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Fluorescence Spectral Studies on Indole-3-Butyric Acid (I-3-BA) in Micellar Media

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ABSTRACT

Indole-3-Butyric acid (I-3-BA) is a plant hormone in the auxin family and is an ingredient in many commercial horticulture plant rooting product. It is a growth regulating hormone and is biologically and analytically an important molecule. Micellar solubilization of I-3-BA in nonionic and ionic surfactants heteromicroenvironment is monitored by fluorescence and absorption spectral techniques has been reported by the authors. The influence of surfactant, concentration and working experimental conditions on the fluorescence spectra of I-3-BA is thoroughly evaluated and discussed. The increase in fluorescence intensity in micellar media can be attributed to the increase in quantum efficiency suggests that the suspended hydrophobic I-3-BA molecules have been solubilized. The solubilizing action has been supplemented and confirmed by few theoretically calculated spectral parameters like, empirical fluorescence coefficient (k_f), quantum yield (ϕ_f), molar extinction coefficient (ε) and Stokes' shift values.

Keywords: Surfactants, I-3-BA, Fluorescence, Solubilization.

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INTRODUCTION

Molecular fluorescence spectroscopy is an important tool for studying the micellar systems of aromatic or highly unsaturated organic molecules present at trace concentrations, especially in biological and environmental samples and surfactants. The high sensitivity and selectivity of the fluorimetric method as compared to other available procedures makes it a better technique of analysis in many fields relevant to physical, chemical, biological and medical sciences.

Micelles are dynamic microheterogenous structure containing surfactant molecules and constitute an important research subject [1-6]. They have been the subject of numerous investigations because of their importance as model system for mimicking biomembranes [7-8]. I-3-BA is a plant hormone and a very important biomolecule. F. Garcia Sanchez et al.[9] developed a micellar liquid chromatographic method with fluorimetric detection for the determination of the plant growth regulator I-3-AA. A novel method for determination of I-3-AA and I-3-BA in an extract from mung bean sprouts using HPLC with chemiluminescence (CL) detection is described by Zhijun Xi et al. [10]. F. Garcia Sanchez et al.[11] have determined I-3-BA in plant culture medium by fluorescence derivatization. JL Vilchez et al.[12] developed a method for simultaneous determination of 4-(indole-3-butyric acid (IBA) and α-Naphthalene acetic acid (NAA) in mixtures by first- derivative spectroscopy and applied to commercial formulations. Room temperature- phosphorescence of IBA in micellar system of non-ionic surfactant Tween-40 was studied by Long wen-quig et al.[13]. Blagoja Andonovski et al.[14] have made the UV study of the protonation of indole and some 3-caboxylalkyl indoles in H_2SO_4 media.

We report here, the investigation carried out on I-3-BA a plant growth hormone and discuss the importance of surfactant micelle to solubilize I-3-BA molecules employing fluorescence and absorption spectral techniques. Some spectral parameters have also been calculated theoretically and interpreted which help in understanding the importance of surfactants in transportation of this growth hormone I-3-BA to various parts of the plant by the sap.

MATERIALS AND METHODS

Analytically pure I-3-BA was a Merck sample. The following surfactants were employed : (A) Nonionic (i) TX-100 : Polyoxyethylene tert-octyl phenyl ether (ii) Tween-80 : Polyoxyethylene sorbitain monooleate (iii) Tween-20 : Polyoxyethylene sorbitain monolaurate (B) Anionic (i) SLS : Sodium lauryl sulphate (ii) DBSS : Dodecylbenzyl sodium sulphonate (iii) DSSS : Dioctyl sodium sulphosuccinate (C) Cationic (i) CPC : Cetylpyridinium chloride (ii) CTAB : Cetyltrimethyl ammonium bromide (iii) MTAB : Myristyltrimethyl ammonium bromide. All the surfactants were either of Sigma (USA) or BDH (UK) products. The stock solution of I-3-BA was prepared in distilled methanol. All the experiments were performed around 23-25°C in aqueous medium containing 1% (v/v) methanol keeping the final concentration of I-3-BA at 3×10^{-5} M for

fluorescence studies. For absorption studies the concentration of I-3-BA was kept at 1 x 10^{-4} M throughout the experiments.

All the fluorimetric experiments were carried out with Perkin Elmer Fluorescence Spectrophotometer (Model No. 204 A) with a synchronized strip chart recorder (Model no. 056). A Xenon lamp was used as a light source. For recording the fluorescence excitation and emission spectra, its slit width was kept at 10 nm and a cell of 1 cm path length was used. The absorption measurements were made with Hewlet Packard (HP) 8452, and diode array spectrophotometer respectively.

The purity of the surfactants was checked by determining their CMC values the help of surface tension measurements, employing drop-weight method. The values obtained coincided with the recorded values. The absolute fluorescence quantum yield (ϕ_f) of I-3-BA was calculated relative to anthracene solution as standard. Fluorescence emission of anthracene is in the same range as that of I-3-BA. Approximate corrections were made to compensate for different absorption of the compound and the standard. Each time the total intensity of fluorescence emission was measured for the standard and the sample from the area of the fluorescence spectrum recorded over the whole range of emission under identical conditions. Molar extinction coefficient data have been reported as its logarithm ($\log \varepsilon$). The Stokes' shift data have also been calculated and are expressed in nanometers.

RESULTS AND DISCUSSION

The metholic solution of I-3-BA showed maximum excitation peak at 285 nm and the maximum emission peak at 365 nm. On addition of TX-100 and Tween-80 the fluorescence intensity decreased significantly as a consequence of fluorescence quenching without any appreciable change in the shape of the emission band. Tween-80 at its higher concentrations caused a blue shift of 15 nm. Tween-20 caused a gradual enhancement in the fluorescence intensity on increasing its concentration. Anionic surfactants caused an enhancement in the fluorescence intensity. A blue shift of 5 nm occurred with DBSS. The cationic surfactants CTAB and MTAB on addition to I-3-BA solution caused an enhancement in fluorescence intensity with a blue shift of 10 nm in the peak position, except with CPC which caused a significant decrease in emission intensity in presence and absence of surfactants are exhibit in Table 1. The fluorescence spectral changes on addition of SLS are given in Fig. 1. The absorbance of I-3-BA was found to be maximum at 265 nm. The effect of all the three classes of surfactants on absorption spectra showed a similar trend of enhancement in peak height. There occurred a blue shift of 5 nm in TX-100 and Tween-80 whereas that of 20 nm in DBSS and CPC micellar media.

The fluorescence quantum yield values and empirical fluorescence coefficient values obtained showed parallel trends to emission intensity of I-3-BA. Molar extinction coefficient values for all the nonionic and ionic surfactants showed an increasing trend. The stokes' shift at

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room temperature was from 5164 cm^{-1} to 7690 cm^{-1} on dilution of I-3-BA solution. All the theoretically calculated spectral parameters are illustrated in Table 2 and Table 3 respectively.

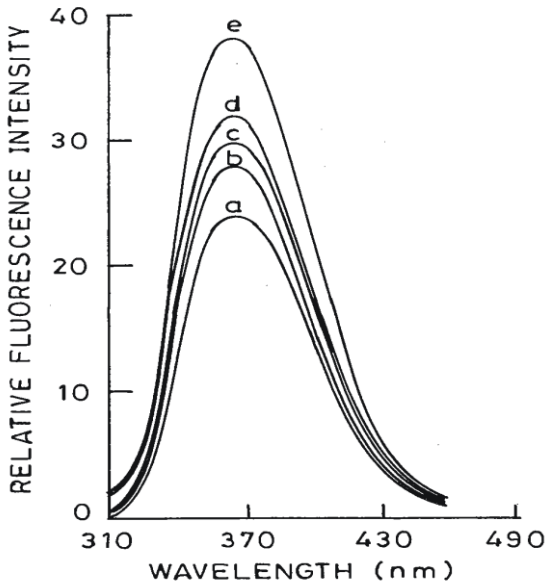

Fig. 1. Influence of addition of SLS on fluorescence intensity of 3 x 10^{-5} M I-3-BA solution
(a) No Surfactant; (b) 0.03 mM SLS; (c) 1.0 mM SLS; (d) 7.0 mM SLS; (e) 9.0 mM SLS

Table – 1: Fluorescence intensity of I-3-BA in absence and presence of surfactant
λ_{ex} = 285 nm λ_{em} = 365 nm P.M. Gain = 2 Sensitivity Range = 0.3

Name of surfactant	Relative Fluorescence intensity in absence of surfactant	cmc's of surfactant (mM)	Max. Concentration of surfactant used (mM)	Relative fluorescence intensity	λ_{em} (nm)
TX-100	24	0.26	0.5	21	360
Tween-80	24	0.1	7.0	15	350
Tween-20	24	0.05	7.0	32	365
CPC	24	0.6	1.0	0	365
CTAB	24	0.90	8.0	35	355
MTAB	24	3.6	9.0	36	355
SLS	24	8.2	9.0	38	365
DSSS	24	0.91	7.0	36	365
DBSS	24	0.81	9.0	36	360

The results obtained can be explained on the basis of solubilization by microheterogenous environment of micelles present in the surfactant solution at or marginally above CMC. Enhancement in the fluorescence intensity of the compound on adding surfactant can be attributed to the increase in the quantum efficiency of fluorescence. Furthermore the quantum yield of fluorescence was higher in anionic and cationic surfactants was higher in non-polar medium, because of the lesser effect of other deactivation processes which compete with fluorescence [15].

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Table 2:Absorption maxima λ_a , fluorescence maxima λ_{em} , molar extinction coefficient $\log \varepsilon$ and quantum yield (ϕ_f) of I-3-BA at different concentration of SLS

S.No.	Concentration of SLS used (Mm)	Absorption maxima λ_a (nm)	Molar extinction coefficient ($\log \varepsilon$) ($dm^3\ mol^{-1}\ cm^{-1}$)	Fluorescence maxima λ_{em} (nm)	Quantum yield ϕ_f
1.	0.00	265	4.2312	365	0.22730
2.	0.3	265	4.2512	365	0.22795
3.	1.0	265	4.2688	365	0.23980
4.	7.0	265	4.2764	365	0.24582
5.	9.0	265	4.3089	365	0.25663

Table -3: Stokes' shift data of I-3-BA at room Temperature P.M. Gain = 2; Sensitivity range = 0.3

S.No.	Concentration of I-3-BA (M)	F.I.	λ_{ex} (nm)	F.I.	λ_{em} (nm)	Stokes' Shift (cm^{-1})
1.	1×10^{-3}	17	300	29	355	5164
2.	7×10^{-4}	11	295	36	360	6120
3.	5×10^{-4}	12	295	37	360	6120
4.	3×10^{-5}	30	295	93	365	6501
5.	1×10^{-5}	22	290	59	365	7085
6.	7×10^{-5}	17	285	48	365	7690
7.	5×10^{-5}	14	285	37	365	7690
8.	3×10^{-5}	9	285	24	365	7690
9.	1×10^{-5}	4	285	9	365	7690

On adding the surfactants to the aqueous solution of the compound, the surfactant micelles get adsorbed at the interfaces and remove the hydrophobic groups from contact with water, thereby reducing the free energy of the system. But in transferring the hydrophobic groups from solution, to the micelle in the solvent, may experience some loss of freedom confined to the micelle and, in the case of ionic surfactants, from electrostatic repulsion from other similarly charged surfactant molecules in the micelle. These forces increase the free energy of the system and thus oppose micellization. Whether micellization occurs in a particular case, if so, at what concentration of monomeric surfactant, therefore depends on the balance between the factors promoting micellization and opposing it. Thus the increase in quantum yield suggests that the surfactants Tween-20, DBSS, DSSS, MTAB and CTAB have solubilized the suspended solubilizate molecules (I-3-BA).

TX-100 and Tween-80 have quenched fluorescence intensity due to formation of intramolecular hydrogen bond in them. So effective hydrogen bonding does not take place between solubilizate and surfactant micelles. The quenching also indicates that the compound prefers the hydrophobic core to the hydrophilic poly(ethylene oxide), PEO shell, particularly for TX-100. Evidently the fluorescence of compound is significantly weakened in the core like in nonaqueous solvents. This implies that the compound is embedded in the core is not hydrated around the aromatic rings[16]. Quenching can also be caused by non-radiation loss of energy

from the excited molecules. Fluorescence quenching was also observed by the addition of CPC, which may be attributed to the electrostatic preferential interaction between the polar substituent of I-3-BA molecules where it loses the coplanarity. The quenching may also be due to interaction between the π-electron system of the excited state fluorophore and quencher molecule (CPC) due to the presence of nucleophilic pyridine ring in the structure which make it act as a quencher via hydrogen bond between the proton donor and acceptor. This will result in delocalization of the π-electrons of the excited state and hence loss of fluorescence [17].

Sufficient large value of $\log \varepsilon$ are assigned to the $\pi \rightarrow \pi^*$ transitions. The large magnitude of Stokes' shift of I-3-BA is due to hydrogen-bond formation, between solute and solute in ground state. This bond breaks following excitation to S_1 but reform following proton transfer [18]. The hydrogen bonded excited state can be produced via two routes as shown by following scheme in which S represents the solvent molecule and A represents the fluorophore.

$$A + S \xrightarrow{h\nu} AS \rightarrow [AS]^*$$

$$A^* + S \rightarrow [AS]^*$$

The blue shift may be attributed to the protic nature of solvent as here hydrogen-donor-solvent interaction takes place between the solubilizate and solvent [19]. This shift may also be considered to be because of the difference in solvation energy of the solute in the ground state and excited state in different microheterogeneous micellar media.

Absorption is less sensitive to its environment as compared to fluorescence, thus absorption spectra are less affected on addition of surfactants. On addition of all the three kinds of surfactants a gradual enhancement in the absorbance occurred. The values of empirical fluorescence coefficient k_f obtained may be attributed to the increased sensitivity of the fluorescence analysis of the solubilization of organic molecules by surfactants which offer a protective microenvironment, leading to enhanced fluorescence of the solubilizate by shielding the excited state from non-radiative decay that normally occurs in bulk aqueous solution.

Aside from the presentation of the spectral and photophysical date, present kind of study finds application in biochemical and agrochemical analysis. The process of solubilization enhances the rate of transportation of this growth hormone I-3-BA to the various parts of the plant by the sap which otherwise would have been slow.

CONCLUSION

The present analysis indicates that during solubilization of solubilizate I-3-BA into the surfactant system, the incorporation of the solubilizate influence the balance of favourable and unfavourable forces guiding micellization and structural changes occurring due to aggregation, dissociation and hydrogen bonding. Thus we can generalize the present understanding to different kinds of solubilizates.

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