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Luminescent material based on the $[Eu(TTA)_3(H_2O)_2]$ complex incorporated into modified silica particles for biological applications

Ana Valéria S. Lourenço [a], Cláudia A. Kodaira [b,1], Eduardo M. Ramos-Sanchez [c,2], Maria Cláudia F.C. Felinto [d,3], Hiro Goto [e,4], Magnus Gidlund [c,2], Oscar L. Malta [f,5], Hermi F. Brito [a,*]

[a] Instituto de Química, Universidade de São Paulo, CP 26077, 05508-000 São Paulo, Brazil
[b] Luminetech Marcadores Ópticos Ltda — CIETEC, Av. Prof. Lineu Prestes, 2242 Sala 9 2ª andar, 05508-000 São Paulo, SP, Brazil
[c] Departamento de Imunologia, Instituto de Ciências Biomédicas-IV, Universidade de São Paulo, Av. Lineu Prestes, 1730, 05508-900 São Paulo, Brazil
[d] Instituto de Pesquisas Energéticas e Nucleares, Av. Lineu Prestes, 2242, 05508-000 São Paulo, Brazil
[e] Faculdade de Medicina e Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, Av. Dr. Enéas de Carvalho Aguiar, 470, 05403-000 São Paulo, Brazil
[f] Departamento de Química Fundamental, CCEN, Universidade Federal de Pernambuco Cidade Universitária, 50670-901 Recife, Brazil

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ABSTRACT

Amino-functionalized luminescent silica particles were investigated for use in immunoassays. The particles were prepared by the *Stöber method* where the $[Eu(TTA)_3(H_2O)_2]$ complex (TTA: 3-thenoyltrifluoroacetonate) was incorporated into silica particles during the hydrolysis and condensation of TEOS: tetraethylorthosilicate. Then, the amino groups were introduced in the particle surface using APTS: 3-aminopropyltriethoxisilane. The resulting particles were characterized by scanning electron microscopy (SEM), X ray diffraction (XRD) and photoluminescence spectroscopy. In order to demonstrate the viability of the use of luminescent particles as optical markers, an enzyme–substrate reaction was performed using HRP: horseradish peroxidase. It was possible to verify the binding of HRP-oxidized LDL (low density lipoprotein) and anti-oxLDL antibody-luminescent silica particles through the evaluation of the presence of HRP. The bioassay data open a broad field for the development of protein-tagged luminescent particles for use in biomedical sciences.

1. Introduction

Nowadays the development of luminescent complexes incorporating silica, which combine the characteristics of inorganic matrices and optical properties of the rare earth complexes, has been the subject of several studies [1–4]. There has been an increasing interest in this field of research, where silica particles were shown to be very useful platforms for lighting and luminescent markers or probes [5–9]. The trivalent rare earths (RE^{3+}) show unique properties [10] and have been widely applied in biological assays [11]. The criteria that should accomplish a RE^{3+} luminescent probe to be helpful in the design of bioassays in diagnostic or drug discovery (high throughput screening) are defined as brightness, absorption wavelength, luminescence decay, instrumentation crosstalk, stability, lipophilicity/hydrophilicity, photobleaching, quenching phenomenon, conjugation chemistry, and synthesis practicability.

The 4f orbitals are shielded from the chemical environment by the 5s and 5p filled orbitals, producing very narrow bands in their emission and absorption spectra from the compounds and long lifetimes [10,12]. Furthermore, the surface of the silica particles incorporating RE^{3+}-complexes can be easily modified, providing new opportunities for their applications in biological assays, enabling conjugation with biomolecules such as proteins, peptides, sugars, antibodies, etc. [13–15].

Ischemic coronary artery disease consequent of atherosclerotic process may lead to an inadequate blood circulation in the myocardium due to partial or complete obstruction of the coronary arteries [16]. Atherosclerosis is responsible for more than 30% of the total number of recorded deaths in the urban centers. The process is a chronic inflammatory disease characterized by the accumulation of lipid and vascular smooth muscle cell proliferation on the wall of the artery [17,18].

Clinical evidences demonstrate that high plasma concentration of low density lipoprotein (LDL) is a risk factor for atherosclerosis development. Indeed the modified forms of LDL as oxidized LDL (oxLDL) are the main components responsible for the formation of foam cells, contributing to the installation of the atherosclerotic process [19,20].

This study presents a preparation of a luminescent marker based on silica particles incorporating $[Eu(TTA)_3(H_2O)_2]$, where TTA: 3-thenoyltrifluoroacetonate. In addition, the recognition of the conjugation between the marker incubated with anti-oxLDL antibody

* Corresponding author. Tel.: +55 11 3091 3708; fax: +55 11 3091 3146.
E-mail address: hefbrito@iq.usp.br (H.F. Brito).
[1] Tel.: +55 11 3039 8432.
[2] Tel.: +55 11 3091 7382; fax: +55 11 3091 7224.
[3] Tel.: +55 11 3133 9343; fax: +55 11 3133 9018.
[4] Tel.: +55 11 3061 7023; fax: +55 11 3061 8270.
[5] Tel.: +55 81 2126 7459; fax: +55 81 2126 8442.

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and the oxLDL labeled with horseradish peroxidase (HRP) was investigated, using enzyme–substrate reaction.

2. Experimental

2.1. Preparation of the $[Eu(TTA)_3(H_2O)_2]$ complex incorporated with amino-functionalized silica particles

The luminescent amino-functionalized silica particles were prepared using the modified Stöber method [21]. Complex diaquatris (thenoyltrifluoroacetonate) europium(III) (1.9 mmol), named as Eu-TTA, prepared by the method reported elsewhere [22,23] was dissolved in 100 mL of ethyl alcohol (Aldrich). Afterwards, 1.4 mmol of TEOS: tetraethylorthosilicate (Fluka) and 2 mL of hydrous ammonia 28% (Synth) were added to the previous solution at room temperature for 12 h under stirring. Then, the precipitate was centrifuged and washed with water and ethyl alcohol. Subsequently, the resulting particles were redispersed in ethyl alcohol (100 mL) and functionalized with 0.2 mmol of APTES: 3-aminopropyltriethoxisilane (Fluka) and stirred at room temperature for 5 h [24]. Finally, the luminescent amino-functionalized silica particles (named as Eu-TTA-Si) were centrifuged, washed with water and ethyl alcohol and dried at 100 °C overnight.

X ray powder diffraction patterns were recorded on a Rigaku Miniflex using Cu Kα radiation (30 kV and 15 mA) in the range from 5 to 70° (2θ). The scanning electron microscopy (SEM) micrographs were obtained in a Field Emission Scanning Electron Microscope model JEOL JSM 7401F. The solid samples were deposited on a double-sided copper tape, attached to the sample holder. The excitation and emission spectra of the complex and amino-functionalized material at liquid nitrogen temperature (77 K) were recorded at an angle of 22.5° (front face) in a spectrofluorimeter (SPEX-Fluorog 2) with double grating 0.22 monochromator (SPEX 1680) and a 450 W Xenon lamp as excitation source. All spectra were recorded using a detector mode correction. The luminescence decay curves of the emitting levels were measured at room temperature using a phosphorimeter SPEX 1934D accessory coupled to the spectrofluorometer.

2.2. Antigen-antibody (Ag–Ab) interaction on the surface of the luminescent amino-functionalized silica particles

2.2.1. Bioconjugation of Eu-TTA-Si particles with anti-oxLDL antibody

The process for the bioconjugation of anti-oxLDL antibody [19,25] onto the luminescent amino-functionalized silica particles is shown in Fig. 1 [11]. The Eu-TTA-Si particles (1.0 mg) were dispersed in phosphate-buffered saline (PBS) solution (pH 7.4) containing 5% glutaraldehyde — GA (Aldrich) and kept at 4 °C for 12 h. After that, these particles were centrifuged, washed four times with a PBS solution and redispersed in 1.0 mL of PBS solution. At that time, 30 μL of anti-oxLDL antibody solution (1.9 mg mL^{-1}) was added and incubated overnight at 4 °C. Then, 1.6 mg of sodium borohydride ($NaBH_4$ from Merck), was added and left to react for 30 min at 4 °C. To block the unreacted aldehyde sites, 50 μL of glycine (Vetec) solution 0.5 mol L^{-1} was added, and this mixture was maintained for 2 h at 4 °C. The conjugate system Eu-TTA-Si particle-GA-anti-oxLDL was obtained and washed four times with PBS solution to remove the excess of glycine, and afterwards stored at 4 °C.

2.2.2. Conjugation of HRP-GA with oxLDL

Different HRP amounts from Aldrich (2 to 370 units) were added in 1.0 mL of PBS buffer (pH 7.4) and 18.75 μL of glutaraldehyde solution (50%) and kept for 12 h at 4 °C. The HRP concentration (in U mL^{-1}) was found as the difference between initial and final enzyme amount remaining in the solution, at all times the specific activity was expressed in terms of pyrogallol units (one pyrogallol unit will form 1.0 mg purpurogallin from pyrogallol in 20 s with pH 6.0 at 20 °C). The solutions were dialyzed in a molecular porous membrane

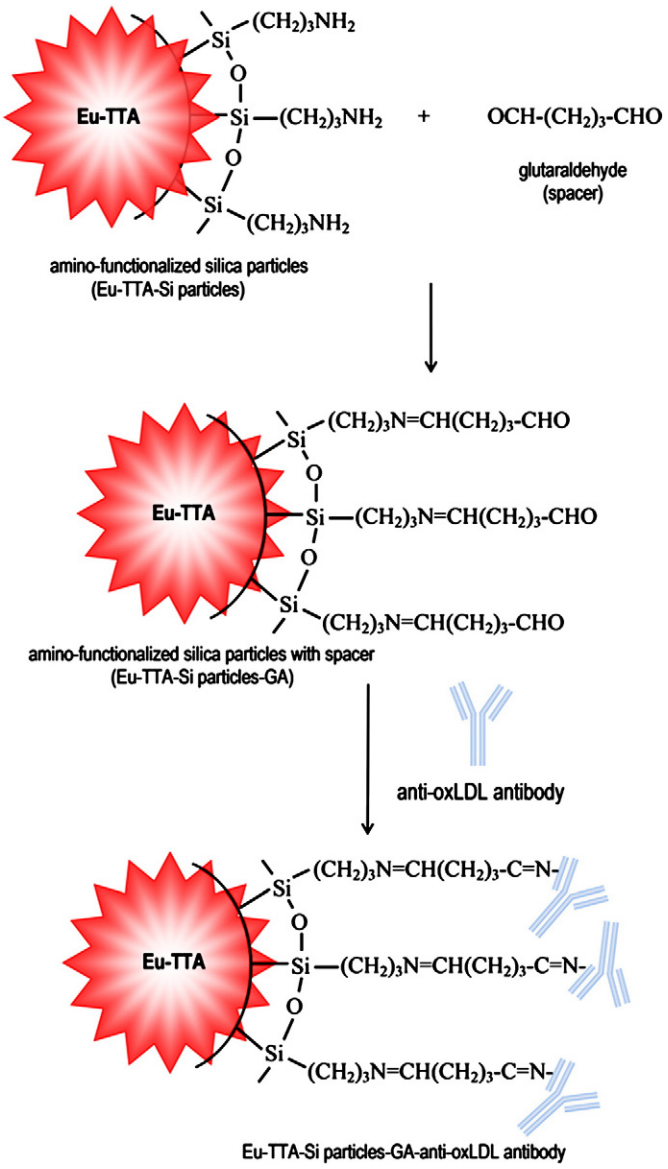

Fig. 1. Representative scheme of the immobilization of anti-oxLDL antibody particles in Eu-TTA-Si using glutaraldehyde as a spacer.

tubing (Spectrum Laboratories, 12–14,000 (molecular weight cut-off)) against PBS solution for 24 h at 4 °C. After this step, 85 μL of oxLDL solution (3.0 mg mL^{-1}), obtained as described in references [26,27], was incubated to the mixture for 12 h at 4 °C. Besides, 1.6 mg of sodium borohydride was added and after 30 min, 50 μL of glycine (0.5 mol L^{-1}) was added and left to react for 2 h at 4 °C. Then this solution was dialyzed against PBS solution for 24 h at 4 °C. The representation of the HRP-GA-oxLDL bioconjugated system is illustrated in Fig. 2.

2.2.3. HRP-GA-oxLDL with Eu-TTA-Si particle-GA-anti-oxLDL antibody reaction

The HRP-GA-oxLDL solution was added to Eu-TTA-Si particle-GA-anti-oxLDL antibody and incubated overnight at 4 °C (Fig. 3). After removal of the supernatant, the solid precipitate was washed fifteen times with PBS. Both the supernatant and washing water were reserved for further analysis to determine unbound HRP-GA-oxLDL.

2.2.4. Measurement of the HRP enzyme concentration

The optical density measurements of the supernatant and the washings were performed on flat bottomed 96-well polystyrene