Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Biosensors and Bioelectronics 22 (2006) 399–404
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Amphiphilic porphyrin film on glass as a simple and selective solid-state chemosensor for aqueous Hg^{2+}

Luisa S. Dolci[a], Ettore Marzocchi[a], Marco Montalti[a], Luca Prodi[a,**], Donato Monti[b,*], Corrado Di Natale[c], Arnaldo D'Amico[c], Roberto Paolesse[b,c]

[a] Dipartimento di Chimica “G. Ciamician”, Università di Bologna, Via Selmi 2, 40126 Bologna, Italy
[b] Dipartimeznto di Scienze e Tecnologie Chimiche, Università di Roma “Tor Vergata”, Via della Ricerca Scientifica 1, 00133 Rome, Italy
[c] Dipartimento di Ingegneria Elettronica, Università di Roma “Tor Vergata”, Via della Ricerca Scientifica 1, 00133 Rome, Italy

Received 27 September 2005; received in revised form 4 May 2006; accepted 4 May 2006

Abstract

Deposition of amphiphilic porphyrin derivatives occurs spontaneously on silanised glass surfaces, in a controlled fashion. The resulting porphyrin films show appreciable fluorescence emission. This emission can be effectively quenched by immersion of the slides into a diluted solution of Hg^{2+} (μM concentration). The initial intensity can be restored by washings with a solution of *N*,*N*,*N*′,*N*′-tetrakis(2-pyridilmethyl)ethylenediammine with no loss of efficiency. A remarkable selectivity is featured toward the detection of Hg^{2+} over Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} counterparts. This protocol can be extended to a flow-through apparatus. The presented results are of importance for the achievement of a solid-state chemosensor for mercuric ions, at micromolar concentration, in water.

Keywords: Fluorescence spectroscopy; Mercury; Porphyrinoids; Sensors

1. Introduction

The detection of heavy metal salts in aqueous solution has important implications in human health. Mercury salts, in particular, are pollutants characterised by extreme toxicity (nephrotoxicity, neurotoxicity) altering the biological activity of important constituents of living cells, such as, for example, proteins or nucleic acids. Hg^{2+}, together with Cd^{2+}, Pb^{2+}, Cu^{2+} and other metal cations are catalogued by the U.S. Environmental Protection Agency as “drinking water contaminants”. The lower concentration limit is fixed by the E.U. in the range between 0.5 and 1 mg/kg of fish (Oehlenschlanger, 2002). When mercury from natural and anthropogenic sources is released to the atmosphere, it may enter the global mercury cycle to be ultimately deposited in aquatic and terrestrial ecosystems. Its bioaccumulation, in fish and shellfish, is at the origin of food intoxications (Clarkson, 1994). Mercury(II) ion or the lipophilic “methylmercury” derivative, target the advanced central nervous system by passing through the blood brain barrier, causing severe lesions at nervous centres (Magos, 1997; Mottet, 1977). Most current methods used for mercury detection are, for example, Atomic Absorption Spectroscopy, Induced Coupled Plasma Spectroscopy or other sophisticated means (Nguyen et al., 1998; Guo et al., 1996). These methods may require time consuming and costly apparatus. Other detection methods, such as potentiometric, amperometric (Bonfil et al., 2000; Manivannan et al., 2002; Seehra et al., 2002; Zejli et al., 2004) or, more recently, coupled electrochemical-bioenzymatic means have been also exploited (Han et al., 2001; Krawezyck et al., 2000; Bertocchi et al., 1999).

The construction of efficient and specific luminescent chemosensors for heavy and transition metal ions constitute an active area of researches (Prodi et al., 2000a; Prodi, 2005; Mello and Finney, 2005; Fabbrizzi and Poggi, 1995). The possibility to rely on selective and reversible chemical systems should constitute an important step toward the development of easy to handle and cheap facilities. Selective and sensitive fluoroionophore for Hg^{II}, Ag^{I} and Cu^{II} in acetonitrile, has been developed by

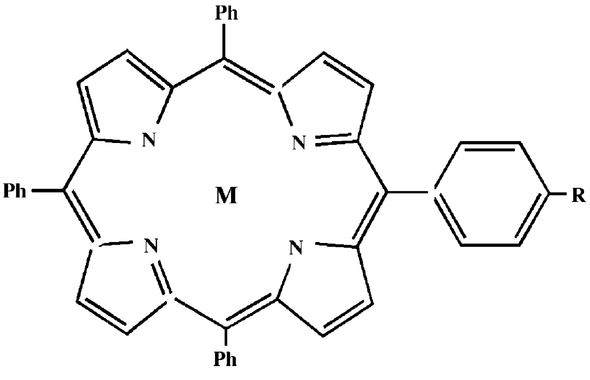
---

[*] Corresponding author. Tel.: +39 06 72594738; fax: +39 06 72594328.
[**] Corresponding author. Tel.: +39 05 12099481; fax: +39 05 12099455.
E-mail addresses: luca.prodi@unibo.it (L. Prodi), monti@stc.uniroma2.it (D. Monti).

0956-5663/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2006.05.013

Rurack et al. (2000). It is based on a thia aza crown-boron dipyrromethene conjugate, whose fluorescence emission can be actively enhanced by complexation to the former thiophilic metal cations. Moreover, in water-acetonitrile media, a minor effect is observed in the presence of Cu^{II}, and the simultaneous detection of Hg^{II} and Ag^{I} is possible by virtue of their clearly distinguishable lifetimes. Fabbrizzi et al. recently reported on an antracenyl-based fluorescent sensor. The remarkable feature of the investigated system resides on the possibility of switching the selectivity toward different ions (e.g. Cu^{II} or Zn^{II}) by simply varying the bulk pH of the aqueous medium (Fabbrizzi et al., 2002). A porphyrin-based supramolecular sensitizer for mercury(II) ion has been recently developed. This is based on a porphyrin/cyclodextrin supramolecular inclusion complex, whose fluorescence emission is strongly quenched by the presence of Hg^{II} at a very low concentration level. Other metal ions such as Ag^{I}, Cu^{II}, Pb^{II} and Cd^{II}, however, affect the emission of the sensitizer, acting as a likely interferents (Yang et al., 2002). The development of a functional, fluorescent, ion-selective chemosensor for Hg^{2+} has been reported by Prodi et al. (2000b). It is based on a hydroxyquinoline fluorophore, linked to a two azacrown macrocycles. The system shows a good selectivity toward mercuric ion in methanol–water solvent mixture, even in the presence of 100-fold excess of alkali metal ions. A “turn on” fluorescent sensor for the selective detection of mercuric ion has been developed (Nolan and Lippard, 2003). It relies on a fluorescein-based moiety coupled to a dithia aza receptor, and it shows enhancement of fluorescence emission, upon binding of thiophilic Hg^{2+}. Of the first row metal ions considered, only Cu^{2+} interferes. Chemodosimeters, i.e. systems in which the ion detection occurs due an irreversible chemical reaction, are widely employed for the detection of Hg^{2+} ions (Liu and Tian, 2005). Important developments have been achieved by Ros-Lis and co-workers, who developed a regenerative chemodosimeter. The chromofluorogenic signaling can be simply restored by addition of a spectroscopic inhibitor (Ros-Lis et al., 2005). Moreover, the probe can be supported onto a solid matrices achieving to a promising tool for rapid screening analysis. The development of a hybrid sensor (reversible, i.e. sensor versus irreversible, i.e. chemodosimeter) based on a fluorescent dye has been recently reported (Mokhir and Krämer, 2005). It features double discrimination by either fluorescence quenching and subsequent reactivity (hydrolysis) upon binding of Cu^{2+}, Ni^{2+}, Fe^{3+} or Eu^{3+}.

Immobilisation of chemosensors on a solid support constitutes an important step for the development of systems for rapid in situ sensing with high efficiency and selectivity. Early attempts were made by Czolk et al. who built a porphyrin-doped sol–gel system able to detect Hg^{2+} even at μg/l level (Plaschke et al., 1995). The system, however, suffered from severe interferences by other heavy metal ions and showed also limited stability in working conditions. Recently, Palomares et al. (2004) published on a heterogeneous colorimetric sensor for mercuric salts, based on a mesoporous nanocrystalline TiO_2 film, sensitised with a ruthenium dye. The system showed remarkable selectivity for Hg^{2+} with a low detection limit. Very recently, Bronson and co-workers reported on the construction of an interesting solid-state chemosensor, selective for Cd^{2+}, by a polymer-aided monolayer formation of a quinoline-linked azacrown receptor (Bronson et al., 2005). The interaction of this architecture results, upon metal ion co-ordination, in an increase of fluorescence by inhibition of photoinduced electron transfer (PET) and photoinduced proton transfer (PPT) deactivation routes. This system, although capable to reach quite low detection limits (below 1 μM), suffers from potential interferences by Zn^{2+} and Cu^{2+}.



1H₂: R = $OCH_2CH_2CH_2NMe_3Cl$; M = 2H

Fig. 1. Porphyrin studied in the work.

As a part of our studies devoted to the construction of chemical sensors based on porphyrin films as sensible material (Paolesse et al., 2002), we recently reported on the deposition of ordered film of amphiphilic porphyrin derivatives, (**$1H_2$**, Fig. 1) from aqueous solutions, on glass surfaces (Monti et al., 2004a).

These porphyrin films present, beside a good mechanical stability, an appreciable fluorescence emission. This property can be a crucial feature in the sensing of heavy or transition metal ions that typically quench the luminescence, often via deactivation to the triplet state by heavy metal atom effect. In this work, we wish to report our results on the use of these simple systems for the selective and efficient detection of Hg^{2+} ions in water.

2. Materials and methods

2.1. General

Porphyrin **$1H_2$** has been prepared by following procedures reported in the literature (Monti et al., 1998; Borocci et al., 2001). UV–vis absorption spectra were taken on a Perkin-Elmer Lambda 16 spectrophotometer. Uncorrected emission and corrected excitation spectra were obtained with a Perkin-Elmer LS 50 Spectrofluorimeter (λ_{exc} 420 nm; λ_{em} 654, 720 nm,). The fluorescence lifetime (uncertainty within ± 5%) were obtained by an Edinburgh single-photon counting apparatus, with a flash lamp filled with D_2. Absorption and emission spectra were acquired by placing the porphyrin layered glass slide (optical density of ca. 0.2 absorbance units) into a quartz cuvette (10 mm path) filled with doubly distilled (Millipore grade) water.