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A CROWN ETHER FLAVIN MIMIC

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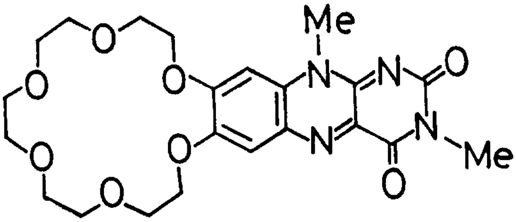
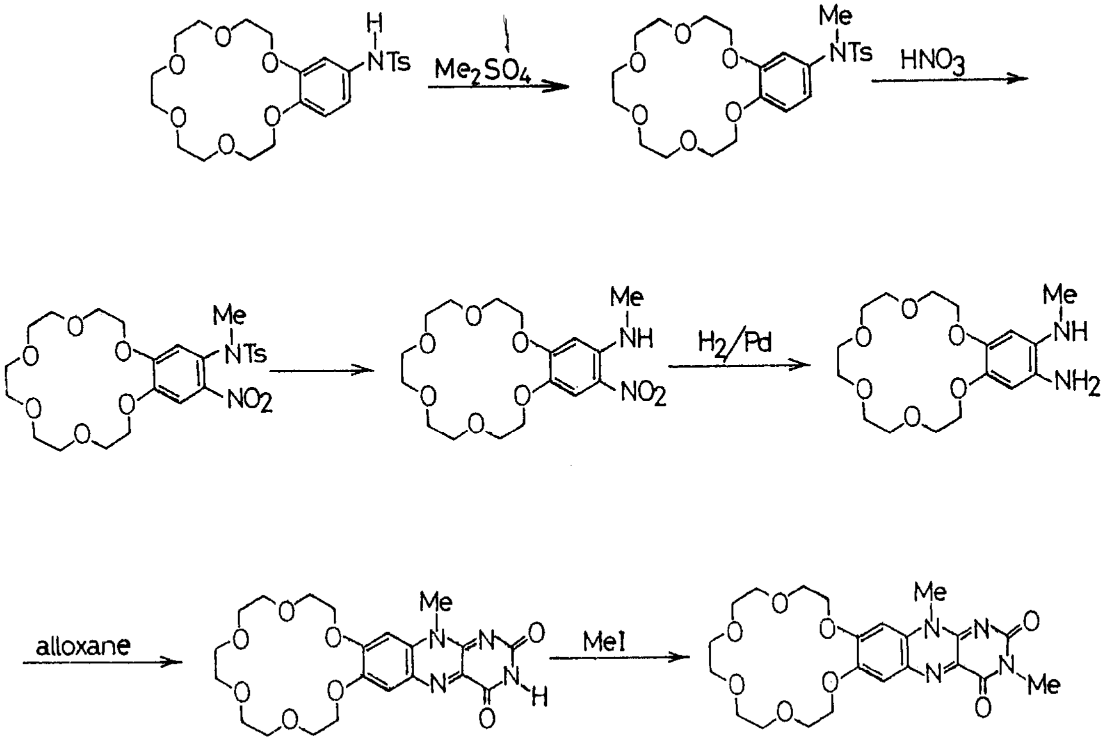
A flavin analogue bearing a crown ring as a recognition site was synthesized. The addition of alkali metal cations sensitively affected the absorption spectrum, the fluorescent emission, and the reaction rate with NADH model compounds.

Coenzymes are prosthetic groups in enzymes and frequently are capable of catalyzing the enzyme-mediated reactions even in the absence of apoenzymes. Hence, coenzymes have attracted much attention as essential model systems of coenzyme-dependent enzymes. In contrast to holoenzymes having both the catalytic site and the recognition site, however, coenzymes consist only of the catalytic site. One may thus expect that the coenzyme bearing the recognition site within a molecule would behave as a more attractive coenzyme model. The ability of crown ethers to associate with a variety of charged and uncharged substrates bears resemblance to early reaction steps in enzyme-mediated reactions. Therefore, crown ethers may be useful as an expeditious candidate for the recognition site. In fact, crown ether mimics of trypsin and NADH-dependent enzymes have been reported.1-5) Here, we wish to report the first example of a crown ether flavin mimic (CrFl).

CrFl:



CrFl was synthesized according to the following reaction scheme and identified by elemental analysis and mass spectrum(M^+ 476): mp 280-284°C.



The absorption spectrum of CrFl in methanol at 30°C showed bimodal absorption maxima at 460 nm (ε 23300: S1 band) and 363 nm(ε 9650: S2 band) which are characteristic of the flavin spectrum, but the S1 band was relatively stronger than the S2 band as compared with the typical flavin spectrum. The addition of chloride salts of alkali metal cations(0.1-2.0 mM) to the methanolic solution of CrFl brought forth the decrease in the absorbance of the S1 band. In particular, potassium chloride provided the greatest decrease, which is attributed to the fitness of the 18-crown-6 ring to K^+ ion. Analyzing the plots of the absorbance against metal concentrations, we estimated the association constants with the crown ring: Na^+ 5700, K^+ 10000-38600,[6)] Rb^+ 3400, and Cs^+ 6600 $M.^{-1}$ Under the identical measurement conditions, the S1 band of 3-methyllumiflavin(LFl) was not affected at all by added alkali metal cations.

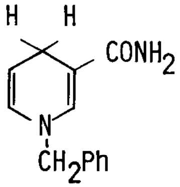
The fluorescence spectrum of CrFl in methanol at 30°C(excitation wavelength 370 nm) gave the emission maximum at 530 nm. The relative intensity(I/Io) sensitively decreased with increasing metal concentrations(0.1-1.0 mM). In particular, Cs^+ and Rb^+ efficiently quenched the fluorescent emission of CrFl($I/Io \approx 0.8$). A similar fluorescent quenching by heavy alkali metal cations has been found for naphthalene-containing crown ethers.[7,8)]

Table 1. Second-order rate constants for the reaction of CrF1 and LF1 with NADH model compounds (30°C)

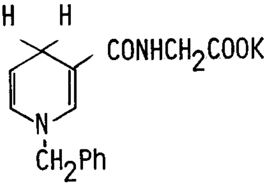
Entry	Medium (conc. M)	Flavin	NADH model	k_2 ($M^{-1}s^{-1}$)
1	water ($[K^+]=0.005$)	LF1	Bz1NAH	22.3
2	water ($[K^+]=1.00$)	LF1	Bz1NAH	22.9
3	water ($[K^+]=0.005$)	CrF1	Bz1NAH	1.91
4	water ($[K^+]=1.00$)	CrF1	Bz1NAH	3.48
5	MeOH-water(7:3)	LF1	Bz1NAH	0.749
6	MeOH-water(7:3) ($[K^+]=0.200$)	LF1	Bz1NAH	0.691
7	MeOH-water(7:3)	CrF1	Bz1NAH	0.096
8	MeOH-water(7:3) ($[K^+]=0.200$)	CrF1	Bz1NAH	0.202
9	MeOH ($[K^+]=0.002$)	LF1	Bz1NAHCOOK	0.810
10	MeOH ($[K^+]=0.002$)	CrF1	Bz1NAHCOOK	0.274

From the plots of I/Io vs. $[M^+]$ we estimated the association constants with the crown ring9): K^+ 8000, Rb^+ 5200, and Cs^+ 5700 $M.^{-1}$ In constrast, the fluorescent spectrum of LF1 was not affected at all by the addition of 0.1-1.0 mM alkali metal cations.

The influence of added metal cations on the oxidizability of CrF1 was estimated on the basis of the reaction with NADH model compounds, Bz1NAH and Bz1NAHCOOK, under aerobic conditions. The results are summarized in Table 1.



Bz1NAH



Bz1NAHCOOK

The second-order rate constants(k_2) for CrF1 were generally smaller than those for LF1 owing to the electron-donating nature of the crown group. Entries 1-4 indicate that the addition of KCl(1.00 M) enhances the rate constant of CrF1 by 1.8-fold, whereas such a salt-originated rate enhancement was not observed for LF1. A similar trend was also seen in a mixed solvent of methanol-water(entries 5-8). Therefore, the K^+ ion complexed in the crown ether ring is capable of strengthening the oxidizability of CrF1. Similarly, the rate constants for CrF1 were enhanced by the addition of NH_4^+, Rb^+, Cs^+, and Ca^{2+}.

We synthesized BzlNAHCOOK, expecting that the carboxylate salt of this NADH model may be recognized by the crown ring of CrFl. The reaction of BzlNAHCOOK with CrFl was speeded up by 3.4-fold relative to that of BzlNAH, but the reaction of BzlNAHCOOK with LFl was also speeded up (2.3-fold). It is not clear from the kinetic measurements, therefore, whether the carboxylate salt is recognized by CrFl.

Further applications of a crown ether flavin mimic are now in progress in this laboratory

REFERENCES

1) Y. Chao and D. J. Cram, J. Am. Chem. Soc., 98, 1015(1976).
2) T. Matsui and K. Koga, Tetrahedron Lett., 1978, 1115.
3) T. J. van Bergen and R. M. Kellogg, J. Am. Chem. Soc., 99, 3882(1977).
4) J.-P. Behr and J.-M. Lehn, J. Chem. Soc., Chem. Commun., 1978, 143.
5) For a comprehensive review see S. Shinkai, Progr. Polym. Sci., 8, 1(1982).
6) The plot for K^+ was biphasic. This is due to the change in the spectrum pattern which was observed only for K^+. The detail will be described in a full manuscript.
7) L. R. Sousa and J. M. Larson, J. Am. Chem. Soc., 99, 307(1977).
8) J. M. Larson and L. R. Sousa, J. Am. Chem. Soc., 100, 1944(1978).
9) Na^+ did not give a dependence analyzable according to the theoretical equation.

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