Research Paper

Subphthalocyanines as electron mediators in biosensors based on phenol oxidases: Application to the analysis of red wines

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ABSTRACT

The electron mediator properties of three subphthalocyanines (SubPcs) [hexa-chloro boron subphthalocyanine (ClSubPc), tri-*tert*-butyl boron subphthalocyanine (*t*-BuSubPc) and hexa-phenoxy boron subphthalocyanine (PhOSubPc)] in Tyrosinase and Laccase biosensors (deposited on ITO glass) for the detection of catechol and hydroquinone were evidenced. A particularly remarkable performance was observed in the PhOSubPc-Tyr sensor, which takes account of the π-π interactions between subphthalocyanine rings and the active sites of the enzymes. Mediated electron transfer between redox enzymes and the ITO electrode improved the limits of detection by one order of magnitude, reaching 10^{-7} mol·L^{-1} values. Studies at increasing scan rates confirmed the improvement of the charge transfer rates caused by the presence of the SubPcs. A bioelectronic tongue formed by an array of the SubPc based biosensors has been able to discriminate red wines according to their Total Polyphenol Index.

1. Introduction

Phenol detection plays an important role in the environment and food industry. Conventional methods for phenol detection can be improved by means of electrochemical sensors and sensitive biosensors for the high-throughput screening of phenols in different samples [1]. Enzymatic electrochemical biosensors based on phenol oxidases have shown to be a good alternative for analyzing phenols due to their high sensitivity and selectivity [2–4].

In electrochemical biosensors, an electron mediator can be used to facilitate the transfer of electrons from the enzyme to the electrode [5].

Phthalocyanines, which are tetrapyrrolic compounds, are efficient electrocatalysts for the non-enzymatic detection of phenols through axial ligand substitution [6] or through $\pi-\pi$ interactions [7,8]. They can also be used in combination with other electrocatalytic materials and synergistic effects have been observed [9–12]. In addition, phthalocyanines have been shown to be excellent electron mediators in biosensor functioning facilitating the transfer of electrons from the active site of immobilized phenol oxidases to the working electrode surface [13–15]. The wide family of phthalocyanines can offer a variety of derivatives that deserve to be tested as electrocatalytic materials. Subphthalocyanines (SubPcs) consist of three isoindole units with a boron atom at the center and show a three-dimensional delocalized 14 π-electron system. As a family of Pc-related macrocycles, SubPcs possess high thermal stability and high solubility in common organic solvents. The non-planar cone-shaped structure of these complexes, as well as the presence of axial ligand prevent aggregation in solvents [16,17]. The structure of SubPcs can be modified in some ways. The halogen atom in the axial position can easily be displaced by nucleophiles [18,19]. The variation of peripheral substituents is possible in either the synthesis stage by preparing substituted phthalonitriles [20] or by modifying the subphthalocyanine core [18]. The interesting ring-enlargement reaction is unique for the subphthalocyanine macrocycle [21].

In addition, subphthalocyanine analogues with an extended π-system: (subphthalocyanines [22–24], subazaphenalenephthalocyanines [25] and planar bi- and trinuclear subphthalocyanines [26,27]), have been obtained and identified.

SubPcs were widely investigated as prospective materials in different application fields, among them organic light-emitting diodes [28], organic photovoltaic [29–32] and non-linear optics [33]. Subphthalocyanines have attracted much attention in the field of sensors due to their optic properties, related with an intense Q absorption band. Colorimetric and fluorometric cyanide sensors based on subphthalocyanines with electron-withdrawing substituents (nitro- and fluorine- groups) were successfully prepared [34]. An ion selective electrode for the salicylate ion, based on phenoxy-substituted subphthalocyanines has recently been described [33].

Besides that, SubPcs have interesting redox properties which are mostly driven by the nature of the macrocyclic aromatic core [16]. According to their fully π-conjugated macrocyclic structure, and their similarities with other pyrrolic compounds such as phthalocyanines, one could expect subphthalocyanines to show strong electrocatalytic properties towards phenols of interest in the food industry. Likewise, electron mediator properties in biosensors, linked to the pyrrolic structure, can be foreseen. However, such properties should differ from those observed in phthalocyanines due to the different electronic density of the aromatic structure, their conic structure or by the fact that the ligand in the axial position can be displaced by phenols [18,19].

These new sensors with dissimilar electrocatalytic properties, can be applied in the field of multisensor systems (the so-called electronic tongues), where an array of sensors with cross-selectivity is coupled to pattern recognition software [35]. Arrays based on phthalocyanines have been successfully used to analyze wines with different qualities or different organoleptic characteristics [36]. The cross-selectivity of the systems could be improved by introducing sensors modified with subphthalocyanines in the array.

In this work, the electron mediator properties of SubPcs in electrochemical biosensors containing Tyrosinase and Laccase, are evaluated. The sensors are prepared on ITO glass and these films are used as the working electrode in voltammetric experiments. Special attention is paid to the influence of the nature of peripheral substituents on sensor properties. For this purpose, the electro-catalytic and electron mediator properties towards phenols of a series of subphthalocyanines (see structures in Fig. 1), possessing electron-withdrawing (Cl), electron-releasing (t-Bu) and (OPh) substituents with intermediate properties are investigated. The sensing properties are tested towards catechol (1,2-dihydroxy-benze) and hydroquinone (1,4-dihydroxybenze), two dihydroxybenzene isomers, with many industrial applications which are widespread in the environment [36,37]. Kinetic parameters and detection limits will be calculated and discussed.

Finally, a multisensor system formed by an array of nine sensors and biosensors based on combinations of subphthalocyanines and phenyl oxidases is coupled to a pattern recognition software to obtain a bioelectronic tongue (bioET). The possibility of using such a system to discriminate red wines according to their Total Polyphenol Index, is evaluated.

2. Material and methods

All reagents and solvents were purchased from Sigma-Aldrich and used as purchased. Deionized water from MilliQ (resistivity 18.2 MΩ·cm) was used in all electrochemical experiments.

Three subphthalocyanines were included in the study: tri-tert-butyl boron phthalocyanine (t-BuSubPc), hexa-phenoxy boron subphthalocyanine (PhOSubPc) and hexa-chloro boron subphthalocyanine (ClSubPc). t-BuSubPc and PhOSubPc were obtained using the previously described approach [20,38]. The synthesis of the ClSubPc is reported here for the first time. Details are given in the following sub-section.

2.1. Synthesis of hexachlorosubphthalocyanine boron chloride (ClSubPcs)

A mixture of 1,2-dicyano-4,5-dichlorobenzene (1.0 g, 0.5 mmol) and boron trichloride (2.5 mL; 1 mol·L^{-1} solution in CH_2Cl_2) were refluxed in o-DCB (4 mL) under argon. The reaction was controlled with thin-layer chromatography (eluent C_6H_6 using Merck Aluminium oxide F_{254} neutral flexible plates) and absorption spectroscopy. The reaction mixture was cooled to room temperature and n-hexane was added. The precipitate was filtered, washed with n-hexane and solved by toluene. Then the solvent was evaporated to give ClSubPc as a purple powder. An additional portion of target complex was obtained by evaporation of the filtrate and subsequent recrystallization from MeOH. The yield of ClSubPc: (50.0 mg). UV–vis (λ_{max}(toluene)/nm(I/Imax)): 553 (49%); 573 (100%). ^1H NMR δH (400.13 MHz, THF-d$_8$) (7.99, s, 1H). MS-MALDI-TOF m/z: 637.1871 ([M], 100%). The molecular mass calculated: 637.3345. IR spectroscopy (diamond) 1098 cm^{-1} (C-Cl st), 738 cm^{-1} (B-Cl st), 1386–1651 cm^{-1} (γ-pyrrole), 1343 cm^{-1} (B-N st), 1679 cm^{-1} (C = N st), 906 cm^{-1} (1,2,4,5-tetra-substituted benzene).

2.2. Preparation of sensors and biosensors

Sensors based on SubPcs were prepared using a Spin coater model 1H-D7 (Mikasa Co., Tokyo, Japan). ITO glass slides (2 cm^2 of surface, surface resistivity 60 Ω/sq), provided by DICRYL SA (Spain), were used as a substrate. Prior to the film deposition, substrates were washed in an ultrasonic bath with acetone and rinsed twice with deionized water (MilliQ). Once cleaned, 100 µL of 2.5·10^{-5} mol·L^{-1} toluene solutions were spread onto the substrate and deposited at 700 rpm during 180 seconds (slope of 60 s) and dried for 10 minutes in a furnace at 50 °C. The sensors
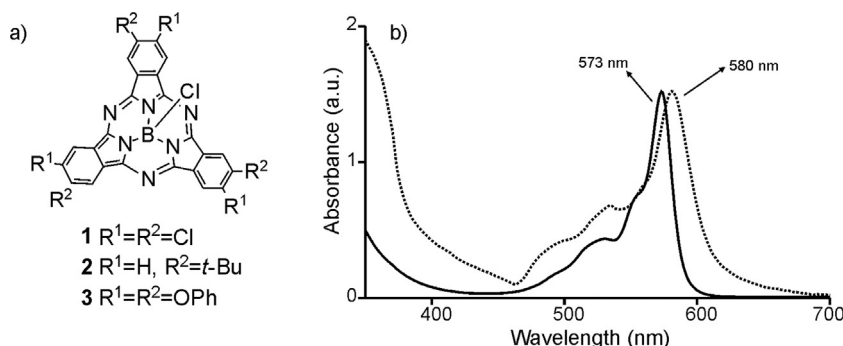

Fig. 1. (a) Structural formula of subphthalocyanines 1-3 and (b) UV–vis spectra of ClSubPc toluene solution (5.0·10^{-5} mol·L^{-1}) and spin coated film onto ITO substrate.