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Nano optical sensor binuclear Pt-2-pyrazinecarboxylic acid –bipyridine for enhancement of the efficiency of 3-nitrotyrosine biomarker for early diagnosis of liver cirrhosis with minimal hepatic encephalopathy

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ABSTRACT

A new, precise, and very selective method for increasing the impact and assessment of 3-nitrotyrosine (3-Nty) as a biomarker for early diagnosis of liver cirrhosis with minimal hepatic encephalopathy (MHE) disease was developed. The method depends on the formation of the ion pair associate between 3-nitrotyrosine and the optical sensor binuclear Pt-2-pyrazinecarboxylic acid (pca)-Bipyridine (bpy) complex doped in sol-gel matrix in buffer solution of pH 7.3. The binuclear Pt (pca)(bpy) has +II net charge which is very selective and sensitive for $[3\text{-Nty}]^{-2}$ at pH 7.3 in serum sample of liver cirrhosis with MHE diseases. 3-nitrotyrosine (3-Nty) quenches the luminescence intensity of the nano optical sensor binuclear Pt(pca) (bpy) at 528 nm after excitation at 370 nm, pH 7.3. The remarkable quenching of the luminescence intensity at 528 nm of nano binuclear Pt(pca) (bpy) doped in sol-gel matrix by various concentrations of the 3-Nty was successfully used as an optical sensor for the assessment of 3-Nty in different serum samples of (MHE) in patients with liver cirrhosis. The calibration plot was achieved over the concentration range $1.85 \times 10^{-5} - 7.95 \times 10^{-10}$ mol L^{-1} 3-Nty with a correlation coefficient of (0.999) and a detection limit of (4.7×10^{-10} mol L^{-1}). The method increases the sensitivity (93.75%) and specificity (96.45%) of 3-Nty as a biomarker for early diagnosis of liver cirrhosis with MHE in patients.

1. Introduction

Minimal hepatic encephalopathy (MHE) is a condition in which patients with liver cirrhosis that has normal mental and neurological status on standard clinical examination exhibit a number of neuropsychiatric and neurophysiological defects. MHE is present in 25–80% of cirrhotic patients without overt hepatic encephalopathy (HE) (Dhiman et al. (1995); Sharma et al. (2007); Romer et al. (2007); Das et al. (2001)). Cirrhotic patients with MHE more frequently develop episodes of overt HE than those without MHE (Das et al. (2001); Hartmann et al. (2000); Teh et al. (2007)). It is probable that MHE is a marker of advanced liver failure, because it is associated with shorter survival time, especially among patients with high concentrations of venous ammonia after oral glutamine load (Romero-Goemez et al. (2002); Amodio et al. (1999)). For this reason, MHE has been proposed as an indication for liver transplantation. Currently, the "gold standard" for diagnosis of MHE is the psychometric hepatic encephalopathy score (PHES) (Ferenci et al. (2002)). However, PHES is time consuming and needs adjusting for age and educational level. As a consequence, MHE is not routinely diagnosed in most clinical settings because of lack of simple procedures, and most patients with MHE remain undiagnosed and untreated. Hence, there is a need for a simple diagnostic test that can be performed routinely in the laboratory to detect MHE in patients with liver cirrhosis. It would be very useful in clinical practice to have some peripheral biomarker that could be measured in blood samples and reflect the presence of MHE in cirrhotic patients. Luminescent nano optical sensors doped dinuclear Pt complexes in sol-gel matrix have more advantages over the present ones; the sensor can provide constant signal response for 2 years which is a 24-fold better stability compared to the life time warranted for the chromatographic and colorimetric methods (Crow and Ischiropoulos, 1996; Frost et al. (2000); Gaut et al. (2002); Roy et al. (2015)). Sensor is stable over all measurements which prevent the source of error in the measurement process and it gives a low standard deviation values. The higher stability of the present sensor can be attributed to the doping of the optical sensor in sol-gel matrix. Square-planar platinum(II) complexes have attracted great attention in the past two decades because of

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their interesting spectroscopic properties and potential applications in optoelectronic devices (Gill and Kaur, 2004; Lu et al. (2004); Lu et al. (2002); Qiu et al. (2009)), chemosensors (Wang et al. (2008); Ji et al. (2009a, 2009b)), photocatalysis (Zhang et al. (2009); Du et al. (2008); Du et al. (2006)) and nonlinear optical materials (Du et al. (2007); Shao et al. (2010); Ji et al. (2010); Shao et al. (2009)). The square-planar Pt(II) coordination of these complexes reduces the D_{2d} distortion that is likely to result in radiationless decay process (Ji et al. (2009a, 2009b); Zhou et al. (2007); Tarran et al. (2014)), which enhances the emission of these complexes. In addition, the planar structure of cyclometalated Pt (II) complexes enables their association via π–π or dz^2–dz^2 interactions of metal ions and ligands to alter the nature and energy of HOMO and LUMO of the complex reflected in their optical and electrochemical properties [Bercaw et al. (2010); Aoki et al. (2011)]. When Pt–Pt bond is formed due to overlap of the dz^2–dz^2 metal ions orbitals, the σ^* orbital becomes the complex HOMO, explaining the high luminescence intensity and possibility to undergo two-electron oxidation into Pt(III) complexes (Katlenoka and Balasheva, 2014; Chakraborty et al. (2013); Sicilia et al. (2012)). The work aims to improve the sensitivity and the specificity of the serum biomarker (3-Nty) 3-nitrotyrosine for early diagnosis of the MHE. This depends on the quenching of the luminescence intensity of the nano binuclear Pt complex optical sensor doped in sol gel at 528 nm (λ_{ex}=370, in phosphate buffer of pH 7.3) by different concentrations of 3-Nty secreted in the blood serum of patients with MHE.

2. Experimental

2.1. Apparatus

All fluorescence measurements were recorded with a Meslo-PN (222-263000) Thermo Scientific Lumina fluorescence Spectrometer in the range (190–900 nm). The absorption spectra are recorded with Thremo UV-Visible double-beam spectrophotometer. All pH measurements are made with a pHs-JAN-WAY 3040 ion analyzer. The TEM image of the nano optical sensor binuclear Pd complex doped in sol-gel matrix was measured (19.12 ± 5.8 nm) by using the JEOL JEM-1230 available at NRC, Dokki, Cairo, Egypt. FTIR measurements (KBr pellets) were carried out on a Unicam-Mattson 1000 FT-IR spectrometer. NMR measurements were performed on a Spectrospin-Bruker 300 MHz spectrometer. Samples were dissolved in $(CD_3)_2SO$ and TMS was used as an internal reference. Elemental analyses were performed on Perkin-Elmer 2400 CHN elemental analyzer. Mass spectrometry measurements of the solid complexes (70 eV, EI) were carried out on a Finnigan MAT SSQ 7000 spectrometer, available at National center for research, NRC, Dokki, Cairo, Egypt.

2.2. Materials and methods

2.2.1. Materials

NaCl, KCl, $CaCl_2$, 3-nitrotyrosine, 2-pyrazinecarboxylic acid, bipyridine and $[PtCl_4]^{2-}$ were purchased from sigma. tetraethoxysilane (TMOS) and diethoxydimethylsilane (DEDMOS) were purchased from Sigma–Aldrich (Saint Louis, USA). Amino acids; tyrosine, tryptophan, phenylalanine, valine, Citrulline and Methionine were purchased from Sigma. A stock solution of 3-nitotyrosine (5.0×10^{-3} mol L^{-1}) was prepared in acetonitrile. The working standard solution (1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7} mol L^{-1}) of 3-nitotyrosine was freshly prepared by appropriate dilution of the stock solution with phosphate buffer of pH 7.3. A stock solution (1×10^{-2} mol L^{-1}) of the optical sensor was prepared by dissolving the required weight of the binuclear Pt (pca) (bpy) in DMSO. The working standard solution (5.0×10^{-4} mol L^{-1}) of the optical sensor was prepared by appropriate dilution of the stock with deionized water, S. data 1. The phosphate buffer of pH 7.3 was prepared by mixing 100 mL 0.1 mol L^{-1} KH_2PO_4 with 74 mL of 0.1 mol L^{-1} NaOH then completed to 200 mL by distilled water. The luminescence intensity was measured at $\lambda_{ex}/\lambda_{em}$=370/528 nm. Human samples were obtained from the New Al-Kasr-EL-Aini Teaching Hospital Cairo University and Ain Shams Specialized Hospital, Ain shams University, Cairo, Egypt in accordance with WHO (World Health Organization) approved protocol for human specimen collection and for the use of this material and related clinical information for research purposes. (All patients are consented and approved the using of their clinical samples in the research work).

2.2.2. General procedures

Nano sensors prepared previously (Attia, 2010; Attia et al. (2010), Attia and Aboaly, 2010) by sol-gel method were suffers from some disadvantages in which the cracking was taken place for the product and the casted optical sensor, which is converted into small species. Some modification was introduced to the method in which the sol after reflux was transferred into deep freezer at zero temperature to expel the trapped gases and decreases the cracking process of the optical sensor. Even though, the cracking still takes places. To overcome this problem, the preparative method was modified by adding a plasticizer (DEDMS) at room temperature. The method of preparation in details: 10 mL of binuclear Pt(pca)(bpy) (5.0×10^{-4} mol $^{-1}$ L) in ethanol. Part of this solution (8 mL) was mixed with TEOS (4 mL), DEDMOS (4 mL) and deionized water (2 mL). Glass vials (diameter 24 mm, height 48 mm) were filled with 9 mL of this solution and were covered with Parafilm. After 2 days, three small holes were pierced in the Parafilm. After 6 days, thin film was prepared from the partly hydrolyzed and condensed solution by spin-coating on small quartz slide (substrate) (width 8.5 mm; height 25 mm) to fit in a cuvette for the measurement of the luminescence intensity. The substrate was cleaned first by putting it in distilled water with a surfactant. In the next step, it cleaned ultrasonically for 30 min in distilled water with surfactant and then ultrasonically for 10 min in acetone, and finally it boiled for 10 min in 2-propanol. Before spin-coating, the substrate was rinsed with 2-propanol and spun dry. Then, the partially hydrolyzed and condensed sol-gel solution was dropped on the substrate with a syringe through a 2 cm filter and spin-coated at 1000 rpm for 1.0 min, see TEM image, S. data 2, (Attia et al. (2014)).

2.2.2.1. Sample preparation. All samples were collected between May 2014 and March 2015 and patients were excluded if they had clinical evidence of overt HE, decompensate diabetes with high levels of glycosylated hemoglobin, renal dysfunction, concomitant neurological disease, severe cardiovascular disease. After collection of the whole blood samples, allow the blood to clot by leaving it 20 min undisturbed at room temperature. Remove the clot by centrifuging at 4000 rpm for 15 min. Then decant the supernatant which is designated as serum. The serum is transferred into a clean polypropylene tube then kept at −20 °C. The separated serum samples contain (i) control subjects (10 samples), (ii) patients without MHE (17 samples), and (iii) patients with MHE (16 samples).

2.2.2.2. Proposed method. A thin film nano optical sensor is placed in the diagonal position in the cell of the spectrofluorimeter, then an appropriate amount (100 μL) of various standard concentrations of the 3-Nty in buffer solution of pH 7.3 was added in the cell and completed to 3.0 mL by the phosphate buffer. The luminescence spectra were then recorded at the $\lambda_{ex}/\lambda_{em}$=370/528 nm.