RECOGNITION OF FLAVIN MONONUCLEOTIDE (FMN) BY A MELAMINE DERIVATIVE HAVING A GUANIDINIUM ION *VIA* A MULTISITE BINDING

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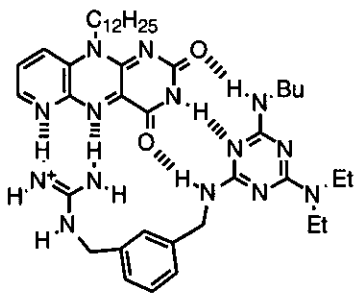
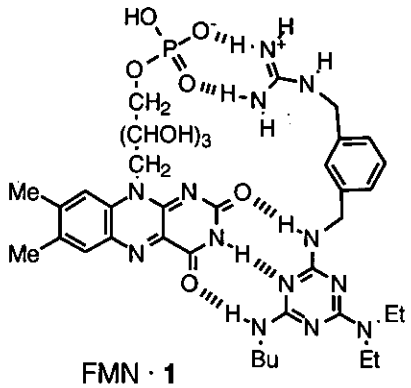
Abstract - A melamine derivative bearing a guanidinium ion was found to act as a flavin mononucleotide (FMN) receptor *via* complementary hydrogen bonding. The receptor extracts FMN in H_2O into $CHCl_3$ and acts as an effective carrier for the transport of FMN through a liquid membrane of $CHCl_3$.

Molecular recognition by hydrogen bonding plays a crucial role in biological systems, that is of intense current interest in biomimetic chemistry.[1] Design of the receptor molecule for a coenzyme is of primary importance for construction of an artificial enzyme,[2] since the functionalized receptor is able to provide a noncovalently assembled system. Effective binding of a target molecule by its receptor would be achieved by increasing the number of interaction sites. For example, we reported that a melamine derivative bearing a guanidinium ion (**1**) binds 6-azaflavin *via* five hydrogen bonds with the binding constant of 1.4×10^5 M^{-1} in $CHCl_3$, whereas that for 6-azaflavin•**2** is only 150 M^{-1} (three hydrogen bonds at the C(2)=O, N(3)-H, and C(4)=O of the isoalloxazine ring).[3] We also reported that the receptor (**1**) regulates the reactivity of the flavin by the hydrogen bonding at the N(1) or N(5) atom of the isoalloxazine ring.[4] Meanwhile a

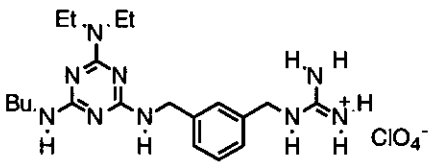
Figure 1 Proposed complexes of 6-azaflavin•**1** and FMN•**1**, and structures of related compounds



6-Azaflavin · **1**



FMN · **1**



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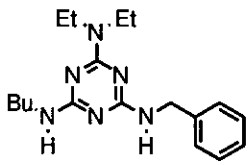

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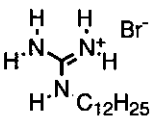

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guanidinium ion is known to bind a phosphate *via* hydrogen bonding.[5] It is also known that FMN is bound by a two-component monolayer having diaminotriazine and guanidinium groups *via* complementary hydrogen bonding on water.[6] These suggest that **1** is able to bind FMN strongly by forming three hydrogen bonds at the uracil moiety and two at the phosphate group as shown in Figure 1. Construction of the CPK molecular models of FMN•**1** indicates that formation of the complex is geometrically possible.

In this paper, we wish to report that **1** acts as an efficient FMN receptor for the extraction and transport of FMN in a two-phase system of H_2O and $CHCl_3$. The relevant compounds used are also shown in Figure 1.[7] The extraction experiment was performed as follows; a two-phase mixture of a H_2O solution (3 ml) containing a flavin (5.0×10^{-5} M) and a $CHCl_3$ solution (3 ml) containing a receptor (2.0×10^{-4} M) was stirred for 1 h at 25 °C. The concentration of the flavin in the H_2O phase was determined spectrophotometrically by monitoring the absorption maximum of the flavin at 445 nm. The results are shown in Table 1. FMN is extracted by $CHCl_3$ in the

Table 1 Extractability of FMN and riboflavin

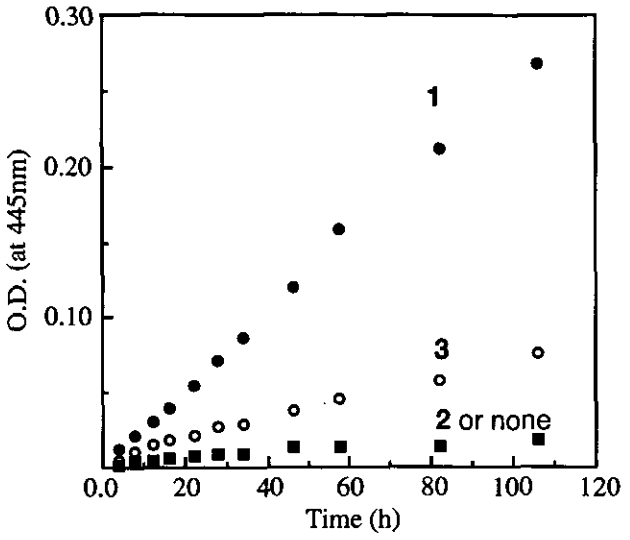
Receptor	Extractability (%)	
	FMN	Riboflavin
1	68 ± 2	0.0
2	0.0	0.0
3	27 ± 3	0.0
2+3	25 ± 4	0.0
CTABr	9.0 ± 0.0	—
none	0.0	0.0

Extractability = $([Flavin]_o - [Flavin]_{aq})/[Flavin]_o \times 100$
CTABr: cetyltrimethylammonium bromide

presence of the receptors except **2**, whereas riboflavin is not extracted at all. The effect of the receptors on the extractability of FMN is in the order of **1**> **3** > CTABr > **2**. The extractability of **1** is much larger than that of each components separately added (**2** + **3**), indicating importance of intramolecular combination of the binding sites to obtain the effective binding. This also suggests that the hydrogen bonded complex between FMN and **1** is fairly lipophilic. The larger extractability of **3** than that of CTABr implies that the hydrogen bonding accompanied by charge neutralization as seen in the phosphate-guanidinium ion is more effective for lipophilicity than the ion pairing between the phosphate moiety and CTABr. The selective extraction of FMN by **1** would provide a convenient and useful methodology for separation of FMN from water-soluble flavins.

A similar tendency for FMN binding by the receptors was also observed in a transport experiment through a liquid membrane of chloroform with a dual cylindrical apparatus ([FMN] = 2.5 x 10^{-3} M in source phase and [receptor] = 1.5 x 10^{-4} M in $CHCl_3$ phase).[8] The increase in concentration of FMN in the receiving phase was followed as described above. As can be seen in Figure 2, **1** is a most effective carrier. In this case, yellow color of FMN in the H_2O source phase immediately transfered to the $CHCl_3$ membrane after stirring. This suggests that the rate of release of FMN from FMN•**1** in the $CHCl_3$ into the receiving phase is rate-determining. From the slopes of Figure 2, the relative transport abilities of **1**, **3**, and **2** (and blank) are estimated to be 15 : 4.0 : 1.0. The fact that a hydrophilic FMN is changed by a receptor molecule to a lipophilic one would be of interest in connection with FMN transport in biological systems. Recently Retello *et al.* have reported that phenylboronic acid acts as a riboflavin carrier due to formation

Figure 2 Transport of FMN by receptors at 25 °C.



Org.phase ($CHCl_3$, 50 ml) ; [receptor] = 1.5 x 10^{-4} M
sourse phase (dist. H_2O, 4 ml) and receiving phase (dist. H_2O, 40 ml)

of hydrophobic boronate ester at the ribose moiety, although they did not refer to FMN.[9]

The stoichiometry of FMN•**1** complex was examined as follows; a mixture of $CHCl_3$ (3 ml) containing **1** (15 mg) and H_2O (3 ml) containing FMN (14 mg) was stirred for 1 h at room temperature to result in precipitation. The precipitates were collected by filtration, and washed with water (3 ml) to remove free FMN, followed by $CHCl_3$ (3 ml) to remove free **1**, and dried in vacuo (13 mg, yellow powder). The ^1H nmr spectrum (DMSO-d_6 : D_2O = 3 : 1 v/v) indicated that the complex consists of an equal mole ratio of FMN and **1** based on integration of each components.[10] Although the attempt to crystallize the powdered complex has not yet been successful, we consider at present a 1 : 1 mole ratio of FMN and **1** as shown in Figure 1.

The present study demonstrates that the melamine derivative having a guanidinium ion (**1**) acts as an FMN receptor *via* multi-site interactions.[11] The selective binding of FMN by the receptor molecule should lead to potential applications including separation of FMN and sophisticated flavin model systems, since functional groups can be easily introduced into the melamine skeleton.

ACKNOWLEDGEMENT

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REFERENCES AND NOTES

1 A. D. Hamilton, 'Bioorganic Chemistry Frontiers,' Vol 2, ed. by H. Dugas, Springer-Verlag, Berlin, 1991, p.117; J. Rebek, Jr., *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 245.

2 Y. Murakami, J. Kikuchi, Y. Hisaeda, and O. Hayashida, *Chem. Rev.*, 1996, **96**, 721.

3 T. Kajiki, N. Tamura, T. Nabeshima, and Y. Yano, *Chem. Lett.*, **1995**, 1063.

4 N. Tamura, T. Kajiki, T. Nabeshima, and Y. Yano, *J. Chem. Soc., Chem. Comm.*, **1994**, 2583.

5 C. H. Hannon and E. V. Anslyn, 'Bioorganic Chemistry Frontier,' Vol. 3, ed. by H. Dugas, Springer-Verlag, Berlin, 1993, p. 193.

6 K. Taguchi, K. Ariga, and T. Kunitake, *Chem. Lett.*, **1995**, 701.

7 Compounds (**1**, **2**, and **3**) were supplied from our previous study (ref. 4). Commercially available riboflavin and Na salt of FMN (Wako Pure Chem.) were used without purification.

8 T. Nabeshima, T. Inaba, N. Furukawa, T. Hosoya, and Y. Yano, *Inorg. Chem.*, 1993, **32**, 1407.

9 E. Lambert, E. C. Breinlinger, and V. M. Rotello, *J. Org. Chem.*, 1995, **60**, 2646.

10 Chemical shifts (from TMS) of 7-CH_3 of FMN (2.40 ppm) and $C\underline{H}_3(CH_2)_3$ group of **1** (0.87 ppm) were employed for the integration.

11 Y. Aoyama, K. Mizokami, and H. Toi, *Chem. Lett.*, **1990,** 651; H. Furuta, D. Magda, and J. L. Sessler, *J. Am. Chem. Soc.*, 1991, **113**, 798.

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