BIOORGANIC CHEMISTRY 15, 269–282 (1987)

# Coenzyme Models

## 46. "Remote Control" of Flavin Reactivities by an Intramolecular Crown Ring Serving as a Metal Binding Site: Relationship between Spectral Properties and Dissociation of the 8-Sulfonamide Group[1]

SEIJI SHINKAI,[2] KEI KAMEOKA, KAORI UEDA, OSAMU MANABE, AND MASAYOSHI ONISHI

*Department of Industrial Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852, Japan*

*Received February 10, 1987*

Crown ether flavin mimics ($CrSO_2NHFl$ and $NCrSO_2NHFl$) which have a flavin moiety serving as a catalytic site and a crown ether moiety serving as a metal binding site at the two sides of a sulfonamide group were synthesized. We have found that the absorption spectra of these flavins are very sensitive to solvent effects; that is, they are yellow to orange in nonpolar solvents like "regular" flavins but imparted a red color to polar solvents characteristic of the intramolecular charge transfer like roseoflavin. This is due to the dissociation of the 8-sulfonamide group in polar solvents. The fluorescence spectra were also sensitive to solvent effects: the quantum yields of neutral $NCrSO_2NHFl$ increased with decreasing solvent polarity. In acetonitrile, $Ca^{2+}$ ion bound to the crown ether cavity in $NCrSO_2NHFl$ facilitated deprotonation of the sulfonamide group to give a new absorption maximum at 452 nm. Correspondingly, the quantum yields for photooxidation of benzyl alcohol by $NCrSO_2NHFl$ increased with increasing $Ca^{2+}$ concentration. These findings indicate that $Ca^{2+}$ ion can control the catalytic activity of $NCrSO_2NHFl$ through the interaction with the sulfonamide group serving as a cap for $Ca^{2+}$ bound to the crown cavity. The changes in the absorption spectra and the quantum yields were not observed for $CrSO_2NHFl$ and a reference flavin, $PhSO_2NHFl$. Therefore, $NCrSO_2NHFl$ acts as a new model system relevant to allosteric enzymes in which binding of an effector to a remote, allosteric site induces activity changes in the active sites. 

## INTRODUCTION

Coenzymes are prosthetic groups in enzymes and catalyze the enzyme-mediated reactions in the active sites. Although some of them are capable of catalyzing the reactions even in the absence of apoenzymes, the activities are mostly controlled through the interactions with apoenyzmes (*1–5*). In particular, allosteric effects by which some catalytic activities of enzymes may be regulated are quite intriguing from a bioorganic viewpoint; that is, binding of an effector to a remote, allosteric site induces activity changes in the active sites (*6*). In order to mimic

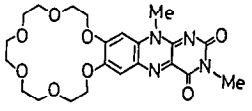
[1] Preliminary communication: S. Shinkai, K. Kameoka, K. Ueda, and O. Manabe, *J. Amer. Chem. Soc.* **109**, 923 (1987).

[2] To whom correspondence should be addressed.

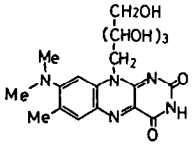


0045-2068/87 $3.00

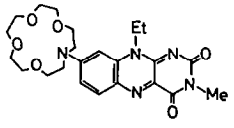
such allosteric functions in synthetic systems, we previously synthesized a crown ether flavin mimic, CrFl[3] (*7–9*). The crown ether cavity in CrFl recognized as a binding site not only spherical metal cations but ammonium cations and others through hydrogen bonding, and the resulting complexes changed the spectral and catalytic behaviors. This suggests that a crown ether family is a potential candidate for binding site of effectors in artificial allosteric enzymes. Meanwhile, roseoflavin, isolated from a culture medium of *Streptomyces* strain No. 768, has a dimethylamino group at the 8-position instead of a methyl group in conventional flavin coenzymes and shows an anti-flavin reactivity (*10–12*). This occurs because the isoalloxazine ring loses its oxidizing ability owing to intramolecular charge-transfer from the 8-dimethylamino group to the pteridine moiety (*13–15*). We previously synthesized a roseoflavin analog with a monoaza-15-crown-5 group at the 8-position (i.e. NCrFl) (*14, 15*). We found that the absorption spectra of this roseoflavin analog are very sensitive to solvent effects and the oxidizing ability is well correlated with the absorption maxima: the shorter the maximum wavelength, the more reactive (*14, 15*). The high reactivity in nonpolar solvents is attributed to the inhibition of intramolecular charge-transfer which results in the charge-separated roseoflavin structure (*15*). The finding suggests a new strategy to design flavins which may exhibit the allosteric functionality; that is, the oxidizing ability of $RSO_2NHFl$ should be greatly reduced, as seen in roseoflavin and its analogs, when the 8-sulfonamide group is dissociated. Furthermore, this dissociation equilibrium could be "remote-controlled" by the metal binding to the crown ether portion. The crown-metal complex in $CrSO_2NHFl$ may act as an electron-withdrawing substituent to facilitate the dissociation of the sulfonamide group (*7–9, 16*). $NCrSO_2NHFl$ is designed so that the dissociation of the sulfonamide group may be facilitated by the interaction as a "cap" with the metal cation bound to the crown cavity (*17–19*).



CrFl

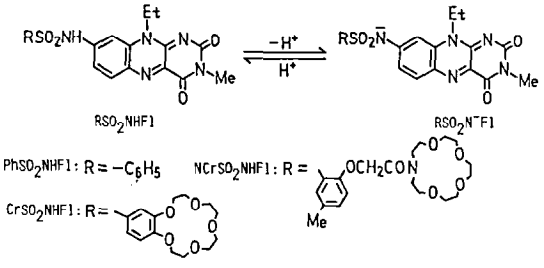


Roseoflavin



NCrFl

[3] Abbreviations used: CrFl, 3,10-dimethyl-1′,4′,7′,10′,13′,16′-hexaoxacyclooctadec-2′-eno[2′,3′-i]isoalloxazine; NCrFl, 3-methyl-8-(1,4,7,10-tetraoxa-13-azacyclopentadec-13-yl)-10-ethylisoalloxazine; $PhSO_2NHFl$, 3-methyl-8-benzenesulfonamido-10-ethylisoalloxazine; $CrSO_2NHFl$, 3-methyl-8-(2,3,5,6,8,9,11,12-octahydro-1,4,7,10,13-benzopentaoxapentadecin-15-sulfonamido)-10-ethylisoalloxazine; $NCrSO_2NHFl$, 3-methyl-8-[2-(1,4,10-tetraoxa-13-azacyclopentadec-13-ylcarbonylmethyloxy)-5-methylbenzenesulfonamido]-10-ethylisoalloxazine; DMF, *N*,*N*-dimethylformamide; THF, tetrahdrofuran.




## RESULTS AND DISCUSSION

### *Phototitration of $PhSO_2NHFl$, $CrSO_2NHFl$, and $NCrSO_2NHFl$*

Both the absorption spectra and the fluorescence spectra showed pH-dependent changes due to the dissociation of the 8-sulfonamide groups. As shown in Fig. 1, $NCrSO_2NHFl$ had an absorption maximum at 436 nm and produced a yellow to orange color in acidic aqueous solution. With increasing pH of the medium the absorption spectrum changed gradually with a few tight isosbestic points, and a new absorption maximum appeared at 485 nm in basic aqueous solution. The color of the basic solution was red. This color is attributable to a charge-transfer band from the 8-sulfonamide anion to the pteridine moiety (Eq. [1]; (*13–15*)). The $pK_a$ was determined to be 5.54 from this pH-dependent spectral change. Acetate buffer (0.01 M) was used in the acidic pH region and phosphate (0.01 M) or borate buffer (0.01 M) was used in the neutral to basic pH region. A similar pH-dependence was also observed for $PhSO_2NHFl$ and $CrSO_2NHFl$, from which their $pK_a$ values were estimated to be 4.85 and 4.93, respectively (Table 1). One may consider, therefore, that these flavins act as analogs of "regular" flavins in acidic aqueous solution while they act as roseoflavin analogs in basic aqueous solution.

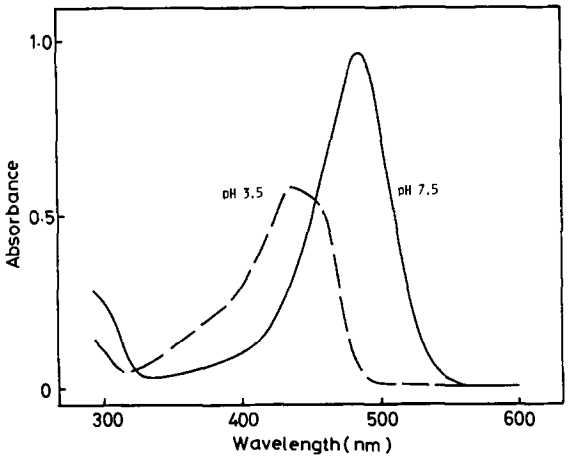
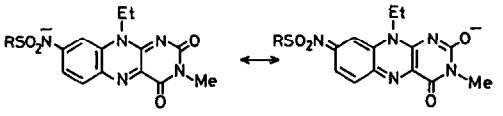



FIG. 1. Absorption spectra of neutral $NCrSO_2NHFl$ and anionic $NCrSO_2N^-Fl$ ($1.17 \times 10^{-5}$ M) at 30°C in an aqueous system.



[1]

It is known that conventional flavins produce a strong green fluorescence at around 520 nm (*7–9, 13–15*). $NCrSO_2NHFl$ gave a similar fluorescence maximum at 505 nm in acidic aqueous solution (Fig. 2). In basic aqueous solution the fluorescence maximum shifted to 552 nm, and the fluorescence intensity ($I$) became significantly weaker than that in acidic aqueous solution. This trend is complementary to the pH effects on the absorption spectra: that is, the excited singlet state of $NCrSO_2N^-Fl$ is quenched more efficiently by the electron-donating, dissociated 8-sulfonamide group. From a plot of $I/I_0$ vs pH we determined the $pK_a$ of the 8-sulfonamide group to be 5.56. The $pK_a$ values for other flavins are also determined by the fluorescence spectra (Table 1). These values are in good agreement with those determined by absorption spectra. The $pK_a$ values are lower by about 5 $pK$ units than that of benzenesulfonamide ($pK_a$ = 10.1; (*20*)). This is due to the electron-withdrawing nature of the isoalloxazine skeleton which is frequently compared with that of the *p*-nitrophenyl group (*21*).

*Solvent Effects on Spectral Properties*

We previously reported that roseoflavin analogs act as a convenient parameter for solvent polarity: the absorption maximum of NCrFl shifts from 498 nm in water to 486 nm (with a distinct shoulder at 457 nm) in benzene (*15*). The solvent effects on the present flavins are more complicated because of the dissociation of the 8-sulfonamide group. They imparted a yellow to orange color to nonpolar solvents as conventional flavins do while they imparted a red color to polar

TABLE 1

Absorption Maxima, Emission Maxima, and $pK_a$ in Aqueous Solution (30°C)

| | Flavin | | |
|---|---|---|---|
| | $PhSO_2NHFl$ | $CrSO_2NHFl$ | $NCrSO_2NHFl$ |
| $\lambda_{max}(\varepsilon_{max})$[a] | | | |
| For neutral species | 433(18,000) | 435(19,300) | 436(24,900) |
| For anionic species | 476(36,200) | 478(37,800) | 485(47,200) |
| $Em_{max}$[b] | | | |
| For neutral species | 504 | 500 | 505 |
| For anionic species | 545 | 540 | 552 |
| $pK_a$ | | | |
| By absorption spectrum | 4.85 | 4.93 | 5.54 |
| By fluorescence spectrum | 4.84 | 5.04 | 5.56 |

[a] [Flavin] = (1.1–2.0) × $10^{-5}$ M.

[b] [Flavin] = (0.59–5.9) × $10^{-7}$ M, excitation 443 nm for $PhSO_2NHFl$; 446 nm for $CrSO_2NHFl$; 452 nm for $NCrSO_2NHFl$. These wavelengths are isosbestic points in the absorption spectra.

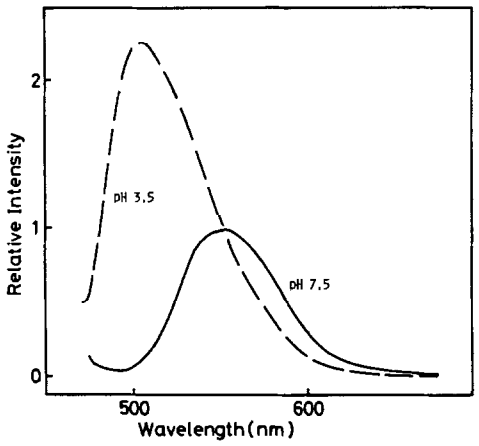



FIG. 2. Fluorescence spectra of neutral $NCrSO_2NHFl$ and anionic $NCrSO_2N^-Fl$ ($5.90 \times 10^{-7}$ M) at 30°C in an aqueous system. Excitation wavelength, 452 nm.

solvents due to the possible dissociation of the 8-sulfonamide group in these solvents. We measured the absorption spectra for neutral $NCrSO_2NHFl$ and anionic $NCrSO_2N^-Fl$ in the presence of trifluoroacetic acid and 1,8-diazabicyclo[5.4.0]-7-undecene, respectively. Then, we measured the absorption spectra of $NCrSO_2NHFl$ in "pure" solvents and determined the fraction of the dissociated species (Table 2). In Fig. 3 the fraction of anionic $NCrSO_2N^-Fl$ is plotted against Dimroth's $E_T$. The 8-sulfonamide group in $NCrSO_2NHFl$ was scarcely dissociated in the solvents less polar than acetonitrile ($E_T$ = 46). The dissociated fraction increased linearly with further increasing $E_T$ (except water, pH 6.5), and the highest value (78.7%) was obtained in methanol.

In roseoflavin analogs, the $h\nu$ values calculated from the maximum absorption frequencies are linearly correlated with $E_T$: the greater the $E_T$, the smaller the $h\nu$ value (*15*). This is probably because Dimroth's $E_T$ would resemble the resonance structure of roseoflavin analogs, although diphenyl betain, used in their study, has a polar ground state while roseoflavin analogs have nonpolar ground states and

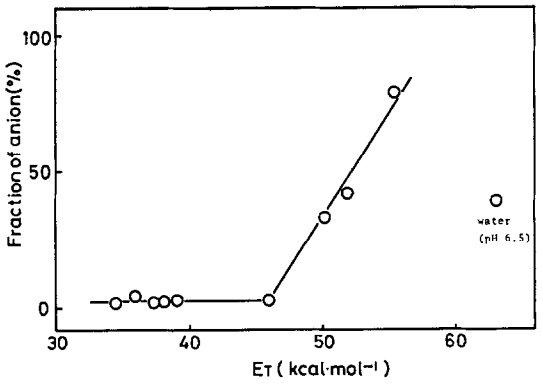



FIG. 3. Fraction of anionic $NCrSO_2N^-Fl$ plotted against Dimroth's $E_T$.

TABLE 2

Absorption Maxima of $NCrSO_2NHFl$ ($1.50 \times 10^{-5}$ M) in Various Solvents (30°C)

| Solvent | $\lambda_{max}$ ($\varepsilon_{max}$) | | Anion(%) |
|---|---|---|---|
| | TFA[a] | DBU[b] | |
| $H_2O$ | 436(24,900)[c] | 485(47,200)[d] | 38.8[e] |
| MeOH | 435(21,000) | 493(45,100) | 78.7 |
| EtOH | 438(21,400) | 500(46,600) | 41.6 |
| 1-BuOH | 439(21,600) | 502(46,500) | 32.7 |
| MeCN | 435(19,000) | 502(48,700) | 2.6 |
| Chloroform | 441(21,500) | 498(48,100) | 2.3 |
| Ethyl acetate | 435(18,400) | 494(48,500) | 2.0 |
| THF | 438(18,600) | 499(43,700) | 2.0 |
| Dioxane | 435(17,500) | 489(47,000) | 4.1 |
| Benzene | 440(17,400) | 494(45,300) | 1.6 |

[a] Trifluoroacetic acid was added: [TFA] = $(2.0–4.3) \times 10^{-5}$ M for aprotic solvents; $(1.0–1.6) \times 10^{-4}$ M for protic solvents. Protic solvents required the high concentration of TFA to suppress the dissociation.

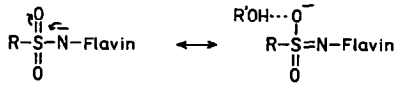
[b] Diazabicyclo[5.4.0]-7-undecene was added: [DBU] = $(3.0–10) \times 10^{-5}$ M for protic solvents; $(1.0–10) \times 10^{-3}$ M for aprotic solvents. Aprotic solvents required the high concentration of DBU to dissociate the 8-sulfonamide group.

[c] pH 3.0.

[d] pH 8.0.

[e] pH 6.5 (no buffer).

polar excited states (*13*). A similar solvent-dependent spectral change may be expected for dissociated $NCrSO_2N^-Fl$ because it has the electronic structure equivalent to roseoflavin (Eq. [1]). We plotted the $h\nu$ values calculated from the maximum absorption frequencies of $NCrSO_2N^-Fl$ against $E_T$ (Fig. 4). Interestingly, the plot provided a curve with a minimum at around $E_T = 50$. This implies that the polar excited state is more stabilized, as in roseoflavin analogs, at $E_T < 50$ but it is rather destabilized at $E_T > 50$. We noticed that Fig. 4 is quite complementary to Fig. 3; that is, $NCrSO_2N^-Fl$ gives the negative slope, like roseoflavin analogs (*15*), for the solvents which cannot facilitate the dissociation of the 8-sulfonamide group, whereas it gives the inverse, positive slope for the solvents which are polar enough to dissociate the 8-sulfonamide group. Conceivably, the latter solvents would strongly interact with $-SO_2-$ through, for example, hydrogen-bonding (Eq. [2]). As a result, the anionic charge on nitrogen is more withdrawn by the $-SO_2-$ moiety and cannot stabilize the polar excited state as expected from the solvent polarity.




[2]

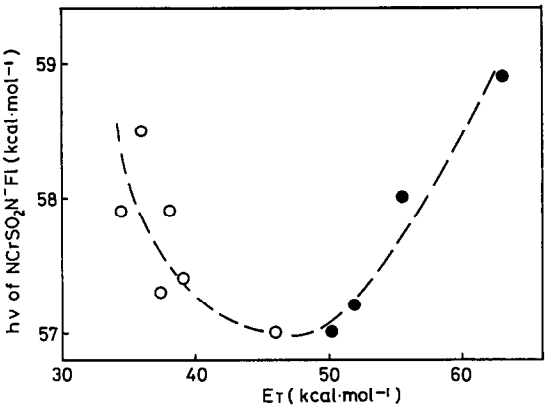



FIG. 4. $h\nu$ calculated from the maximum absorption frequencies of dissociated $NCrSO_2N^-Fl$ plotted against Dimroth's $E_T$. Open circles denote aprotic solvents and solid circles denote protic solvents.

On the other hand, the maximum absorption frequencies of neutral $NCrSO_2NHFl$ are less sensitive to the solvent effect.

The fluorescence properties of roseoflavin analogs were found to be exquisitely sensitive to the polarity of the solvent (*14*, *15*). The yield of fluorescence increased as the solvent polarity was decreased; for example, the quantum yield of fluorescence emission increased by over 33-fold upon moving from water ($\Phi_f$ = 0.018) to benzene ($\Phi_f$ = 0.61) (*15*). The emission maxima of roseoflavin analogs also shifted to shorter wavelengths as the solvent polarity was decreased (*15*). We mainly examined the solvent effects on the fluorescence properties of neutral $NCrSO_2NHFl$ (Table 3). The solvent effects on dissociated $NCrSO_2N^-Fl$ were fairly complicated because of fluorescence quenching by the added base (e.g., 1,8-diazabicyclo[5.4.0]-7-undecene). Examination of Table 3 reveals that (i) in aqueous solution the neutral species gives a $\Phi_f$ somewhat greater than the dissociated

TABLE 3

Fluorescence Emission Maxima and Fluorescence Quantum Yields of $NCrSO_2NHFl$ (30°C)

| Solvent | $Em_{max}$(nm)[a] | $\Phi_f$(%)[a] | $\Phi_f$ of NCrFl[b] (%) |
|---|---|---|---|
| Water (no buffer) | — | — | 1.8 |
| Water (pH 3.5) | 505 | 2.8 | — |
| Water (pH 7.5) | 558 | 2.1 | — |
| MeOH | 545 | 12 | 2.1 |
| EtOH | 535 | 14 | — |
| 1-BuOH | 536 | 22 | — |
| MeCN | 498 | 30 | 6.1 |
| Benzene | 498 | 51 | 61 |

[a] Trifluoroacetic acid (0.0273–0.464 nM) was added.
[b] Cited from Ref. (*15*).

species, (ii) the $\Phi_f$ values of the neutral species increase as the solvent polarity is decreased and the $\Phi_f$ in benzene (51%) is comparable with that of NCrFL (61%) or even with that of fluorescein (80%) (*22*), and (iii) the emission maxima also shift to shorter wavelengths as the solvent polarity is decreased. Trends (ii) and (iii) are in line with those of NCrFl. These findings suggest that the excited singlet state of $NCrSO_2NHFl$ has considerable dipolar character in polar solvents. Therefore, the dipolar excited state is destabilized in nonpolar solvents, resulting in hyposochromic shifts in the emission maxima and greatly enhances fluorescence intensities. Figure 5 shows a plot of $\Phi_f$ against $E_T$. The plot provided a good linear relationship ($r = 0.98$) expressed by

$$\Phi_f = -0.017E_T + 1.07. \quad [3]$$

The finding supports that $NCrSO_2NHFl$ also acts as an excellent fluorescence probe for the solvent polarity.

*Effects of Added Metal Salts on the Photooxidation Activity*

In an aqueous system the absorption spectra of $CrSO_2NHFl$ and $NCrSO_2NHFl$ were unaffected by the addition of alkali and alkaline earth metal cations (~1 M). This means that the "Lewis acidity" of these metal cations is not strong enough to induce the dissociation of the 8-sulfonamide group. In acetonitrile the absorption spectra of $PhSO_2NHFl$ and $CrSO_2NHFl$ were scarcely affected by the addition of these metal cations (as perchlorate salts). In contrast, the absorption spectrum of $NCrSO_2NHFl$ changed significantly upon the addition of alkaline earth metal cations, and $Ca(ClO_4)_2$ induced the largest spectral change, giving rise to a new absorption maximum at 452 nm (Fig. 6). This is probably due to the "lariat effect" (*17–19*) by which the dissociation of the 8-sulfonamide group is facilitated. We believe that the 8-sulfonamide anion serves as a cap for the $Ca^{2+}$ ion bound to the crown cavity (Eq. [4]). A continuous variation method indicated that $NCrSO_2NHFl$ and $Ca^{2+}$ form a 1 : 1 complex. The association constant ($K$) was estimated to be $4.27 \times 10^4$ $M^{-1}$ from a plot of $OD_{452}$ vs $[Ca(ClO_4)_2]$. It is known that conventional flavins interact with certain metal cations such as $Mg^{2+}$ and $Zn^{2+}$ in aprotic solvents (e.g., acetone and acetonitrile) (*23–25*). However, the $K$ values are so small ($K$ = ca. $10^2$ $M^{-1}$) that the spectral changes are observed only in the

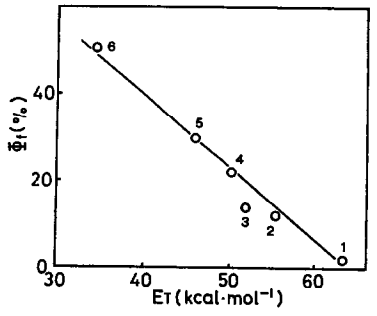



FIG. 5. Plot of $\Phi_f$ vs $E_T$. 1, Water; 2, methanol; 3, ethanol; 4,1-butanol; 5, acetonitrile; 6, benzene.

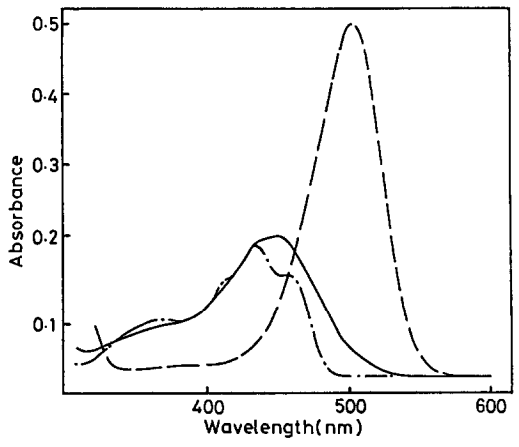
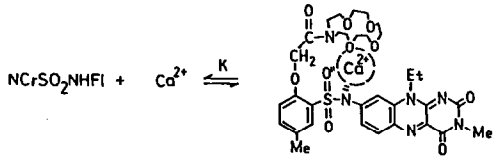



FIG. 6. Absorption spectra of $NCrSO_2NHFl$ ($1.03 \times 10^{-5}$ M) in acetonitrile at 30°C. (---) Neutral $NCrSO_2NHFl$ ([$CF_3COOH$] = $2.32 \times 10^{-5}$ M); (---) dissociated $NCrSO_2N^-Fl$ ([1,8-diazabicyclo[5.4.0]-7-undecene] = $1.70 \times 10^{-5}$ M); (—) $NCrSO_2N^-Fl \cdot Ca^{2+}$ complex ([$Ca(ClO_4)_2$] = $7.65 \times 10^{-3}$ M).

presence of a large excess of the metal cations (ca. 0.10 M or higher). The large $K$ values were attained only when additional metal-binding sites were introduced intramolecularly into the isoalloxazine skeleton (*7–9, 26–28*). The large $K$ values observed for $NCrSO_2NHFl$ are therefore attributed to the synergestic effect of the crown ring and the sulfonamide cap.




[4]

In order to examine the potential correlation between the metal-induced spectral change and the oxidizing ability, we carried out the anaerobic photooxidation of benzyl alcohol to benzaldehyde (*24, 26*). The results are summarized in Table 4. In an aqueous system the quantum yields ($\Phi_{ox}$) for the oxidation by neutral $PhSO_2NHFl$ and $NCrSO_2NHFl$ (at pH 3.5) were greater by 16- to 640-fold than those for the corresponding dissociated species (at pH 7.5). On the other hand, the $\Phi_{ox}$ for 3-methyl-10-ethylisoalloxazine was scarcely affected by the medium pH. Usually, photooxidation by flavins proceeds via the triplet state (*15, 29–31*). We previously confirmed that photooxidation by roseoflavin analogs, isoelectronic to the present flavins, also proceeds via the triplete state (*15*). Therefore, the result suggests, together with the spectral data, that the oxidizing ability of these flavins in the triplet state is primarily governed by the extent of the intramolecular charge-transfer; that is, the dissociated 8-sulfonamide group definitely deactivates the isoalloxazines as oxidizing agents.

Interestingly, we found that in acetonitrile the $\Phi_{ox}$ for $NCrSO_2NHFl$ increases with increasing $Ca^{2+}$ concentration but that for $PhSO_2NHFl$ increases only

TABLE 4

Quantum Yields for Photooxidation of Benzyl Alcohol by $PhSO_2NHFl$, $NCrSO_2NHFl$, and 3-Methyl-10-ethylisoalloxazine

| Solvent | $[Ca(ClO_4)_2]$ (mM) | $\Phi_{ox}$ (%) | | |
|---|---|---|---|---|
| | | $PhSO_2NHFl$ | $NCrSO_2NHFl$ | 3-Methyl-10-ethylisoalloxazine |
| Water (pH 3.5) | 0 | 0.64[a] | 0.60 | 3.34 |
| Water (pH 7.5) | 0 | ca. $1 \times 10^{-3}$[a] | 0.04 | 2.47 |
| MeCN | 0 | 0.04 | 0.05 | 0.08 |
| MeCN | 0.199 | 0.07 | 0.08 | — |
| MeCN | 1.01 | 0.07 | 0.13 | — |
| MeCN | 2.55 | 0.08 | 0.20 | — |

[a] Neutral $PhSO_2NHFl$ was sparingly soluble in water, so that the photooxidation was carried out in 40 vol% aqueous acetonitrile.

slightly. This finding is in good accord with the $Ca^{2+}$ effect on the absorption spectra; that is, $Ca^{2+}$ ion bound to the crown cavity of $NCrSO_2NHFl$ can induce the spectral change and enhance the photooxidizing ability as well. Conceivably, $Ca^{2+}$ ion suppresses the intramolecular charge-transfer through the interaction with the 8-sulfonamide anion serving as a cap for this metal cation. As a result, the isoalloxazine ring is activated as an oxidizing agent. The result may be described in the following way: the binding of $Ca^{2+}$ to the crown "subunit" induces the coordination of the sulfonamide group and "controls" the extent of the intramolecular charge-transfer which is essential to the photooxidizing ability. We also used other alkaline earth metal cations such as $Mg^{2+}$ and $Ba^{2+}$, but these metal cations were not so effective as $Ca^{2+}$ in the millimolar concentration region. Probably, $Ca^{2+}$ which has an ion diameter (1.98 Å) similar to $Na^+$ (1.90 Å) would interact with the monoaza-15-crown-5 ring most strongly.

## CONCLUSIONS

The present paper demonstrated that isoalloxazines which have the sulfonamide group at the 8-position undergo the sensitive solvent effects due to the deprotonation equilibrium of the sulfonamide group. The absorption and fluorescence spectral properties can be rationalized in terms of the intramolecular charge-transfer from the 8-sulfonamide to the pteridine moiety. More important are the metal effects: in $NCrSO_2NHFl$ the crown ether moiety serving as an allosteric site can alter the oxidizing ability of the isoalloxazine serving as a catalytic site. The close imitation of natural control mechanisms suggests that in a sense $NCrSO_2NHFl$ is a well-constructed miniature of "allosteric" enzymes.

## EXPERIMENTAL

*Materials.* It is known that 8-chloroflavin derivatives undergo nucleophilic substitution (*14, 15, 32*). We synthesized $RSO_2NHFl$ by the reaction of 3-methyl-8-chloro-10-ethylisoalloxazine (*15*) with the corresponding sulfonamides in the presence of base.

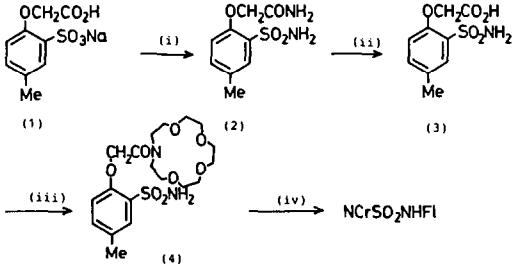
*$PhSO_2NHFl$.* Sodium salt of benzenesulfonamide (2.58 g, 14.4 mmol) and 3-methyl-8-chloro-10-ethylisoalloxazine (1.00 g, 3.61 mmol) were heated at 90°C in 60 ml of dimethyl sulfoxide. After 1 day the reaction mixture was poured into water. The aqueous solution was made alkaline (pH 9 with $Na_2CO_3$); the insoluble precipitate was removed by filtration. The filtrate was acidified by HCl to afford the yellow precipitate. The product was isolated by a preparative TLC method (silica gel-chloroform : acetone = 5 : 1, v/v): mp 290–292°C, one spot on TLC, yield 5.4%, mass spectrum $M^+$ (*m/e*) 411; IR(KBr) $\nu_{c=o}$ 1610 and 1670 $cm^{-1}$, $\nu_{so_2}$ 1130 $cm^{-1}$.

*Anal.* Calcd for $C_{19}H_{17}N_5O_4S \cdot H_2O$: C, 53.14; H, 3.99; N, 16.31%. Found: C, 52.58; H, 4.35; N, 15.76%. The several yellow or orange spots were found on the TLC plate, and the yield was low. This indicates that the substitution of the 8-chloro with amines is not a clean reaction.

*$CrSO_2NHFl$.* Sodium salt of 4′-sulfonamidobenzo-15-crown-5 (0.68 g, 1.84 mmol) and 3-methyl-8-chloro-10-ethylisoalloxazine (0.170 g, 0.613 mmol) were heated at 90°C in 10 ml of DMF. The progress of the reaction was followed by a TLC method (silica gel-chloroform: methanol = 10 : 1, v/v). After 1 day the reaction mixture was cooled and evaporated to dryness *in vacuo*. The yellow residue was subjected to a TLC separation: mp 248–250°C, one spot on TLC, yield 33%, mass spectrum $M^+$ (*m/e*) 601; IR(KBr) $\nu_{c=0}$ 1650 and 1700 $cm^{-1}$, $\nu_{SO_2}$ 1360 $cm^{-1}$, $\nu_{c-o-c}$ 1160 and 1180 $cm^{-1}$.

*Anal.* Calcd for $C_{27}H_{31}N_5O_9S \cdot H_2O$: C, 52.34; H, 5.37; N, 11.30%. Found: C, 52.12; H, 5.09; N, 11.11%.

*$NCrSO_2NHFl$.* This isoalloxazine was synthesized according to Scheme 1. The methyl group was introduced in order to avoid the formation of the *o,p*-mixture in the sulfonation of phenoxyacetic acid.




SCHEME 1. (i) $SOCl_2$ and then $NH_3$; (ii) hydrolysis; (iii) *t*-BuCOCl + monoaza-15-crown-5; (iv) 3-methyl-8-chloro-10-ethylisoalloxazine.

Sodium 2-carboxymethyloxy-5-methylbenzenesulfonate (**1**) (5.0 g, 18.6 mmol) was dissolved in 20 ml of thionyl chloride and heated at 70°C for 1 day in the presence of several drops of DMF. Thionyl chloride was evaporated *in vacuo* to dryness. In order to remove a trace amount of thionyl chloride we added toluene and evaporated the solution once again. The residue (oil) was dissolved in benzene and mixed with 18 ml of 28% $NH_3$. After 3.5 h the precipitate was collected by filtration. Recrystallization from ethanol gave 2-carbamoylmethyloxy-5-methylbenzenesulfonamide (**2**): mp 193–194°C, yield 42%; IR (KBr) $\nu_{NH_2}$ 3200 and 3400 $cm^{-1}$, $\nu_{c=o}$ 1680 $cm^{-1}$.

*Anal.* Calcd for $C_9H_{12}O_4N_2S$: C, 44.26, H, 4.95; N, 11.47; S, 13.13%. Found: C, 44.71; H, 4.91; N, 11.11; S, 12.99%.

(**2**) (1.50 g, 6.14 mmol) was dissolved in 10 ml of water containing NaOH (0.65 g, 16.3 mmol) and the solution was refluxed for 1 h. After cooling the solution was acidified with concd HCl. 2-Carboxymethyloxy-5-methylbenzenesulfonamide (**3**) was recovered as the white precipitate: mp 169–170°C, yield 83.7%, IR(KBr) $\nu_{NH_2}$ 3200 and 3400 $cm^{-1}$, $\nu_{c=o}$ 1720 $cm^{-1}$.

*Anal.* Calcd for $C_9H_{11}O_5NS$: C, 44.08; H, 4.52; N, 5.71; S, 13.07%. Found: C, 44.34; H, 4.48; N, 5.65; S, 13.39%.

Pivaloyl chloride (0.75 g, 6.14 mmol) was added dropwise to 20 ml of the THF solution containing (**3**) (1.24 g, 5.06 mmol) in an ice-bath. After 2.5 h the THF solution (15 ml) containing monoaza-15-crown-5 (1.28 g, 5.49 mmol) and triethylamine (0.65 g, 6.42 mmol) was added dropwise. The reaction was continued at room temperature for 4 h and at reflux temperature for 1 day. The precipitate (triethylamine hydrochloride) was removed by filtration; the filtrate was evaporated to dryness. The residual yellow oil was subjected to a TLC separation (silica gel-methanol : chloroform = 1 : 10, v/v). Recrystallization from methanol gave 2-(1,4,7,10 - tetraoxa - 13 - azacyclopentadec - 13 - ylcarbonylmethyloxy) - 5 - methylsulfonamide (**4**): mp 108–112°C, yield 41%; IR(KBr) $\nu_{NH}$ 3280 $cm^{-1}$, $\nu_{c=o}$ 1650 $cm^{-1}$; $\nu_{c=o-o}$ 1120 $cm^{-1}$.

*Anal.* Calcd for $C_{19}H_{30}O_8N_2S$: C, 51.11; H, 6.77; N, 6.27; S, 7.18%. Found: C, 50.43: H, 6.93; N, 6.01; S, 6.50%.

(**4**) (0.90 g, 2.02 mmol) and 3-methyl-8-chloro-10-ethylisoalloxazine (0.586 g, 2.02 mmol) were dissolved in 30 ml of DMF and heated at 90°C for 4 days in the presence of powdered $K_2CO_3$ (0.84 g, 6.08 mmol). The precipitate was removed by filtration; the filtrate was concentrated *in vacuo* to dryness. The brown residue was subjected to a TLC separation (silica gel-methanol: chloroform = 1 : 15, v/v). Recrystallization from ethanol containing several drops of concd HCl gave $NCrSO_2NHFl$ as the yellow crystal: mp 179–182°C, yield 19.8%; IR(KBr) $\nu_{NH}$ 3430 $cm^{-1}$, $\nu_{c=o}$ 1650 $cm^{-1}$, $\nu_{c-o-c}$ 1140 $cm^{-1}$; mass spectrum (FAB) $M^+$+1 701.

*Anal.* Calcd for $C_{32}H_{40}O_{10}N_6S$: C, 54.85; H, 5.75; N, 11.99; S, 4.58%. Found: C, 54.85; H, 6.07; N, 11.62; S, 4.46%.

*Determination of quantum yields.* Integrated fluorescence quantum yields were determined relative to fluorescein in 0.1 M NaOH ($\Phi_f$ = 0.86) in exactly the same geometry (*22*). The excitation wavelength was 313 nm.

A standard actinometer (potassium trioxalatoferrate (III)) was used for the quantum yield determination on the photochemical reaction of flavins and benzyl

alcohol (*9, 33*). A sample solution in a 1-cm quartz cell was deaerated and 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 a glass filter (Corning 7-37). The quantum yields were determined by comparing the light intensities with the reduced flavins in exactly the same geometry.

## ACKNOWLEDGMENTS

We thank Professor M. Takagi and Professor G. W. Gokel for helpful discussions. This research was supported by a grant from the Ministry of Education of Japan.

## REFERENCES

1. WALSH, C. (1979) Enzymatic Reaction Mechanism, Freeman, San Francisco.
2. BRUICE, T. C., AND BENKOVIE, S. J. (1969) Bioorganic Mechanisms, Benjamin, New York.
3. JENCKS, W. P. (1969) Catalysis in Chemistry and Enzymology, McGraw–Hill, New York.
4. BRESLOW, R. (1980) *Acc. Chem. Res.* **13,** 170.
5. For comprehensive reviews on coenzyme model catalyses see SHINKAI, S. (1982) *Progr. Polym. Sci.* **8,** 1; SHINKAI, S. (1984) Enzyme Chemistry, p. 40, Chapman & Hall, London.
6. For allosteric effects in organic chemistry see the following papers and references cited therein. REBEK, J., JR., COSTELLO, T., MARSHALL, L., WATTLEY, R., GADWOOD, R. C., AND ONAN, K. (1985) *J. Amer. Chem. Soc.* **107,** 7481; REBEK, J. J., COSTELLO, T., AND WATTLEY, R. (1985) *J. Amer. Chem. Soc.* **107,** 7487.
7. SHINKAI, S., ISHIKAWA, Y., SHINKAI, H., TSUNO, T., AND MANABE, O. (1983) *Tetrahedron Lett.* **24,** 1539.
8. SHINKAI, S., ISHIKAWA, Y., SHINKAI, H., TSUNO, T., MAKISHIMA, H., UEDA, K., AND MANABE, O. (1984) *J. Amer. Chem. Soc.* **106,** 1801.
9. SHINKAI, S., NAKAO, H., UEDA, K., MANABE, O., AND OHNISHI, M. (1986) *Bull. Chem. Soc. Japan.* **56,** 1632.
10. OTANI, S., TAKATSU, M., NAKANO, M., KASAI, S., MIURA, T., AND MATSUI, K. (1974) *J. Antibiot.* **27,** 88.
11. KASAI, S., KUBO, Y., YAMANAKA, S., HIROTA, T., SATO, H., TSUZUKIDA, Y., AND MATSUI, K. (1978) *J. Nutri. Sci. Vitaminol.* **24,** 339.
12. KASAI, S., MIURA, T., AND MATSUI, K. (1975) *Bull. Chem. Soc. Japan.* **48,** 2877.
13. SONG, P.-S. WALKER, E. B., VIERSTRA, R. D., AND POFF, K. L. (1980) *Photochem. Photobiol.* **32,** 393.
14. SHINKAI, S., KAMEOKA, K., HONDA, N., UEDA, K., AND MANABE, O. (1985) *J. Chem. Soc. Chem. Commun.*, 673.
15. SHINKAI, S., KAMEOKA, K., HONDA, N., UEDA, K., MANABE, O., AND LINDSEY, J. (1986) *Bioorg. Chem.* **14,** 119.
16. TAKAGI, M., AND UENO, K. (1984) *Top. Curr. Chem.* **121,** 39.
17. SCHULTZ, R. A., DISHONG, D. M., AND GOKEL, G. W. (1982) *J. Amer. Chem. Soc.* **104,** 625.
18. GUSTOWSKI, D. A., ECHEGOYEN, L., GOLI, D. M., KAIFER, A., SCHULTZ, R. A., AND GOKEL, G. W. (1984) *J. Amer. Chem. Soc.* **106,** 1633.
19. DISHONG, D. M., DIAMOND, C. J., CINOMAN, M. I., AND GOKEL, G. W. (1983) *J. Amer. Chem. Soc.* **105,** 586.
20. HINMAN, R. L., AND HOOGENBOOM, B. F. (1961) *J. Org. Chem.* **26,** 3461.
21. HEMMERICH, P., VEEGER, C., AND WOOD, H. C. S. (1965) *Angew. Chem.* **4,** 671.
22. DAWSON, W. R., AND WINDSOR, M. M. (1968) *J. Phys. Chem.* **72,** 3251.

*23.* HEMMERICH, P., MÜLLER, F., AND EHRENBERG, A. (1965) Oxidase and Related Redox Systems, p. 157, Wiley, New York.
*24.* FUKUZUMI, S., KURODA, S., AND TANAKA, T. (1985) *J. Amer. Chem. Soc.* **107,** 3020.
*25.* SHINKAI, S., NAKAO, H. TSUNO, T., MANABE, O., AND OHNO, A. (1984) *J. Chem. Soc. Chem. Commun.,* 849.
*26.* SHINKAI, S., NAKAO, H., HONDA, N., MANABE, O., AND MÜLLER, F. (1986) *J. Chem. Soc. Perkin Trans. I,* 1825.
*27.* SHINKAI, S., HONDA, N., ISHIKAWA, Y., AND MANABE, O. (1985) *J. Chem. Soc. Perkin Trans. I,* 565.
*28.* TRABER, R., WERNER, T., SCHNEIR, R., KRAMER, H. E., KNAPPE, W.-R., AND HEMMERICH, P. (1980) *in* Flavins and Flavoproteins (Yagi, K., and Yamano, T., Eds.), p. 431, Japan Scientific Soc. Press, Tokyo.
*29.* EBERLEIN, G. A., AND POWELL, M. F. (1984) *J. Amer. Chem. Soc.* **106,** 3309.
*30.* ROKITA, S. E., AND WALSH, C. T. (1984) *J. Amer. Chem. Soc.* **106,** 4589.
*31.* YONEDA, F., SHINOZUKA, K., HIROMATSU, K., MATSUSHITA, R., SAKUMA, Y., AND HAMANA, M. (1980) *Chem. Pharm. Bull.* **28,** 3576.
*32.* GALVERT, J. G., AND PITTS, J. N., JR. (1966) *Photochemistry,* pp. 734, 738, Wiley, New York.