SPIROPYRAN-ZINC INTERACTION CHARACTERIZED BY FLUORESCENCE SPECTROMETRY AND CAPILLARY ELECTROPHORESIS WITH LASER-INDUCED FLUORESCENCE DETECTION

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Abstract: Zinc is an important biogenic element which is able to connect with indicators such as a spiropyran. The spiropyran is known for its fluorescent character and selectivity towards zinc ions (and some other metal ions, e.g. cadmium). This complex, especially with zinc, gives a high fluorescence intensity which was analyzed by fluorescence spectra and by capillary electrophoresis with laser induced fluorescence detection. In addition, the influence of visible light irradiation on zinc release was tested. The spiropyran specificity to zinc was also investigated by coupling with copper and cadmium.

Key Words: the spiropyran, zinc, fluorescence intensity, vis irradiation

INTRODUCTION

Zinc is a very important biogenic element which plays many roles in human body and other living organisms. In terms of the human body, it is a cofactor of lots of enzymes and an important component of insulin molecule and insulin metabolism. Further, zinc ions participate in metabolism of saccharides, proteins, and phosphorus (Maret 2013). It is predominantly an intracellular element. It is possible to detect and determine zinc using many methods, e.g. UV spectroscopy, atomic force microscopy (Taranath et al. 2015), methods using extractions, atomic absorption spectrometry or spectrophotometry (Smith et al. 1979). Zinc ions can be also detected by fluorescence probes such as FluoZin (Kimura andKoike 1998). Here, a sensoris meant a molecule which binds other molecules, elements, hormones, and other substances and is able to transmit them under certain conditions (Pijanowska et al. 2003).

One of the newly synthesized receptors is a spiropyran. The spiropyran is a receptor which is fluorescent and photoregenerable. It is selective towards zinc(II) ions. Creation of a complex is connected with a structure isomerization in a response to electromagnetic irradiation. After creation of metastable merocyanine, the phenolic oxygen with a negative charge causes the ability to bind metal ions (e.g. zinc) (Natali et al. 2010).

Due to the fluorescent character of the spiropyran, it is possible to use fluorescence spectrometry to measure fluorescence intensity. The advantage of fluorescence intensity detector is that it has much larger range of application than absorbance scan and differences between single measurements are more perceptible and it is much easier to evaluate them (Strickler andBerg 1962). The next step to complete information of fluorescence intensity is using capillary electrophoresis with laser induced fluorescence detection (CE-LIF). This method is universal, highly effective and very sensitive and accurate (Huang et al. 2006).

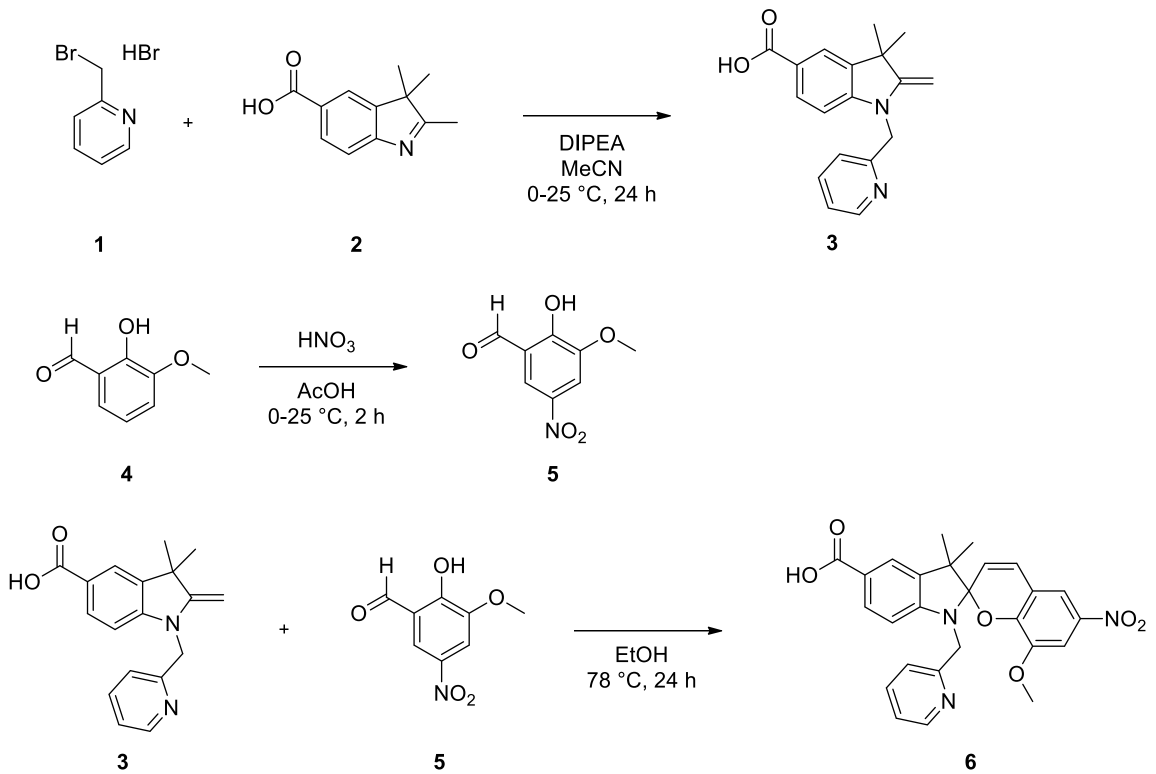
In this study the photosensitive spiropyran was synthesized. Effect of zinc complexation (and other metals) and effect of visible light (vis) irradiationwere characterized by fluorescence spectra. The fluorescence signal was analyzed also by CE-LIF.

MATERIAL AND METHODS

The spiropyran synthesis

All chemicals of ACS purity were obtained from Sigma-Aldrich (St. Louis, USA) unless otherwise stated. The spiropyran was prepared from commercially available starting material in three steps (Figure 1). First, 2,3,3-trimethyl-3H-indole-5-carboxylic acid **(1)** was alkylated with 2-bromomethyl pyridine **(2)** in presence of *N*,*N*-diisopropylethylamine (DIPEA) leading to compound **3** (Natali et al. 2010). In parallel, *ortho*-vanilin**(4)** was nitrated with nitric acid in acetic acid providing 3-methoxy-5-nitrosalicylaldehyde **(5)** (Darwish et al. 2014).Then a condensation reaction of compounds **4** and **5** in ethanol led to desired the spiropyran **6** (Natali et al. 2010).

Figure 1 The scheme of the spiropyran synthesis



Fluorescence spectrometry analysis

Dimethyl sulfoxide (DMSO) was chosen as an appropriate solvent for fluorescence spectrometry analysis. Before analysis DMSO was deprived of zinc ions by using Chelex 100 Resin (Bio-Rad, USA). The amount of Chelex in DMSO constituted of about 10% of the total volume. The whole solution was then once more treated by Chelex. Then spiropyran (1 mg/ml) in DMSO was mixed with equal moles of $ZnCl_2$, $CdSO_4$ or $Cu(NO_3)_2$. The fluorescence spectra were measured on fluorimeter Infinite M200 PRO microplate reader (Tecan, Austria). The data were compiled by fluorimeter software i-control 1.9 (Tecan). All samples were monitored using excitation wavelength λ_{ex} 490 nm.

Visible light irradiation

White LED (6000 K, Roithner Lasertechnik, Austria) was used to illumine the samples before measurement. Different times of irradiation were tested (1 and 5 min).

Capillary electrophoresis with laser-induced fluorescence detection (CE-LIF)

Samples of spiropyran with and without zinc were analyzed by CE-LIF. The capillary electrophoresis separation was implemented on 7100 CE System (Agilent, Germany) with a fluorescence detector (ZetaLIF, Picometrics, France) and a solid-state laser (λ_{ex} = 488 nm) as an excitation source. The uncoated fused silica capillary (Polymicro Technologies, USA) with inner diameter 75 µm, the total length 64 cm, and effective length 43cm was used. All data evaluation was performed with Agilent ChemStation software. The 20 mM borate buffer (pH 9.2) was used as a background electrolyte. The hydrodynamic injection by 30 mbar for 5 s and the separation voltage of 25 kV was employed.

RESULTS AND DISCUSSION

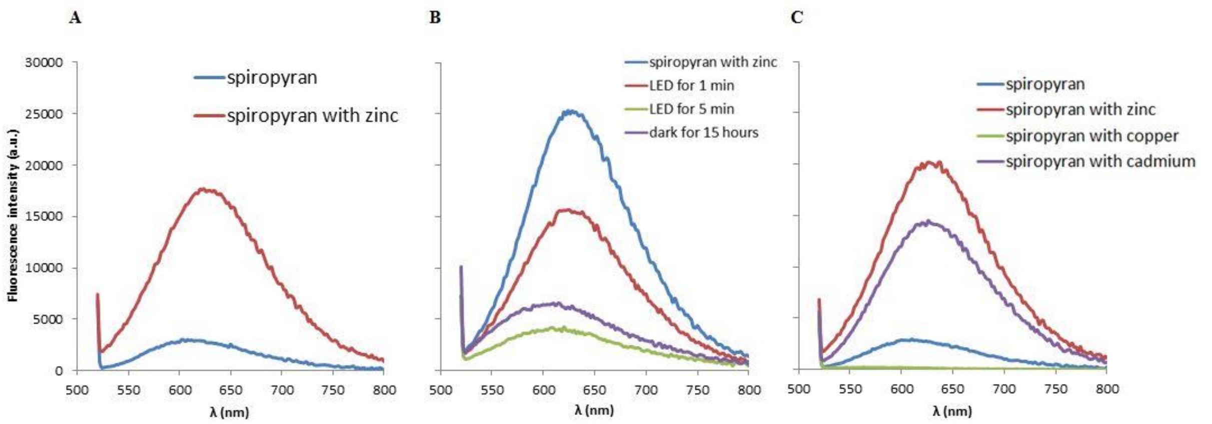
The spiropyran interaction with zinc ions

First of all, the fluorescence intensity of spiropyran itself with fluorescence intensity of the spiropyran complexed with zinc was compared. The fluorescence intensity maximum for spiropyran was at 605 nm and for the complex at 625 nm. As it is obvious (Figure 2A), the spiropyran complexed with zinc has much higher fluorescence intensity than the spiropyran alone. In the presence of zinc ions, the fluorescence intensity increased six times. Compared to commonly used zinc-sensitive fluorescent probes (e.g. FluoZin-3(de Silva et al. 1997)), the here presented probe belongs to the group of stimuli-responsive materials and therefore after irradiation by visible light, the captured zinc ions are released.

Visible light irradiation

Subsequently the influence of vis light irradiation on zinc release and re-complexation was investigated (Figure 2B). Samples of complexes were illuminated by white LED for 1 and 5 min causing decrease of fluorescence intensity by 40 and 80 %, respectively. This observation corresponds to zinc ions release from the complex. Then the sample was placed in lightproof box in order to survey re-complexation. As was shown in Fig 2B, not even after 15 hours the fluorescence intensity did not reach the original value. This fact indicates very slow or partial re-complexation of the zinc ion to the spiropyran.

Figure 2 Development of fluorescence intensity depending on the conditions



Legend: A – difference between fluorescence intensity of the spiropyran (blue) and the spiropyran with zinc (red), B – fluorescence intensities depending on enlightenment changes, C – differences between fluorescence intensity of the spiropyran with other metals

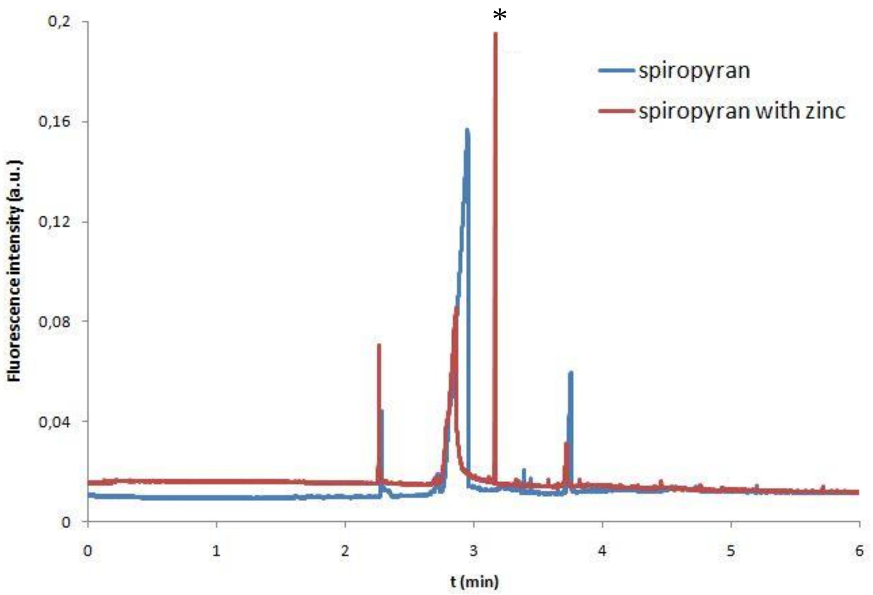
Specificity study

Finally, the spiropyran interaction with other metals was investigated (Figure 2C). In case of copper, no fluorescence signal was observed, because copper does not react with the spiropyran at all. The situation changes in case of cadmium which forms a complex with fluorescence intensity higher than the spiropyran alone however intensity reaches about 70% of fluorescence intensity in comparison with the spiropyran–zinc complex. These results are in agreement with the work by Natali et al. where the interaction with a variety of ions (mono-, di-, and tri-valent) was tested; however the exact explanation of the selectivity was not wholly explained yet.

CE-LIF analysis

Preliminary results obtained by CE-LIF shoed (Figure 3) that the solution of spiropyran alone led to formation of peaks in migration times of 2.3 min, 2.7 min and 3.7 min. In case of the spiropyran-zinc complex, another peak with migration time of 3.1 min appeared (*).

Figure 3 The typical electropherogram of the spiropyran and the spiropyran with zinc



CONCLUSION

Aim of this study was to investigate the influence of presence of zinc ions and influence of illumination on the spiropyran fluorescence intensity.

As found, the spiropyran formed a highly fluorescent complex with zinc ions. This complex dissociated by LED illumination, but its reassociation in darkness was much slower. The spiropyran was also able to bind cadmium but not to copper. As observed, it is convenient to use the spiropyran as a probe of zinc, because it is easy to determine this complex and utilize this knowledge in other studies. Future work includes not only detail investigation of the probe sensitivity and selectivity

ACKNOWLEDGEMENTS

This research was carried out under the project CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II.

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