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SPECIFIC INHIBITION OF FLAVIN CATALYSES BY A "MOLECULAR HINGE"

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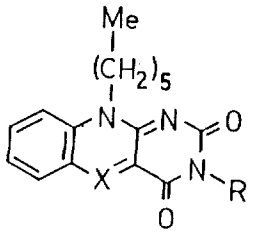
Abstract: An artificial receptor having both a 2,6-diamidopyridine hydrogen bonding site and a naphthalene stacking site selectively quenched (5-deaza)flavin fluorescence and inhibited flavin-mediated photo-oxidation of 1,4-butanedithiol.

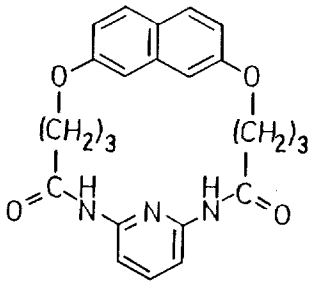
Recent advances in molecular recognition have shown that strong and selective complexation of a substrate can be achieved by incorporating complementary binding groups within a synthetic cleft or cavity.[1,2] In this paper we report a study on the molecular recognition of flavins and the effect of complexation on their fluorescence and photo-redox properties.

The versatility of flavin coenzymes as biological electron transfer catalysts is due, in part, to modification of their rodex properties by different enzyme active sites.[3] Synthetic models containing elements of a protein environment in the form of covalently linked hydrophobic or chiral features have been reported.[4-6] However, the design and synthesis of complementary receptors represent a novel approach to the modification of flavin reactivity via non-covalent interactions. The flavin receptors were based on the hydrophobic interaction with the phenyl moiety as well as the hydrogen-bonding interaction with the pteridine moiety.[3] This suggests that the two-site binding strategy, which has been applied to the recognition of nucleotide bases,[7] is also useful to modification of flavin reactivity. Incorporation of suitable amide and hydrophobic groups within a macrocyclic framework leads to receptors capable of simultaneous hydrogen bonding and aromatic stacking to a substrate.[8]

First, we estimated the influence of added quenchers (2-4) on the fluorescence intensity of 1a-1d in chloroform. The fluorescence intensity of 1b and 1d, having a methyl group at N(3) position, was scarcely affected by the addition of these quenchers. The fluorescence intensity of 1a and 1c,



1a: X=N, R=H
1b: X=N, R=Me
1c: X=CH, R=H
1d: X=CH, R=Me



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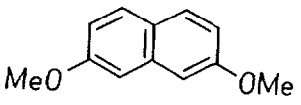

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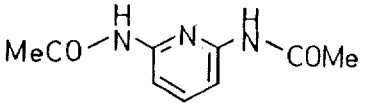

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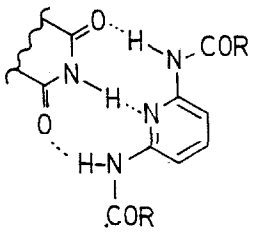
having an acidic proton at N(3) position, was not affected by the addition of 3 (at least at ~4x 10^{-4} M) but was significantly quenched by the addition of 2 and 4 (Fig. 1). Continuous variation plots (at [1] + [quencher] = 4.50 x 10^{-5} M) gave a clear break point at 0.5, supporting the view that fluorescence quenching is due to the formation of a 1:1 complex. We thus determined the association constants (K) according to the Benesi-Hildebrand equation (Table 1).

The association of 1a and 1c with 2 and 4 is primarily due to hydrogen bonding interactions between the pteridine moiety of isoalloxazine and the 2,6-diamidopyridine moiety of 2 and 4 (as shown in 5). It is known that the strong flavin (with H at N(3) position) fluorescence disappears at high pH.[9] This is attributed to the dissociation of 3-NH to 3-N^- (pK_a ca. 10). Hence, the fluoresence decrease observed for 2 and 4 is attributed to the "partial dissociation" of 3-NH. In contrast, stacking interactions between the isoalloxazine ring and naphthalene ring are less important in the association in chloroform solvent where hydrophobic interactions are scarcely expected. As shown in Fig. 1 and Table 1, however, I/I_o (relative fluorescence intensity) for 2 is always smaller than that for 4. The difference is explained only by the occurrence of pseudo-intramolecular quenching of the singlet excited state by the naphthalene ring of bound 2. Thus, the naphthalene ring in 2 can quench the flavin fluorescence in an intramolecular manner (as shown in 6).



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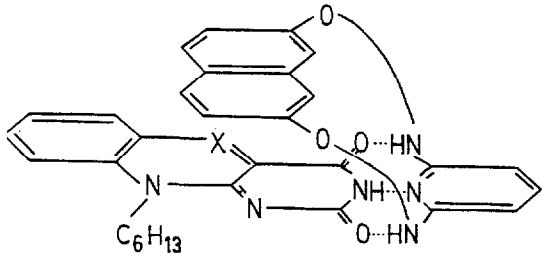

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In order to obtain an insight into the influence of these specific quenchers on flavin reactivities, we carried out flavin-mediated photo-oxidation of 1,4-butanedithiol to the disulfide in chloroform. The reaction was carried out in an anaerobic Thunberg cuvette immersed in a thermostatted water-bath (further details of the reaction conditions are recorded in a footnote to Table 2). The absorbance of flavins decreased with photo-irradiation time and the plots of log A/A_o vs. time were approximated by the first-order equation for up to 25 % reaction. The results (Table 2) indicate that the significant rate inhibition is observed only for 1a + 2 (3.4 times slower than that in the absence of 2). The finding supports the idea that receptor 2 acts as an efficient inhibitor for flavin-mediated photo-oxidation as long as the 3-NH group of flavins is not modified.

In summary, we have shown that flavins form strong complexes to our two-site receptors. In addition, binding leads to a substantial quenching of the flavin fluorescence emission and inhibition of flavin-mediated redox reactions.

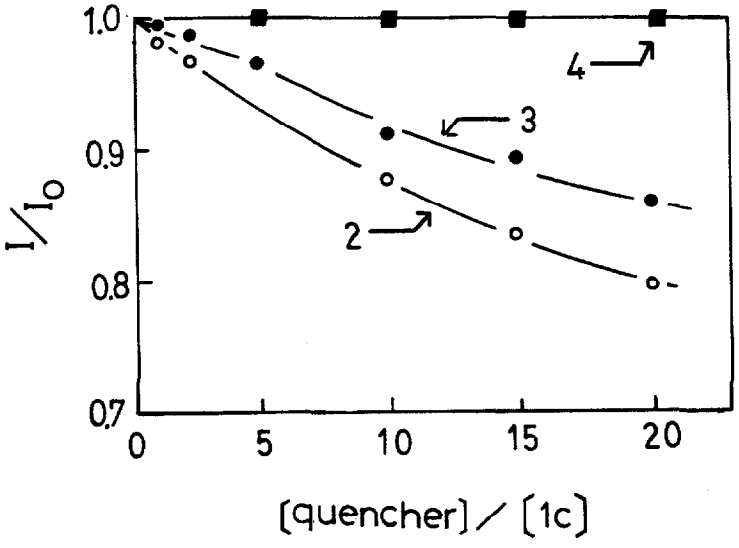

Fig. 1. Fluorescence quenching of 1c by a molecular hinge (2) and its analogs. 1a showed similar plots. The measurement conditions are recorded in a footnote to Table 1.

Table 1. Association constants (K, M^{-1}) determined by fluorescence quenching[a)]

Flavin	Quencher		
	2	3	4
1a	3500(0.67)[b)]	n.e.	2800(0.75)[b)]
1b	n.e.	n.e.	n.e.
1c	4500(0.66)[b)]	n.e	4500(0.71)[b)]
1d	n.e.	n.e.	n.e.

a) Chloroform, 25 °C, excitation 370 nm, emission 507 nm for 1a and 1b and 457 nm for 1c and 1d, [1] = 2.00×10^{-5} M, [quencher] = (2.5-40) $\times 10^{-5}$ M. N.e. denotes "no effect".

b) The number in parenthesis indicates the relative fluoresence intensity (I/I_o) at [quencher]/[1] = 20.

Table 2. Apparent first-order rate constants (k, min^{-1}) for photo-oxidation of 1,4-butanedithol[a)]

Flavin	Quencher		
	None	2	4
1a	0.013	0.0038	0.014
1b	0.010	0.011	0.0092

a) Chloroform, 30 °C, 17-W fluorescent lamp, N_2, [1] = 2.00×10^{-5} M, [2 or 4] = 4.00×10^{-4} M. [1,4-butanedithiol] = 5.00×10^{-3} M. The distance between the lamp and the reaction cell was 14 cm. The data are average values of four runs (relative error, less than 15 %).

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