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UV-Visible spectral studies on amphiphilic isoalloxazines in single and mixed solvent systems

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ABSTRACT

UV-visible spectral studies on amphiphilic isoalloxazines have been performed in single and mixed solvent systems. 10-Hexadecyl-3-methylisoalloxazine gave S1 peak as well-resolved three-banded type C in aqueous solution at room temperature, which has been believed to appear only in nonpolar solvents or in enzymatic hydrophobic pockets.

Key Words: Amphiphilic Isoalloxazines, UV-visible spectroscopy.

INTRODUCTION

Isoalloxazines (or flavins), a cofactor of flavoenzymes, have usually two characteristic absorption maxima in the UV (330 nm, S2 peak) and visible regions (440 nm, S1 peak) [1-3]. It has been established from the solvent effects that the absorption maximum of S2 can serve as an indicator of solvent polarity since it shifts to shorter wavelengths in apolar solvents and the absorption maximum of S1 is scarcely affected by solvent polarity, but the solvent effect is sensitively reflected by the spectral shape: a simple gaussian-type peak in aqueous solution (type A), two or three shoulders in dipolar solvents (type B) and a well resolved three band fine structure (type C) in apolar solvents (Figure 1) [1-6]. The spectral changes have been considered to reflect the degree to which hydrogen bonding to isoalloxazine plays a role in solvent-isoalloxazine interactions. At extremely low temperature, the fine structure appears even in dipolar solvents [5]. In apolar solvents, both the blue shift of S2 and the split of S1 occur simultaneously.

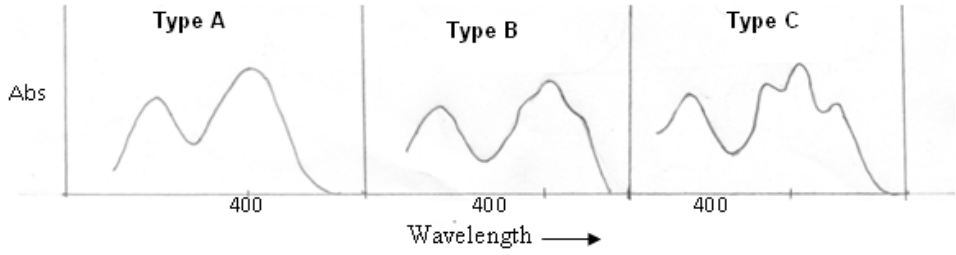


Figure 1

This basic information on solvent effects has enabled to use the isoalloxazine family as an environment probe in enzymatic and membrane biology [7,8]. The present study is a step towards understanding the solvent effects of flavoenzymes.

MATERIALS AND METHODS

Melting points have been determined on a Thomas Hoover Unimelt capillary melting apparatus and are uncorrected. The absorption spectra of isoalloxazines were recorded on a Shimadzu UV-260 spectrophotometer at 28±1^{o}C temperature and absorption maxima have been expressed in nanometers. The concentration was usually 5.00×10^{-5}M. The isoalloxazine derivatives [10-hexyl-3-methylisoalloxazine (**1**), 3-dodecyl-10-hexylisoalloxazine (**2**) and 10-hexadecyl-3-methylisoalloxazine (**3**) (Table 1)] were synthesized by the acidic cyclocondensation of N-substituted-2-aminoanilines with alloxan monohydrate [9,10] and their further reaction with alkyl halide in the presence of DBU as base [11].

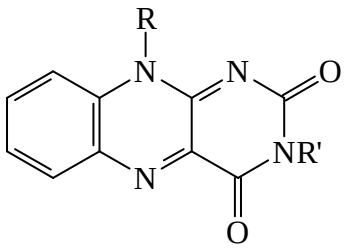
RESULTS AND DISCUSSION

UV-visible studies in Single Solvent Systems

The spectral behaviour of the isoalloxazines has been very similar in organic solvents but significant differences have been found in aqueous solution. The absorption maxima (λ_{max}) of isoalloxazine derivatives **1-3** (Table 1) in single different solvents are summarized in table 2. It has been observed from table 2 that the λ_{max} of S2 of **1** in benzene is considerably shifted to shorter wavelength (328 nm) in comparison with that in water (339 nm) and also there is split in S1 band in benzene in comparison to water. The solvent effect has shown the spectra of **1** in aqueous solution as type A and type C in benzene. On the other hand, S1 of **3** was a well-resolved fine structure (type C) even in aqueous solution.

Interestingly, the presence of C_{12} alkyl chain at position 3 in **2** did not split the spectra into three banded and we end up with type B, indicating the involvement of position 10 in its unusual behaviour. These results suggest that the fine structure observed in the aqueous system cannot be explained in terms of the simple solvent effect, but also some type of aggregation is playing some role.

Table 1. Isoalloxazines synthesized for study



Comp.	R	R'	Mp. (Lit. Mp.)[#] (°C)
1.	C_6H_{13}	CH_3	180 (180) [11]
2.	C_6H_{13}	$C_{12}H_{25}$	98-99 (98-99) [11]
3.	$C_{16}H_{33}$	CH_3	140 (140) [11]

[#]Reference

UV-visible studies in Mixed Solvent Systems

The absorption spectra of isoalloxazines in the mixed solvent system are different from those obtained in single solvent systems. The addition of ethanol or pyridine to the aqueous solution of **3** changes the shape of S1 from type A to type B after reaching a particular concentration (Table **3** & **4**). The absorption maximum (S2) of **1** is shifted from 339 nm in water to 331 nm in ethanol or to 325 nm in pyridine. These results are in agreement with the solvent effects on the absorption spectra of isoalloxazines [2].

Table 2. Absorption maxima (nm) of isoalloxazines in various solvents at 28±1 °C

Medium	1		2		3	
	S1	S2	S1	S2	S1	S2
Water	431	339	427(S)	333	423 441 478	338
Ethanol	438(S)	331	435(S)	329	436(S)	332
DMF	435(S)	329	432(S)	331	437(S)	330
Pyridine	442(S) 420	327	441(S) 420	329	440(S) 422	328
Benzene	442 472	328	443 471	330	442 470	329

S = Shoulder

The fine splitting in the visible region of the isoalloxazine **3** gradually disappeared with increase in ethanol concentration and the spectra in ≥40 vol% ethanol solution were classified as type B. The similar effect has been observed in water-pyridine mixtures where the addition of only 5 vol% pyridine in water makes the spectra weaker than that in water, while it completely changes as type B in ≥ 20 vol% pyridine solution.

Table 3. Absorption maxima (nm) and OD_{max} in ethanol-water mixed solvents of isoalloxazines at 28±1 °C

Ethanol (vol %)	λ_{max} (OD_{max}) of **1**		λ_{max} (OD_{max}) of **2**		λ_{max} (OD_{max}) of **3**	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**
0	434 (0.509)	339 (0.479)	430 (0.439)	337 (0.348)	424 (0.449) 446 (0.513) 475 (0.391)	340 (0.417)
20	434 (0.501)	338 (0.400)	431 (0.421)	335 (0.315)	424 (0.429) 444 (0.518) 479 (0.381)	341 (0.411)
40	435 (0.470)	337 (0.379)	431 (0.410)	334 (0.311)	448 (0.520) 475 (0.371)	339 (0.400)
60	436 (0.468)	334 (0.360)	434 (0.401)	334 (0.301)	438 (0.479)	337 (0.359)
80	437 (0.412)	333 (0.358)	438 (0.399)	330 (0.298)	437 (0.473)	335 (0.319)
100	437 (0.412)	331 (0.350)	439 (0.395)	329 (0.270)	435 (0.481)	332 (0.310)

[isoalloxazine] = 5.00 ×10^{-5} M

These results again suggest the involvement of some aggregation behaviour apart from the solvent effect for splitting. The typical UV-visible spectra of isoalloxazines play important role in the identification of flavin cofactors present in various redox enzymes. The detail study about the aggregation behaviour is in progress.

Table 4. Absorption maxima (nm) and OD_{max} in pyridine-water mixed solvents of isoalloxazines at 28±1 °C

Pyridine (vol %)	λ_{max} (OD_{max}) of **1**		λ_{max} (OD_{max}) of **2**		λ_{max} (OD_{max}) of **3**	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**
5	439 (0.542)	342 (0.388)	429 (0.440)	340 (0.301)	448 (0.768) 481 (0.669)	353 (0.665)
10	440 (0.605)	339 (0.398)	432 (0.449)	336 (0.311)	445 (0.753) 476 (0.660)	350 (0.555)
15	441 (0.448)	339 (0.401)	433 (0.452)	332 (0.319)	447 (0.710) 479 (0.645)	349 (0.505)
20	441 (0.599)	338 (0.501)	435 (0.455)	330 (0.330)	441 (0.659)	348 (0.510)
40	441 (0.612)	333 (0.498)	436 (0.461)	329 (0.333)	442 (0.652)	341 (0.465)
60	442 (0.589)	326 (0.521)	436 (0.462)	326 (0.341)	441 (0.654)	338 (0.311)
80	442 (0.600)	325 (0.511)	439 (0.471)	324 (0.345)	441 (0.642)	330 (0.421)
100	441 (0.601)	325 (0.500)	440 (0.478)	320 (0.361)	440 (0.652)	328 (0.413)

[isoalloxazine] = 5.00 ×10^{-5} M

CONCLUSION

The UV-visible spectral studies have demonstrated that the fine structure is observed for the S1 peak of the 10-hexadecyl-3-methylisoalloxazine (**3**) in aqueous solution. The long alkyl chain present at position 3 of the isoalloxazine does not have significant role to play for the splitting of S1 peak in aqueous solution. These results suggest that the fine structure observed in the aqueous system cannot be explained in terms of the simple solvent effect, but also some type of aggregation is taking place. These findings have significant implications in flavoenzyme chemistry because the surroundings of the binding sites of flavoenzymes are being discussed in terms of the change in the absorption spectra of bound flavin coenzymes.

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