriazine, and orotate head groups. The complementary complexation was confirmed by surface pressure-area isotherm and IR and XPS spectroscopies of the transferred film.

During the past several years, molecular recognition via complementary hydrogen bonding between lipid monolayers on water and aqueous guest molecules has been studied.[1-7] For example, single-component monolayers having diaminotriazine (**T**)[3,4], guanidinium (**G**)[5,6], and orotate (**O**)[7] head groups effectively bind barbituric acid, phosphates, and adenine, respectively. These results have been extended to double-site recognition in which uridine monophosphate (UMP) is bound to a mixed monolayer that contains both of guanidinium and adenine functions.[6] The multi-site recognition is extended in this study to a more complex system of FAD and three-component monolayers. FAD molecule is composed of one isoalloxazine unit, two phosphate units and one adenine unit, which are complementary to triaminotriazine, guanidinium, and orotate, respectively. In addition, related guest molecules such as FMN, AMP and ADP are commercially available and their binding behaviors yield valuable information on the mode of molecular recognition of FAD itself. The monolayer components and guest molecules we employed in this study are shown in Figure 1, together with the mode of molecular recognition at the air-water interface.[4,5,7]

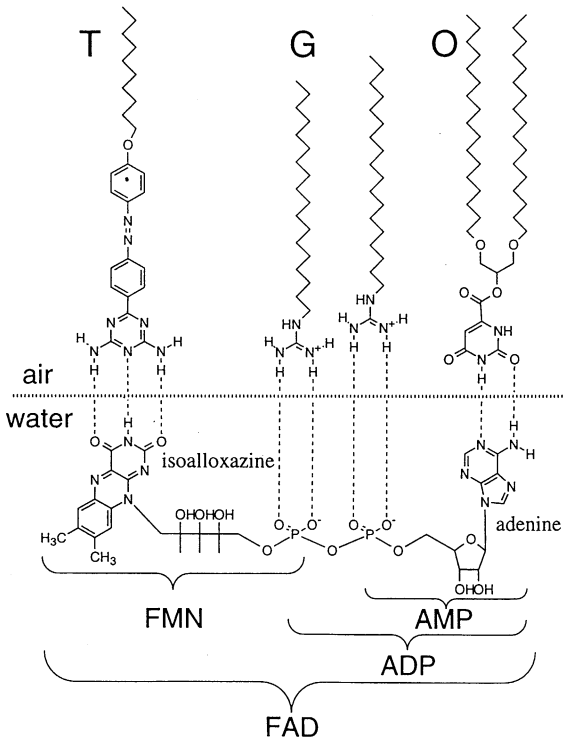

Figure 1. Multi-site binding of mixed monolayers with complementary aqueous guests.

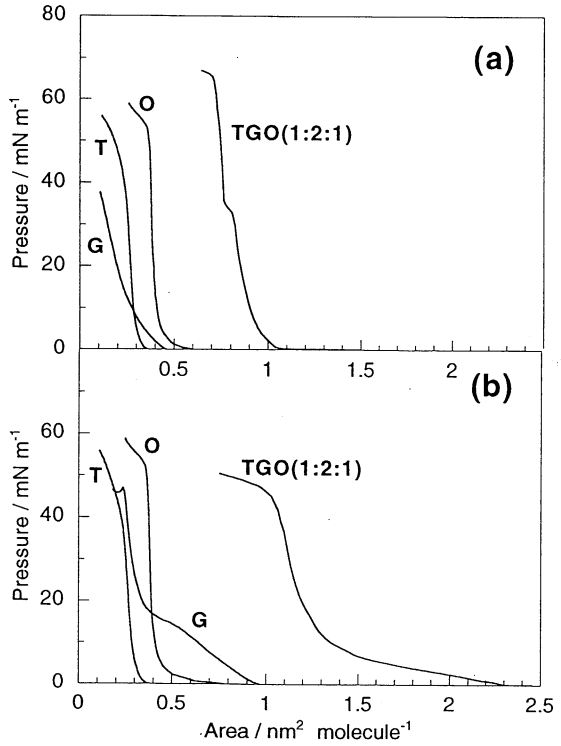

Figure 2. π-A Isotherms of monolayers of **T**, **G**, **O**, and mixed amphiphiles, **TGO**(1:2:1). (a) on pure water, (b) 10^{-5} M FAD.

The oxidized form of FAD (Na_2FAD, Tokyo Kasei Kogyo Co.), flavin mononucleotide (NaFMN, Wako Pure Chem.), adenosine diphosphate and adenosine monophosphate (Na_2ADP and Na_2AMP, Oriental Yeast.) were commercially supplied and used without purification.

The π-A isotherms that are measured as described previously[3-7] on pure water and on aqueous FAD are shown in Figure 2 (a) and (b), respectively. A mixed monolayer of **TGO** (molar ratio, 1:2:1) showed a solid-like phase on pure water, whereas it revealed expanded and condensed phases on aqueous FAD. The limiting area for **TGO**(1:2:1) on pure water, 0.84 nm^2·molecule^{-1} was smaller than the combined limiting areas (1.07 nm^2·molecule^{-1}) that were measured separately for the individual monolayers.[8] This decrease was caused by strong interaction among the component amphiphiles and the consequent component dissolution as mentioned below. This limiting area became much enhanced to 1.33 nm^2·molecule^{-1} in the presence of aqueous FAD. The latter figure is rather close to the minimal cross section of the total alkyl chains for **TGO**(1:2:1) (1.1 - 1.3 nm^2·molecule^{-1}) that was estimated for

Table 1. Elemental ratios of the LB-films measured by XPS[a]

Monolayer	Subphase	C (%)	N (%)	O (%)	P (%)	Guest[b] (%)
TGO(1:2:1)[c]	100 μM FAD	79.94	10.55	8.35	1.03	106
TGO(1:2:1)	1 μM FAD	79.16	10.79	9.14	0.79	80
TGO(1:2:1)	1 μM ADP	81.92	10.29	7.23	0.56	50
TGO(1:2:1)	pure water	85.01	9.16	5.83	-	-
TG(1:1)	1 μM FMN	77.14	14.16	7.96	0.60	55
GO(1:1)	1 μM AMP	85.02	7.01	7.86	0.05	6
TO(1:1)	100 μM FAD	82.50	8.78	8.65	0.05	5

[a] LB films (9 layers with transfer ratio 1) were used. The contents of S and Na were less than 0.15%. [b] Binding of aqueous guest was estimated from N/P ratio (error was within 10%). [c] Calcd for **TGO**·FAD(1:2:1:1): C, 78.52; N, 11.42; O, 9.01; P, 1.05. **TGO**(1:2:1): C, 84.85; N, 10.67; O, 4.28. **TGO**(1:1:1): C, 84.57; N, 10.24; O, 5.20.

perpendicular chain packing (see Figure 1). The π-A curves of individual monolayers of **T** and **O** were not altered in the presence of 10^{-5} M FAD, probably due to low substrate binding. In fact, aqueous riboflavin and adenosine did not show efficient binding to monolayers of **T** and **O**, respectively, at this low substrate concentration.[3,7] In contrast, aqueous FAD caused expansion of a monolayer of **G**. Combined ionic and hydrogen-bonding interactions between guanidinium and phosphate groups contribute to the strong binding, as already discussed.

Table 1 compares elemental ratios of the LB films as determined by XPS analyses. The composition of an LB film from **TGO**(1:2:1) monolayer on aqueous FAD agrees most closely with the calculated value for the composition of **TGO**·FAD(1:2:1:1). The absence of sulfur and sodium indicates that ion exchange of the counterion of **G** (*p*-toluenesulfonate) with FAD is complete.

The LB film displayed distinct IR changes in the carbonyl stretching region upon FAD binding. Peak enhancement at 1734 cm^{-1} and its decrease at 1682 cm^{-1} suggest that the isoalloxazine group of FAD and/or the orotate group of monolayer are involved in the binding, though assignment of these peaks is not yet defined.[9] FAD binding was almost stoichiometric even when the FAD concentration was lowered to 10^{-6} M.

The binding behavior of guest molecules that corresponded to partial structures of FAD was subsequently examined. The binding of ADP to the **TGO**(1:2:1) monolayer at a concentration of 10^{-6} M was 50%, and the binding of FMN to **TG**(1:1) was 55% at 10^{-6} M. Binding of AMP to **TG**(1:1) and of FAD to **TO**(1:1) at the same substrate concentration was much less efficient (6% and 5%, respectively). Lower binding constants of these host-guest combinations reflect the fact that sites of interaction are lessened relative to that of FAD.

An LB film formed from a **TGO**(1:2:1) monolayer on water did not contain sulfur, probably due to neutralization of the guanidinium unit with the deprotonated orotate unit, as reported for the thymine/guanidinium system.[6] Its elemental composition was closer to that of **TGO**(1:1:1) rather than that of the original mixture. In addition, limiting area of 0.84 $nm^2 \cdot molecule^{-1}$ was much smaller than the combined limiting area (1.07 $nm^2 \cdot molecule^{-1}$) of an each component of **TGO**(1:2:1), and was rather close to that of **TGO**(1:1:1).[8] All these data suggest dissolution of one **G** component during the compression process. This was not the case when FAD was present in the subphase. The multi-site interaction of FAD is satisfied with the **TGO**(1:2:1) composition, and this keeps the monolayer composition intact.

We conclude from the preceding results that there exists multiple molecular recognition[4,5,7] such as depicted in Figure 1. This finding should lead to many novel possibilities in molecular recognition and molecular patterning. The latter possibility will be reported elsewhere.

References and Notes

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8 The monolayer of **G**(*p*-toluenesulfonate) did not show a condensed phase on pure water. Therefore, its limiting area of 0.2 $nm^2 \cdot molecule^{-1}$ was assumed to be identical with single chain amphiphiles such as stearic acid.
9 The peak at 1734 cm^{-1} can be assigned to $\nu_{C=O}$ of the imide moiety in O or in FAD. The peak position is different from those of the pure components (O; 1748, FAD; 1714 cm^{-1}) probably due to the hydrogen bonding interaction.