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ON THE UNUSUAL SPECTROSCOPIC BEHAVIORS OF 3-METHYL-10-DODECYLISOALLOXAZINE(AMPHIPHILIC FLAVIN ANALOGUE)

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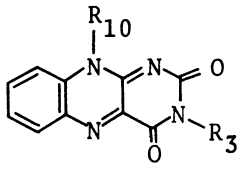
At room temperature in aqueous solution, the visible absorption peak of the title isoalloxazine (III) gives a three-banded fine structure, which has been believed to appear only in enzymatic hydrophobic region, in nonpolar solvents, or at extremely low temperature. On the other hand, another amphiphilic flavin 3-hexadecyl-10-butylisoalloxazine (II) has not such a fine structure. Thus, the unusual fine structure in (III) was attributed to aggregative "stacking" of the isoalloxazine heads, and the difference in the stacking mode between (II) and (III) was discussed.

A flavin family has frequently been employed as a probe in enzymatic and membrane biology, since the light absorption spectra have characteristic dependence upon medium polarity. Oxidized flavins usually have two characteristic absorption maxima at around 330 nm (peak S2) and 440 nm (peak S1). It has been established on the basis of solvent effects that (i) the absorption maximum of S2 can serve as indicator of solvent polarity, shifting to shorter wavelengths in apolar solvents and (ii) the absorption maximum of S1 scarcely shifts but the spectral shape is significantly affected by solvent polarity: a simple Gaussian peak in aqueous solution (type A), two to three shoulders in dipolar solvents (DMF, THF, acetonitrile, etc.) (type B), and a resolved three-banded fine structure in hydrocarbon solvents (benzene, 3-methylpentane, etc.)(type C).[1-4] At extremely low temperature (77°K), the fine structure appears even in dipolar solvents (e.g., 2-methyltetrahydrofuran).[4] In nonpolar solvents, both the blue shift of S2 and the splitting of S1 occur simultaneously. To the best of our knowledge, however, neither of them has ever been reported in aqueous solution except for those in the enzymatic hydrophobic pocket.[5-7] We here report for the first time that 3-methyl-10-dodecylisoalloxazine (III) (amphiphilic flavin analogue) gives the three-banded fine structure in aqueous solution at room temperature, whereas simple isoalloxazine (I) and another amphiphilic flavin analogue (II) with a long alkyl chain at 3-position give the spectra of type A or B. Based on the spectral examination, we attributed the unusual spectral behavior of (III) to the association of isoalloxazine nuclei.



(I) $R_3 = CH_3$, $R_{10} = C_2H_5$

(II) $R_3 = n\text{-}C_{16}H_{33}$, $R_{10} = n\text{-}C_4H_9$

(III) $R_3 = CH_3$, $R_{10} = n\text{-}C_{12}H_{25}$

Based on the spectral measurements, we classified the medium effects as follows. The typical spectra and absorption maxima are recorded in Fig. 1 and Table 1, respectively.

Type A: (I) in aqueous solution.

Type B: (II) in aqueous solution and (I), (II), and (III) in dipolar solvents (DMF, THF, CH_3CN, dioxane etc.).

Type C: (III) in aqueous solution and (I), (II), and (III) in apolar solvents (benzene, toluene, etc.).

The spectral behavior of three isoalloxazines was very similar in organic solvents, but significant differences appeared in aqueous solution. In particular, S1 of (III) in aqueous solution had the well-resolved fine structure which is similar to that of (I) in benzene. It should be noticed, however, that S2 of (I) in benzene considerably shifts to shorter wavelength (328 nm), whereas that of (III) in aqueous solution does not (340 nm). The difference suggests that the origin of the effect which induces the split of S1 of aqueous (III) is somewhat different from simple solvent effects.

Interestingly, the fine structure of (III) disappeared completely in aqueous solutions containing surfactants (CTAB, Brij-35, and SDS) above the critical micelle concentrations, and the spectra were classified as type B (Table 1 and Fig. 1c). The fine structure also disappeared by the addition of ethanol (>45 vol%) or pyridine (>20 vol%). By the addition of small amount of pyridine, a new, broad absorption band appeared at 500-700 nm, which is probably ascribed to a charge

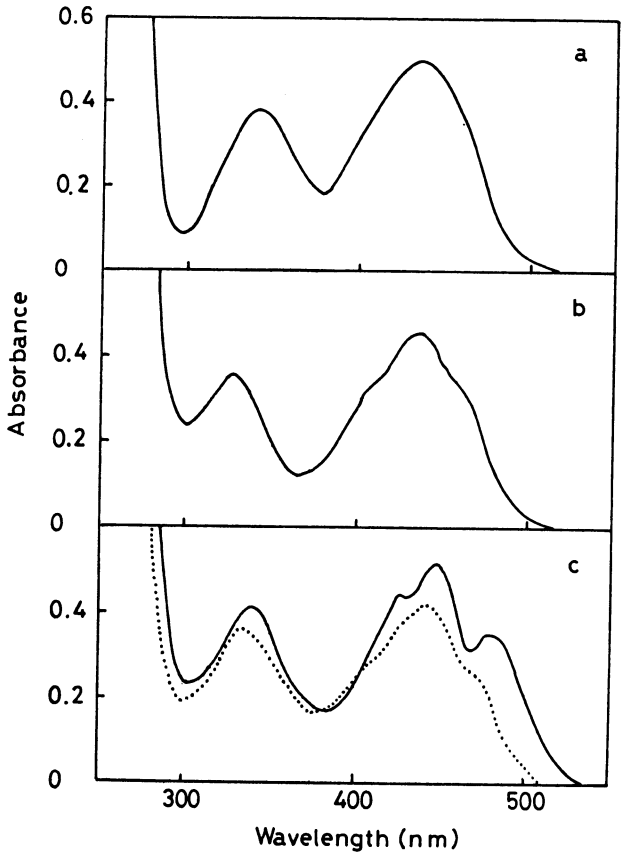


Fig. 1 Absorption spectra of isoalloxazines (5.00×10^{-5} M) at 30°C. (a) (I) in water (type A); (b) (I) in DMF (type B); (c)—(III) in water (type C),···(III) in CTAB (10 mM) (type B).

Table 1. Absorption maxima (nm) of (I)-(III) in typical solvents (30°C).[a)]

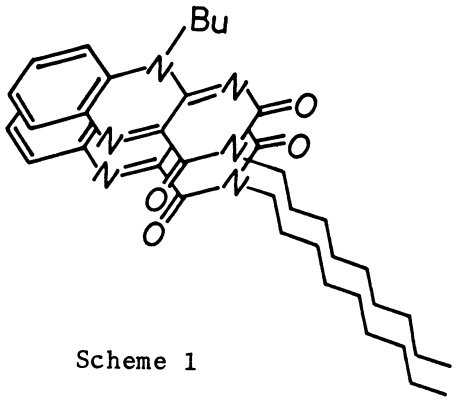
Medium	(I) S2	(I) S1	(II) S2	(II) S1	(III) S2	(III) S1
Water	341,	433	334,	426(S)	340,	427 447 480
CTAB(10 mM)	340,	433	336,	440(S)	338,	440(S)
CH_3CN	328,	433(S)	328,	436(S)	328,	435(S)
Benzene	328,	418 440 466	329,	420 442 466	329,	420 440 467

a) (S) indicates that S1 has shoulders. Three values given for S1 mean each absorption maximum for the fine structure split.

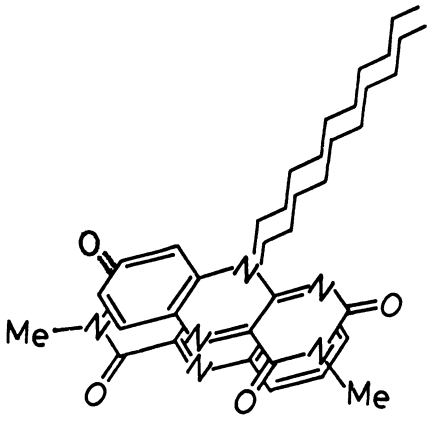
transfer band. Both the new absorption band and the fine structure were observed at pyridine content less than 19 vol%, but they suddenly disappeared at 20 vol% pyridine. The dramatic change in the spectral shape would reflect some critical change in the aggregation mode. The broad absorption band at 500-700 nm was not observed in the aqueous pyridine solution of (I) and (II).

The three-banded fine structure of S1 appears when D-amino acid oxidase (flavoprotein) of hog kidney forms a complex with benzoate.[5)] The phenomenon is accounted for as such that flavin is located in a hydrophobic environment in the protein.[6)] The concept may be partially applied to the present system, but cannot accommodate following facts: (i) the fine structure rather disappears in the micelles with hydrophobic environments, (ii) apolar solvents which may mimic the enzymatic hydrophobic environments cause both the blue shift of S2 and the split of S1, whereas only the latter phenomenon is seen in aqueous (III), and (iii) (III) has the fine structure and (II) has not. As an alternative explanation, we propose that the fine structure is due to "stacking" of isoalloxazine head groups of amphiphilic flavin (III). It is frequently seen that "stacking" of dye molecules induces the splitting of absorption bands and the deaggregation is induced by surfactants above the critical micelle concentration.[7,8)] The effect of the micelles is accounted for by the deaggregative dilution in the micellar phase. (iii) is most difficult to rationalize. We noticed an essential difference between (II) and (III), however. The driving force of the association is hydrophobic interaction between hydrocarbon chains. Provided that the association requires the parallel orientation of hydrocarbon chains, two carbonyls at 2- and 4-position of (II) must always overlap with

those of others (Scheme 1). On the other hand, (III) with a hydrocarbon chain at 10-position can stack in different way (Scheme 2).[9] The absence of the dipole-dipole repulsion would largely facilitate "stacking" of the isoalloxazine nuclei of (III). It is interesting to note that the crystal of 9-bromo-3,7,8,10-tetramethyl-isoalloxazine monohydrate has the structure analogous to Scheme 2.[10]



Scheme 1



Scheme 2

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9) Here, "stacking" is not limited to the dimer. We consider that an aggregate consists of several molecules of (III).
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