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Analytical Biochemistry 355 (2006) 140–144
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Urea hydrogen peroxide determination in whole blood using europium tetracycline probe

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Received 20 February 2006
Available online 2 June 2006

Abstract

We introduce the use of a lanthanide complex, tetracycline–europium, for the clinical diagnosis of urea hydrogen peroxide in human whole blood. The values obtained agree with the urea concentration variation verified in 49 patients, including 12 predialysis, 12 peritoneal, and 15 dialysis subjects, and 10 controls. This method is noninvasive and can help in the identification of renal and cardiac diseases.

Keywords: Lanthanides; Optical sensor; Glucose; Urea hydrogen peroxide; Tetracycline; Energy transfer

Lanthanide complexes have become particularly attractive for improving sensitivity and selectivity of bioanalysis because of their specific fluorescence features. Its Stokes shift is usually large; the fluorescence intensity of the main band of a lanthanide complex is very strong, although its quantum yield is usually lower than those found on conventional fluorophores. Furthermore, the relatively long decay time of lanthanide complexes have greatly facilitated the time-resolved fluorimetry [1–3]. Upon complexation with trivalent europium (Eu) ions, tetracycline (Tc)[1] forms stable chelates which exhibit spectra with broad absorption bands and a narrow emission band centered on 612 nm, characteristic of the $^5D_0 \rightarrow {}^7F_2$ transition within the lanthanide ion [4]. Tetracycline has several proton-donating groups that offer different possibilities of complexation with lanthanide ions depending on the pH solution. With a pH around 7.0, lanthanides are probably bound to oxygen atoms [5].

Rakicioglu et al. [6] observed that the europium fluorescence intensity is increased 15 times when H_2O_2 (HP) is added to a tetracycline–europium (TcEu) solution. The tetracycline–europium probe acts as a luminescent probe for hydrogen peroxide at neutral pH without the need for using an oxidative enzyme such as a peroxidase [7]. Moreover, there are some reports about using tetracycline–europium complex as a fluorescent probe, for example for the determination of glucose [8] and heparin [9].

We recently reported [10] that europium luminescence also increases in the presence of urea hydrogen peroxide (UHP). Urea hydrogen peroxide, or carbamide peroxide, is a stable form of HP and a potential cytotoxic agent [11]. The renal and cardiac levels of UHP and pentodisine are correlated with the levels of renal and cardiac pentosidine produced from the Maillard reaction [12]. Both UHP and HP have potential deleterious effects on various cells,

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[1] *Abbreviations used:* Tc, tetracycline; HP, hydrogen peroxide (H_2O_2); UHP, urea hydrogen peroxide; TcEu, tetracycline–europium; Mops, 3-(*N*-morpholino) propanesulfonic acid; CO, control group; CRF, chronical renal failure; AGE, advanced glycation end products.

0003-2697/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2006.05.015

including those of the kidney and the heart. The knowledge that chronic renal failure (CRF) is related to increased UHP levels in the renal and cardiac matrices enables the control of these toxins to delay functional damage of the heart and kidney in predialysis patients.

The conventional methods for determining UHP levels were very indirect and numerous steps and reagents were involved [12]. This paper describes a fast and easy method to determine the presence of UHP in human blood by measuring europium–tetracycline as fluorescence probe.

Materials and methods

All inorganic salts used in this work have analytical purity and were obtained from Sigma–Aldrich and Molecular Probes. All solutions were prepared in 10 mmol L^{-1} 3-(*N*-morpholino) propanesulfonic acid (Mops; Carl Roth, Germany) buffer (pH 6.9). The tetracycline–HCl used was a secondary pattern kindly provided by Bunker Indústria Farmacêutica Ltda. The urea hydrogen peroxide 98% used in this work was obtained from Aldrich. Solution I consisted of 63 mmol L^{-1} solution of europium chloride ($H_{12}Cl_3EuO_6$) in 10 ml bideionized water with Mops (pH ~6.9). Solution II consisted of 21 mmol L^{-1} solution of tetracycline chloride ($C_{22}H_{24}N_2O_8{\cdot}HCl$) in 10 ml bideionized water with Mops (pH ~6.9). TcEu solution (solution III) was prepared with the mixture of 10 ml of solution I and 10 ml of solution II.

Subject selection and blood sampling

A total of 49 patients were involved in this study: 12 patients were in peritoneal (CAPD), 15 were in dialysis (HD), and 12 were in nondialysis treatment (IR). Healthy control subjects ($n = 10$) with no clinical signs of vascular or renal disease and no family history of renal disease were recruited among blood donors and hospital staff. A written consent for studies was obtained from all patients and subjects from the control group. The local University Ethics Committee on human research approved the study.

Approximately 4.5 cm^3 of venous blood was aseptically collected from each individual. EDTA was used as anticoagulant and blood samples were maintained at 4 °C.

Biochemical analysis

Plasmatic urea was determined by colorimetric enzymatic test using Labtest kit (Labtest Diagnóstica, Lagoa Santa, MG/Brazil) in a semiautomated spectrophotometer (Photometer 5010; Boheringer Mannheim). The main reaction was urea being hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia produced in the first reaction combines with 2-oxoglutarate and NADH in the presence of glutamate–dehydrogenase to yield glutamate and NAD^+. The decrease in NADH absorbance per unit of time is proportional to the urea concentration.

A total of 200 µl of whole blood of each subject was mixed with 200 µl of TcEu stock solution. The fluorescence measurements were performed immediately after by exciting the samples in a 1-mm-thick cuvette with a 300-W xenon lamp and a 0.25-m Jarrel ash monochromator fixed at 400 nm with repetition rate of 20 Hz obtained by a mechanical chopper. The sample emissions were analyzed by a 0.5-m monochromator (Spex) and a PMT detector. The signal was amplified with an EG&G 7220 lock-in and processed by a computer. The relative errors in the emission measurements are estimated to be less than 10%.

Results

The absorption spectra of the TcEu complex in the presence of UHP are shown in Fig. 1a. It is possible to observe that, in the presence of UHP, the maximum wavelength of the absorption band is blue-shifted from 399 to 389 nm.

When the TcEu stock solution is excited to around 400 nm, the energy absorbed by the tetracycline is transferred from the triplet state to the central Eu^{3+} ion and the typical emission spectra of the main band centered at 612 nm ($^5D_0 \rightarrow {}^7F_2$) is observed. The addition of urea hydrogen peroxide to TcEu solution causes a large increase of the Eu emission band as shown in Fig. 1b. Nevertheless no effective changes were observed with the addition of

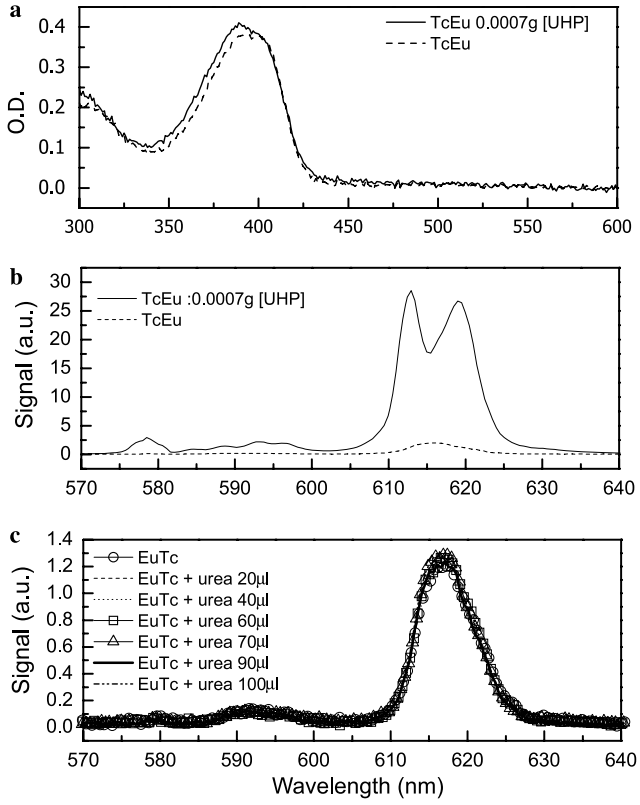

Fig. 1. (a) Absorption spectra of TcEu (1 ml stock solution III) in the presence of UHP. (b) Urea hydrogen peroxide concentration effect on TcEu–UHP complex emission spectra. (c) Effect of the addition of urea on the Eu emission band.