INTERACTION OF FLUORESCENT PROBES WITH MOLECULES
CAPABLE OF HYDROGEN BONDING AND MICELLES

PhD Thesis Abstract

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Debrecen, 2007
**Introduction**

Fluorescent compounds are valuable probes in the study of both structural and functional aspects of biological systems.

As the spectrum, lifetime and anisotropy of fluorescence strongly depend upon intermolecular interactions, intrinsic fluorescence or fluorescent labeling provides information on the local surroundings of the fluorophore, conformational changes, binding of ligands or other association processes.

In order to develop the most suitable dye for different purpose, it is important to reveal how the molecular structure and microenvironment influence the fluorescence properties and the deactivation rate constants of the excited probe.

The rapidly increasing number of publication in this field proves the importance of fluorescence techniques.

**Literature overview and research objectives**

*Ionic liquid*

The air and moisture stable room temperature ionic liquids have currently received considerable attention as novel media for chemical synthesis, homogeneous catalysis and separation techniques. Their unique properties offer a great potential for industrial application as nonvolatile, nonflammable, environmentally benign alternatives to conventional organic solvents. In spite of the many publications dealing
with ionic liquids, there was no systematic study in the literature on the aggregation of ionic liquids in water. It was also unknown whether an ionic liquid could modify the characteristics of the micelles of conventional surfactants.

2-Hydroxy Nile Red (HONR)

A previous study has demonstrated that the photophysical properties of HONR fluorescent probe are very sensitive to the polarity of the microenvironment. This dye seemed to be an excellent probe for the study of ionic liquid micelles because of its low solubility and fluorescence quantum yield in water.

Because of the remarkable solvent effect on the photophysical properties of HONR, we studied whether this dye can be utilized for the characterization of the local polarity in micelles.

The phenolic OH group of the dye can provide a binding site for hydrogen-bond acceptors such as nitrogen heterocyclic compounds and amines. Before the utilization of HONR as fluorescent probe in biological systems, it is essential to know how the interaction with a
In this dissertation we reveal the effect of the basicity of the additives and solvent polarity on the kinetics and mechanism of the excited HONR quenching.

Ellipticine and 6-methyl-ellipticine

Ellipticine and its derivatives exhibit high cytotoxic activity against various tumor cells. Several ellipticine derivatives have reached the phase II of clinical trials. Despite the importance of ellipticine in the biomedical field no systematic studied have been performed to unravel the effect of hydrogen-bond acceptors on their photophysical properties. Hence, we studied the fluorescence properties of ellipticine in the presence of hydrogen-bond acceptors such as organic nitrogen compounds, fluoride and acetate anions.

Olivacine, a compound differing from ellipticine only in the position of a methyl group emits dual fluorescence in methanol. This phenomenon was explained in the literature by a long distance, solvent-
assisted excited-state proton transfer from the pyrrole to the pyridine moiety.

In this dissertation, we focus on the solvent effect on the photophysical properties of E and ME and unveil the origin of dual fluorescence and the kinetics of light initiated processes in methanol.

**Lumichrome**

The major product of riboflavin photodecomposition and biodegradation is lumichrome. The tautomerization of this probe was described in literature only in excited state in concentrated acetic acid and pyridine.

![Lumichrome (Lc)](image)

The main goal of this part of the dissertation has been to find a compound, which could cause the tautomerization of lumichrome even in the ground state.

**Experimental methods**

The UV-visible absorption spectra were recorded with a Unicam UV 500 spectrophotometer. Corrected fluorescence spectra were obtained on a Jobin-Yvon Fluoromax-P spectrofluorometer.
Fluorescence lifetimes were measured with a time-correlated single-photon counting technique. The light of a Picoquant diode laser (wavelength 400 nm or 632 nm), or a LED (330 nm) excited the samples and the emission was collected at 90° angle through an Oriel 0.25 m monochromator located in front of a Hamamatsu R3809U-51 microchannel plate photomultiplier. The output signal was connected to a Picoquant Timeharp 100 computer board module (channel width 36 ps). The fluorescence decay traces were collected until 10⁴ counts in the peak channel were reached. Data were analyzed by a nonlinear least-squares reconvolution method using Picoquant FluoFit software.

Electric conductivity was measured with a Consort C832 apparatus, which was calibrated with KCl solution.

**Conclusions**

**Ionic liquid**

a) We have shown that 1-butyl-3-methylimidazolium octyl sulfate ionic liquid forms micelle above 0.031M concentration in aqueous solution.

b) Markedly different behavior has been found when the cation of the ionic liquid contained n-octyl moiety. 1-methyl-3-octylimidazolium chloride produced inhomogeneous solution above 5mM concentration.

c) Our results demonstrate that 1-methyl-3-octylimidazolium chloride begins to be incorporated into sodium dodecyl sulfate
micelles at fairly low concentration. Thus, ionic liquids could be used to modify the properties of the conventional micelles.

d) HONR dye proved to be a sensitive probe for the study of the ionic liquid aggregation and the subtle changes of the interfacial layer in aqueous solution.

2-Hydroxy Nile Red (HONR) interaction with organic nitrogen compounds

a) The fluorescent properties of HONR proved to be highly sensitive to the basicity of hydrogen-bond acceptors and to solvent polarity.

b) We revealed the detailed mechanism and the kinetics of the fluorescence quenching by organic nitrogen-compounds with the combined analysis of the steady-state and time-resolved spectroscopic data.

c) The shortening of the fluorescence lifetime of the excited hydrogen-bonded complexes with increasing solvent polarity is due partly to the acceleration of the internal conversion and ion pair formation.

d) The dissociation of the excited hydrogen-bonded complex into excited HONR and ground-state base was able to compete with the energy dissipation only when 2,4,6-trimethylpyridine was used as hydrogen-bond acceptor in toluene.

e) The bimolecular quenching of the excited hydrogen-bonded complex played a significant role in apolar solvents.
f) Photoinduced proton transfer is an important reaction in polar media such as acetonitrile and dichloromethane.

**Ellipticine and 6-methyl-ellipticine**

a) The similar photophysical characteristics of E and ME in all solvents prove that long distance, solvent assisted excited-state proton transfer does not occur.

b) Our results evidenced that the long-wavelength fluorescence in methanol is due to photoinduced protonation by the solvent.

c) The interaction of ellipticine with fluoride proved to be very sensitive to the polarity of the microenvironment. In contrast with the behaviour in toluene and dichloromethane where ellipticine forms only 1:1 H-bonded complex with $F^-$, interaction of ellipticine with two fluoride ions caused deprotonation in acetonitrile even in ground state.

d) We showed that the H-bonding with two fluoride anions has much higher deprotonation power than one of the strongest organic base DBU (1,8-diazabicyclo[5.4.0]undec-7-ene).

**Lumichrome**

a) I established that lumichrome was able to tautomerize in the presence of a trace amount of fluoride or acetate ions even in the ground state in acetonitrile.
b) Addition of water significantly influenced the reactivity of fluoride ion with lumichrome. The more water was in the solution, the more fluoride ion was needed to bring about tautomerization.

c) Because fluoride and acetate ions have a large effect on both the absorption and fluorescence spectra of ellipticine and lumichrome in non-hydroxylic solvents, both of these heterocyclic compounds can be utilized as highly sensitive fluorescent probes.
Publications related to this dissertation:

1. Zsombor Miskolczy, Krisztina Sebők-Nagy, László Biczók, Sinem Göktürk
   „Aggregation and micelle formation of ionic liquids in aqueous solution”

2. Krisztina Sebők-Nagy, Zsombor Miskolczy, László Biczók
   „Interaction of 2-Hydroxy Substituted Nile Red Fluorescent Probe with Organic Nitrogen Compounds”
   Photochemistry and Photobiology 81, 1212 (2005)

3. Zsombor Miskolczy, László Biczók
   „Anion-induced changes in the absorption and fluorescence properties of lumichrome: A new off-the-shelf fluorescent probe”

4. Zsombor Miskolczy, László Biczók
   „Fluorescent Properties of Hydrogen-bonded Ellipticine: A Special Effect of Fluorid Anion”

5. Zsombor Miskolczy, László Biczók, István Jablonkai

**Other publications:**

6. Csaba Gábor Ágoston, Zsombor Miskolczy, Zoltán Nagy and Imre Sóvágó;
   „The effect of ring size of fused chelates on the stability constants and spectroscopic properties of nickel (II) and palladium (II) complexes of peptides”

7. Zsombor Miskolczy, József Nyitrai, László Biczók, Krisztina Sebők-Nagy, Tamás Körtvelyesi
   „Photophysical Properties of Novel Cationic Naphthalimides”

8. Rozália Vanyúr, László Biczók, Zsombor Miskolczy
   „Micelle formation of 1-alkyl-3-methylimidazolium bromide ionic liquids in aqueous solution”

9. Zsombor Miskolczy, László Biczók, István Jablonkai
“Dual fluorescence of 1-hydroxy-substituted Nile Red dye: Excited-state proton transfer along intramolecular hydrogen bond”

Lectures and posters related to this dissertation:


5. Miskolczy Zsombor, Biczók László
„Biológiai fontosságú vegyületek alkalmazása fluoreszcenciás jelzőanyagként”
Munkabizottsági Ülés, Gyöngyöstarján, 2005. október 20-21

6. Zsombor Miskolczy, László Biczók, Krisztina Sebők-Nagy (poszter)
„Interaction of 2-Hydroxy Substituted Nile Red Fluorescent Probe with Organic Nitrogen Compounds”

7. Biczók László, Miskolczy Zsombor, Megyesi Mónika (poszter)
„Compounds of biological importance as fluorescent probes“

Other lectures and posters:

8. Zsombor Miskolczy, Sándor Darabont (poszter)
„A La_{2/3}Ca_{1/3}Mn_{1-x}In(Al)_xO_{3-d} állítása és jellemzése szerkezeti és mágneses szempontából“
VIII. Nemzetközi Vegyészkonferencia, Kolozsvár, Románia, 2002.nov.15-17

9. Miskolczy Zsombor, Ágoston Csaba, Nagy Zoltán, Sóvágó Imre:
„Csatolt kelátyúrűk hatása a peptidek Cu(II), Ni(II) és Pd(II) komplexeinek termodinamikai stabilitására és spektrális paramétereire“
10. Zoltán Nagy, Anikó Magyari, Zsombor Miskolczy and Imre Sóvágó (poszter)
„Ternary complexes of palladium(II) with thioether and imidazole ligands”

11. Zoltán Nagy, Anikó Magyari, Zsombor Miskolczy and Imre Sóvágó (poszter)
„Complexation of monofunctional palladium(II) species with thioether and imidazole ligands”
IX. International Symposium on Inorganic Biochemistry, Szklarska Poreba, Poland, 2003.szept.4-7