Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 162 (2016) 98–104

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Determination of phenformin hydrochloride employing a sensitive fluorescent probe

Lin Shi, Jian-Hong Xie, Li-Ming Du *, Yin-xia Chang, Hao Wu

Analytical and Testing Center, Shanxi Normal University, Shanxi, Linfen 041004, PR China

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 3 March 2016
Accepted 6 March 2016
Available online 9 March 2016

Keywords:
Cucurbit [7]uril
Phenformin hydrochloride
Palmatine
Fluorescent probe

ABSTRACT

A complexation of non-fluorescent phenformin hydrochloride (PFH) with cucurbit [7]uril (CB [7]) in aqueous solution was investigated using the fluorescent probe of palmatine (PAL) coupled with CB [7]. The fluorescent probe of CB [7]–PAL exhibited strong fluorescence in aqueous solution, which was quenched gradually with the increase of PFH. This effect is observed because when PFH was added to the host–guest system of CB [7]–PAL, PFH and PAL competed to occupy the CB [7] cavity. Portions of the PAL molecule were expelled from the CB [7] cavity owing to the introduction of PFH. Based on the significant quenching of the supramolecular complex fluorescence intensity, a fluorescence method of high sensitivity and selectivity was developed to determine PFH with good precision and accuracy for the first time. The linear range of the method was 0.005–1.9 μg mL^{-1} with a detection limit of 0.003 μg mL^{-1}. In this work, association constants (K) of PFH with CB [7] were also determined. $K_{CB\ [7]–PFH} = (2.52 \pm 0.05) \times 10^5$ L mol^{-1}. The ability of PFH to bind with CB [7] is stronger than that of PAL. The results of a density functional theory calculation authenticated that the moiety of PFH was embedded in the hydrophobic cavity of CB [7] tightly, and the nitrogen atom is located in the vicinity of a carbonyl-laced portal in the energy-minimized structure. The molecular modelling of the interaction between PFH and CB [7] was also confirmed by ^{1}H NMR spectra (Bruker 600 MHz).

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

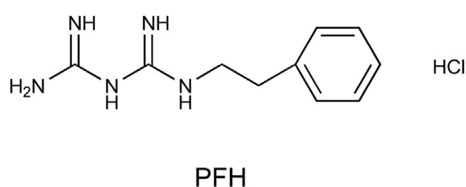
Phenformin hydrochloride (PFH, 1-phenethylbiguanide hydrochloride, Scheme 1) is a biguanide antidiabetic agent that is suitable for treating non-insulin-dependent (type 2) diabetes mellitus [1]. PFH has been banned in most countries and regions because of its relatively large side effects [2,3]: metformin hydrochloride is usually used in its place. However, there are still many manufacturers illegally adding PFH to dietary supplements and diet pills for better curative effects and higher profits. Of course, PFH also has a significant function such as phenformin-loaded micelles [4]. Thus far, PFH has been investigated using high-performance liquid chromatography (HPLC) [5], UV–visible spectrophotometric [6], chemical ionization mass spectrometry [7], capillary electrophoresis (CE) [8], chemiluminescence (CL) [9], gas chromatography [10] and so on. However, applying the HPLC method generally requires expensive equipments, complicated apparatuses, disposal of solvents, preparation for labour-intensive sample and personnel skills in chromatographic techniques. Fluorimetry's main advantage over the HPLC method [5] is its rapidity. Compared with the UV–visible spectrophotometric method [6], the fluorescence method possesses better analytical selectivity, higher capacity against blank interference, improving the limit of detection. In this paper we firstly use the fluorescence method for determination of PFH through fluorescent probe. The proposed method is convenient and suitable for routine determination of the PFH.

Cucurbit [7]uril (Scheme 2) is a cyclic host molecule comprising 7 glycoluril units bridged by 14 methylene groups. Symmetrical CB [7] hosts possess hydrophobic cavities and restrictive polar portals lined with ureido carbonyl groups. CB [7] has attracted increasing attention because of its hydrogen bonding, ion–dipole interactions, and dipole–dipole interactions with a wide range of guest molecules. And CB [7] also has many significant applications in biology and nanoparticles [11–13]. Moreover, the molecular recognition properties of the guest molecules are different owing to the rigid structure with different sizes [14–16]. The CB [7] host has been particularly interesting in recent years because of its superior solubility in aqueous solutions compared with other cucurbit[*n*]uril members and because of its remarkable ability to form host–guest complexes with organic compounds and cations [17].

Palmatine (PAL, Scheme 3) is a natural isoquinoline alkaloid [18], which has many applications, such as probe [19] and pharmacokinetic study [20]. PAL can emit weak fluorescence in aqueous solution. Surprisingly, our group found that the fluorescence intensity of PAL was increased significantly after the addition of CB [7] in aqueous solution [21,22]. When PAL enters the cavity of CB [7], PAL and CB [7] can form a strong fluorescent system of CB [7]–PAL. Thus the significance of using PAL reflects the concentration of non-fluorescent PFH indirectly by

* Corresponding author.
E-mail address: lmd2004@163.com (L.-M. Du).

http://dx.doi.org/10.1016/j.saa.2016.03.006
1386-1425/© 2016 Elsevier B.V. All rights reserved.



Scheme 1. Structure of PFH.

observing the phenomenon of fluorescence quenching when PFH was added into the CB [7]–PAL system. PAL is a vital probe. The reason of fluorescence quenching is that PAL was squeezed out of the cavity of CB [7] by PFH. Thus the fluorescent system of CB [7]–PAL was established to detect the non-fluorescent substance.

Herein, we aimed to propose a novel and sensitive fluorescence spectrophotometric for the analysis of PFH. We employed PAL as a signal probe for the fluorescence detection of PFH and investigated the supramolecular correlation between CB [7] and PFH in aqueous solution. The fluorescent properties of PAL rely on a highly sensitive microenvironment [23]. The distinct decrease of fluorescence intensity was observed when suitable concentration and appropriate amounts of PFH were added to the CB [7]–PAL complex.

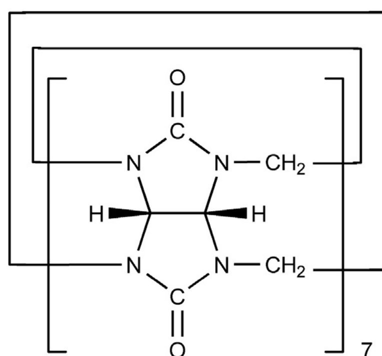
2. Experimental

2.1. Apparatus

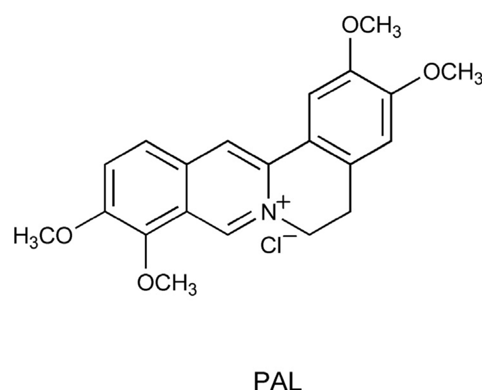
The fluorescence spectra were implemented on a Cary Eclipse spectrofluorometer (Agilent Technologies, USA) equipped with a xenon lamp. The slit widths of both excitation and emission monochromators were set to 5 nm. The scan rate of the fluorescence spectra was 600 nm min^{-1}. UV–visible spectroscopy was performed on a Cary 300 UV–visible spectrophotometer (Varian Associates, USA) with a slit width set at 2.5 nm. All measurements were performed at room temperature using a standard 10 mm path length quartz cell. ^{1}H NMR spectra were analysed by means of a Bruker Ascend™ 600 MHz NMR spectrometer in D_2O.

2.2. Reagents and chemicals

PFH was obtained from the Dalian Meilun Biological Technology Co. Ltd. (Dalian, P.R. China). PFH was dissolved in fresh double-distilled water as a stock standard solution with final concentration of 100 μg mL^{-1}. PAL was acquired from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P.R. China) without further treatment. PAL was dissolved in fresh double-distilled water as



Scheme 2. Structure of CB [7].



Scheme 3. Structure of PAL.

a stock solution of 1.0 mM. CB [7] was prepared and characterized according to the literature [15]. The CB [7] stock solution of 1.0 mM was also dissolved in fresh double-distilled water. All stock standard solutions can be stored stably for a long time at room temperature. Standard working solutions were prepared by deliquating the stock standard solutions with fresh double-distilled water before use. All other reagents used were of analytical grade.

2.3. Spectra measurement procedure

2.3.1. Spectral measurements of CB [7]–PAL inclusion complex

The 0.1 mM PAL standard working solution in the amount of 0.7 mL was placed in a 10 mL colorimetric cylinder by a measuring pipette, and an appropriate amount of 0.1 mM CB [7] standard working solution was then added. The mixture was diluted to cubage of 10 mL with fresh double-distilled water and shaken for 10 min at room temperature, after which fluorescence intensities (or absorption spectra) were determined.

2.3.2. Spectral measurements of CB [7]–PFH inclusion complex

The 0.1 mM CB [7] standard working solution in the amount of 0.7 mL and the 0.7 mL of 0.1 mM PAL standard working solution were poured into a 10 mL colorimetric cylinder. Suitably different amounts of PFH solution were subsequently added to the colorimetric cylinder. The mixture was also diluted and shaken similar to the mixture of CB [7]–PAL. The fluorescence intensity values of the solution ($F_{PAL–CB\ [7]–PFH}$) and the blank solution ($F_{CB\ [7]–PAL}$) were observed at 495 nm using an excitation wavelength of 343 nm.

2.4. Analysis of spiked human urine

The fluorescent probe method was applied in the determination of PFH in human urine samples. Urine samples were processed according to the literature [24]. In short, the urine sample was placed in a centrifuge tube (10.0 mL), spiked with 1.0 mL of drug stock solution and then centrifuged at 5000 rpm for 10 min. The supernate of the spiked urine sample was extracted by solid phase extraction (SPE) using 001 × 7 strong-acid cation exchange resin. The procedure was as follows: 1 mL of the spiked urine sample was transferred into the SPE cartridge. After allowing the sample to pass through to waste under gravity, the resin was washed with 1 mL of 1% sodium hydroxide solution and 2 mL of double-distilled water. The filtrate was discarded. Finally, the analyte was eluted into a colorimetric flask with 3 mL of methanol/water (1:1).

2.5. Analysis of pharmaceutical preparations

The fluorescent probe method was also applied in the determination of PFH in pharmaceutical preparations. A certain number of tablets were

Download English Version:

https://daneshyari.com/en/article/1231081

Download Persian Version:

https://daneshyari.com/article/1231081

Daneshyari.com