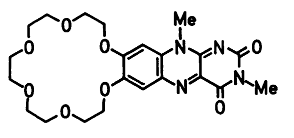
1632 © 1986 The Chemical Society of Japan NOTES *Bull. Chem. Soc. Jpn.*, *59*, *1632—1634 (1986)* [Vol. 59, No. 5

Selective Photooxidation of Alkali Mandelates by a Flavin Bearing a Crown Ring as a Metal Recognition Site[1)]

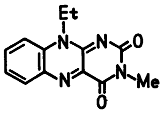
Seiji Shinkai,* Hideki Nakao, Kaori Ueda, Osamu Manabe,
and Masayoshi Ohnishi
Department of Industrial Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852
(Received November 4, 1985)

Synopsis. The quantum yields (Φ) for photooxidation of mandelate salts by a crown-containing flavin (CrFl) and 3-methyl-10-ethylisoalloxazine (MeEtIA) were determined in acetonitrile. MeEtIA photooxidized tetramethylammonium mandelate more selectively than potassium mandelate ($\Phi_{K^+}/\Phi_{Me_4N^+}$=0.42—0.64), while CrFl showed a high selectively for photooxidation of potassium mandelate ($\Phi_{K^+}/\Phi_{Me_4N^+}$=19—42).

The catalytic actions of coenzymes have attracted much attention of bioorganic chemists because they can catalyze the reactions to some extent even in the absence of apoenzymes. Thus, the model investigations on the coenzyme catalyses have provided many clues to elucidate the origin of the enzyme "activity" reasonably.[2,3)] In contrast, there are a few model investigations on the enzyme "selectivety" which is another important aspect of enzyme catalyses. In order to design such an enzyme model with a substrate selectivity one may add a recognition site (e.g., crown ether) to a coenzyme-based catalytic site.[4)] As an approach to model systems for flavoenzymes, we have synthesized a crown ether flavin mimic, 3,10-dimethyl-7,8-(1′,4′,7′,10′,13′,16′-hexaoxacyclooct-2′-eno)isoalloxazine (CrFl).[5)] Here we report that in photooxidation of mandelate salts CrFl not only acts as an excellent oxidizing agent but also shows a remarkable metal selectivity. In order to differentiate the contribution of the crown ring, we used 3-methyl-10-ethylisoalloxazine (MeEtIA) as a reference flavin.



CrFl



MeEtIA

Experimental

A standard actinometer (potassium trioxalatoferrate(III) was used for the quantum-yield determinations on the photochemical reaction of flavins and mandelate salts.[6)] Three-milliliter aliquots of the substrate solutions in a 1 cm quartz cell (10×10×45 mm) were deaerated and were irradiated under nitrogen with a 300 W high-pressure Hg lamp. The extraneous lines of the lamp other than 366 nm were filtered out using a combination of two solution filters ($CuSO_4$ and 5,7-dimethyl-1-azania-4-azacyclohepta-4,6-diene perchlorate) and of a glass filter (Corning 7-37). Light intensities at 366 nm were determined by trioxalatoferrate (III) actinometry.[6)] The quantum yields were determined by comparing the light intensities with the reduced flavins in the exactly same geometry.

Results and Discussion

The lowest singlet state (Sl band) of flavins corresponds to the polarization along the long axis of the molecule.[7)] The addition of $KClO_4$ (0.40 mM)* to CrFl (2.48×10^{-5} M) in acetonitrile (λ_{max} 459 nm, ε_{max} 19700) induced the blue shift of the absorption maximum (λ_{max} 455 nm, ε_{max} 18500). This indicates that the binding of K^+ to the crown ring suppresses the polarization from the benzene moiety to the uracil moiety along the long axis of CrFl. The addition of $NaClO_4$ and $CsClO_4$ also induced the blue shift although the magnitude was smaller than that in the presence of K^+. On the other hand, the absorption spectrum of MeEtIA was scarcely affected by these metal ions under the identical conditions. We estimated the association constants (K) for $MClO_4$+CrFl in acetonitrile at 30 °C from a decrease in the absorbance at 459 nm: $K=6.6\times10^3\ M^{-1}$ for Na^+, $2.5\times10^4\ M^{-1}$ for K^+, $4\times10^3\ M^{-1}$ for Cs^+, and less than $10^2\ M^{-1}$ for Li^+.

Photoexcited flavins can oxidize mandelic acid to benzoic acid via dehydration followed by oxidative decarboxylation ($PhCH(OH)CO_2^- \rightarrow PhCOCO_2^- \rightarrow PhCO_2^- + CO_2$).[8)] We found that alkali and tetramethylammonium salts of mandelic acid are photooxidized by CrFl and MeEtIA in acetonitrile. The absorption bands of the oxidized flavins in the anaerobic solutions disappeared gradually with the photoirradiation time. Introduction of an O_2 stream

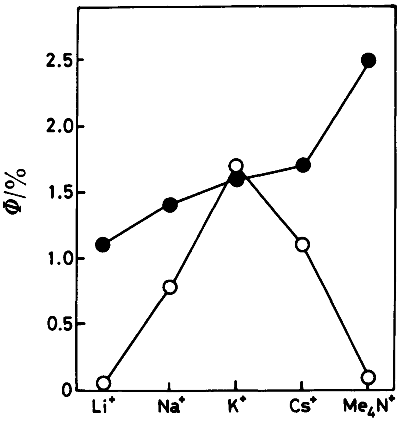


Fig. 1. Plots of Φ vs. alkali and tetramethylammonium cations in acetonitrile–methanol (70 : 30 v/v).
○: CrFl, ●: MeEtIA.

---

* 1 M=1 mol dm^{-3}.

Table 1. Photooxidation of Mandelate Salts ($PhCH(OH)CO_2^-M^+$) by Flavins[a)]

Flavin	M^+	B18C6	Φ/%			
		mM	MeOH(vol%)=0	10	20	30
CrFl	Li^+		*	*	*	0.05
CrFl	Na^+		*	1.7	0.87	0.77
CrFl	K^+		10.5	2.6	—	1.7
CrFl	K^+	0.21	9.4	—	—	—
CrFl	K^+	2.1	8.0	—	—	—
CrFl	Cs^+		4.7	1.6	—	1.1
CrFl	Me_4N^+		0.25	—	—	0.09
MeEtIA	Li^+		*	*	*	1.1
MeEtIA	Na^+		*	1.9	1.6	1.4
MeEtIA	K^+		3.4	2.3	—	1.6
MeEtIA	K^+	0.21	5.1	—	—	—
MeEtIA	K^+	2.1	7.0	—	—	—
MeEtIA	Cs^+		5.0	3.0	—	1.7
MeEtIA	Me_4N^+		8.1	—	—	2.5

a) Room temperature, $[PhCH(OH)CO_2^-M^+]=4.24\times10^{-3}$ M, $[\text{flavin}]=2.00\times10^{-4}$ M. * Denotes that the salt is not soluble sufficiently.

into the final solution regenerated the oxidized flavins quantitatively, indicating that the light-mediated oxidation of mandelate salts occurs without the intervention of side reactions. The results are summarized in Table 1.

The inherent reactivity of CrFl and MeEtIA may be compared by the quantum yields (Φ) for tetramethylammonium (Me_4N^+) mandelate, because Me_4N^+ ion does not interact with the crown ether ring. As shown in Table 1, the Φ values for MeEtIA are greater by 2.8—3.2 fold than those for CrFl. The difference is attributed to the electron-donating effect of the 7,8-polyether groups in CrFl. Examination of Table 1 reveals several interesting aspects characteristic of the crown-metal interaction: (i) The reactivity order for MeEtIA is $Me_4N^+>Cs^+>K^+>Na^+>Li^+$, whereas that for CrFl is $K^+>Na^+\approx Cs^+>Me_4N^+>Li^+$ (Fig. 1), (ii) the Φ for CrFl+potassium mandelate in acetonitrile is greater than MeEtIA+Me_4N^+ mandelate, (iii) the Φ values decrease with increasing methanol concentration and the decrease in CrFl occurs more sensitively than that in MeEtIA, and (iv) the addition of monobenzo-18-crown-6 (B18C6) enhances the Φ for MeEtIA (except that for Me_4N^+ mandelate), whereas it slightly decreases the Φ for CrFl.

It is well-known that decarboxylation is extensively affected by solvent effects[9—11]: The rates are enhanced in dipolar aprotic solvents because of desolvation of carboxylate anions. Hence, the decarboxylation rate ($PhCOCO_2^- \rightarrow PhCO_2^- + CO_2$) should be enhanced in dipolar aprotic solvents. Similarly, the dehydration process ($PhCH(OH)CO_2^- \rightarrow PhCOCO_2^-$) would be facilitated by desolvation of the carboxylate anion. The rate decrease caused by methanol addition is thus ascribed to specific solvation of the carboxylato group through hydrogen bonding. The Φ for MeEtIA+potassium mandelate increased on the addition of B18C6. At the high B18C6 concentration the Φ (7.0%) is comparable with that for Me_4N^+ mandelate (8.1%) which is believed to exist as a free ion pair.[11)] The order of the reactivity for MeEtIA is parallel to the cation size and is rationalized in terms of the formation of loose (e.g., Cs^+) or tight (e.g., Li^+) ion pairs. These findings all support that photooxidation by MeEtIA is governed by solvation of mandelate salts.

Interestingly, the Φ for CrFl+potassium mandelate is unusually enhanced: The Φ augmentation relative to Me_4N^+ mandelate corresponds to 42-fold in acetonitrile and the Φ is even greater than that for MeEtIA+Me_4N^+ mandelate. It is reasonable to propose that the unusual reactivity stems from the crown-metal interaction, because the order of the reactivity is exactly in accord with the spherical recognition pattern for an 18-crown-6 analogue (Fig. 1). Therefore, one reason for the high Φ should be the desolvation of the carboxylato group by the crown-K^+ complexation. However, this effect cannot fully rationalize the fact that the Φ (10.5%) for potassium mandelate is much greater than that for free-ion-like Me_4N^+ counterpart (0.25%). Conceivably, one must take two more important effects into consideration. Firstly, CrFl should become electron-dificient through the binding of K^+ to the crown ether moiety. A similar trend has been observed for the thermal oxidation of 1-benzyl-1,4-dihydronicotinamide by CrFl.[5)] Secondly, one may expect the contribution of the proximity effect between the anionic mandelate and the cationic CrFl·K^+ complex in acetonitrile. The contribution of these two effects is supported by the finding that the addition of B18C6 suppresses the photooxidation of potassium mandelate by CrFl (Table 1). It is likely that added B18C6 binds K^+ competitively and interferes with the "pseudo-intramolecular" reaction process via the CrFl·$K^+\cdots PhCH(OH)CO_2^-$ complex. It seems reasonable to conclude, therefore, that the specific Φ

increase for CrFl+potassium mandelate is achieved by several synergistic effects.

These results indicate that CrFl can imitate several biological concept important in enzyme chemistry. The crown ether moiety not only recognizes metal cations but also "activates" the flavin molecule through complexation with M^+. The close imitation is achieved because CrFl has within a molecule flavin as a catalytic site and crown ether as a recognition site, which are the minimum constituents for enzyme models.

References

1) Coenzyme Models. 44.

2) C. Walsh, "Enzymatic Reaction Mechanism," W. H. Freeman and Co., San Francisco (1979).

3) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Benjamin, New York (1966).

4) R. M. Kellog, *Top. Curr. Chem.*, **101**, 111 (1982).

5) S. Shinkai, Y. Ishikawa, H. Shinkai, T. Tsuno, H. Makishima, K. Ueda, and O. Manabe, *J. Am. Chem. Soc.*, **106**, 1801 (1984).

6) J. G. Galvert and J. N. Pitts, Jr., "Photochemistry," Wiley, New York (1966), pp. 734, 738.

7) P.-S. Song, "Flavins and Flavoproteins," ed by H. Kamin University Park Press, Baltimore (1981), p. 37.

8) W. H. Walker, P. Hemmerich, and V. Massey, *Eur. J. Biochem.*, **13**, 258 (1970).

9) D. S. Kemp and K. G. Paul, *J. Am. Chem. Soc.*, **92**, 2553 (1970).

10) D. H. Hunter, W. Lee, and S. K. Sim, *J. Chem. Soc., Chem. Commun.*, **1974**, 1018.

11) A. J. Parker, *Chem. Rev.*, **69**, 1 (1969).