Inorganica Chimica Acta 435 (2015) 53–59

Contents lists available at ScienceDirect

Inorganica Chimica Acta

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

Development of a selective fluorescent ligand for aluminum ion having a 6-chloro-pyridazine moiety

Hideaki Ichiba [a], Makiko Yonei [a], Tatsuya Sakamoto [a], Hikono Kuwayama [a], Yosuke Hamada [a], Hiroshi Yamada [a], Mayu Onozato [a], Tami Hiruta [b], Hiroaki Nakagawa [c], Takeshi Fukushima [a,*]

[a] Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi-shi, Chiba 274-8510, Japan
[b] Optical Instrument Engineering Department, Hitachi High-Tech Science Corporation, 1040, Ichige, Hitachinaka-shi, Ibaraki-ken 312-0033, Japan
[c] Medical Systems Development Center, Science & Medical System Design Division, Hitachi High-Technologies Corporation, 882, Ichige, Hitachinaka-shi, Ibaraki-ken 312-8504, Japan

ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form 29 May 2015
Accepted 30 May 2015
Available online 14 June 2015

Keywords:
Fluorescent complex
6-Chloro-*N*′-(2-hydroxybenzylidene)pyridazine-3-carbohydrazide
Aluminum ions

ABSTRACT

A functional ligand, 6-chloro-*N*′-(2-hydroxybenzylidene)pyridazine-3-carbohydrazide (CPCH), was designed and synthesized, and metal ions that can induce fluorescence in CPCH were comprehensively screened by a batch method. CPCH showed intense fluorescence at 490 nm (ex. 394 nm) in the presence of Al(III) at weakly acidic pH values (2.0–6.0) because of the formation of a fluorescent CPCH–Al(III) complex. The binding ratio of CPCH to Al(III) was 3:1, as determined by the molar ratio method. The strongest fluorescence intensity of the CPCH–Al(III) complex was observed at pH 3.0, and the detection limit of Al(III) was approximately 200 nmol L^{-1}.

1. Introduction

There is a pressing need to develop fast and reliable methods for the detection of metal ions in environmental (river or seawater) and biological samples (cultured cells). Thus far, a number of fluorescent chelating ligands, which act as chemosensors for the detection of toxic or harmful metal ions, have been developed [1–3]. Most functional ligands reported to date for the efficient capture of metal ions possess Schiff-base moieties in their structures [4–8]. In fact, in our laboratory, we have developed several chelating ligands with a Schiff-base moiety that can coordinate to metal ions such as Zn(II), Ga(III), and Sc(III) to emit fluorescence [9–11]. These chelating ligands have one or two Schiff-base moieties between two aromatic moieties such as quinazoline, vanilline, 2,4-dihydroxybenzene, and *p*-aminosalicylate [9–11].

In the search for novel chelating ligands, 6-chloro-*N*′-(2-hydroxybenzylidene)pyridazine-3-carbohydrazide (CPCH), which has a Schiff-base moiety between 6-chloropyridazine and 2-hydroxybenzylidene (Fig. 1), was designed and synthesized in our laboratory. In the present study, we screened 19 different metal ions for their ability to coordinate with CPCH and generate fluorescence in aqueous media under optimal pH conditions, and synthesized a CPCH–Al(III) complex that showed strong fluorescence under weakly acidic pH conditions. We also investigated the fluorescence characteristics of the formed CPCH–Al(III) complex.

2. Experimental

2.1. Chemicals

Dimethyl formamide (DMF), dimethylsulfoxide (DMSO), aluminum chloride ($AlCl_3{\cdot}6H_2O$), potassium chloride (KCl), and HPLC-grade acetic acid were purchased from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma Co., Ltd. (St. Louis, MO, USA). HPLC-grade acetonitrile (CH_3CN), and HPLC-grade methanol (MeOH) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Ethanol (EtOH, 99.0%) was obtained from Japan Alcohol Trading Co., Ltd. (Chiba, Japan) and was used after distillation. Water was used after purification with a Milli-Q System Auto Pure (Yamato Co., Ltd., Tokyo, Japan). Metal ions were purchased from Wako Pure Chemicals Co., Ltd. (Osaka,

---

Abbreviations: CHEF, chelation enhanced fluorescence; CPCH, 6-chloro-*N*′-(2-hydroxybenzylidene)pyridazine-3-carbohydrazide; DMF, dimethyl formamide; ESI, electrospray ionization; ESIPT, excited-state intramolecular proton transfer; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PET, photo-induced electron transfer.

* Corresponding author. Tel./fax: +81 47 472 1504.
E-mail address: t-fukushima@phar.toho-u.ac.jp (T. Fukushima).

http://dx.doi.org/10.1016/j.ica.2015.05.033
0020-1693/

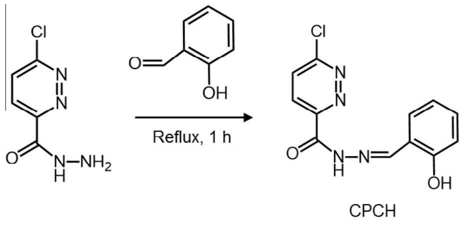

Fig. 1. Synthetic route of CPCH.

Japan) in the form of salts with NO_3^-, SO_4^{2-}, or Cl^- ions and were of atomic absorption grade.

2.2. Equipment

Excitation and fluorescence spectra were measured using a spectrofluorometer (F-7000, Hitachi High-Tech Science Corporation, Tokyo, Japan) with a 1 cm quartz cell. The UV absorption and emission spectra were measured using a JASCO V-650 instrument (Jasco, Tokyo, Japan). All fluorescence and absorption data were obtained in a temperature-controlled room (25 °C). NMR spectra were measured using a JEOL ECP1 (JEOL, Ltd., Tokyo, Japan) (500 MHz) with tetramethylsilane as the internal standard. Infrared spectra were recorded on an FT/IR-4100 (Jasco), and accurate mass spectra were measured using a JMS T100LP (JEOL, Ltd., Tokyo, Japan).

2.3. Synthesis of 6-chloro-N′-(2-hydroxybenzylidene)pyridazine-3-carbohydrazide

6-Chloropyridazine-3-carbohydrazide was synthesized using previously reported protocols [12,13]. Salicylaldehyde (0.15 g, 1.23 mmol) was added to 6-chloropyridazine-3-carbohydrazide (0.15 g, 0.87 mmol), dissolved in MeOH (50 mL), and refluxed for 1 h. On cooling, yellow crystals were generated, which were isolated by filtration. The crystals were washed with MeOH and dried *in vacuo*.

^{1}H NMR (DMSO-d_6) δ (ppm): 8.32 (1H, d, J = 8.9), 8.16 (1H, d, J = 8.9), 8.87 (1H, s), 7.53 (1H, d, J = 5.9), 6.93 (1H, d, J = 8.7), 7.32 (1H, d, J = 8.7), 6.92 (1H, d, J = 7.6), 11.21 (1H, s). ^{13}C NMR (DMSO-d_6) δ (ppm): 117.79, 119.42, 120.65, 130.19, 131.40, 132.01, 133.24, 153.38, 153.77, 159.69, 160.37, and 160.53. IR (KBr) cm^{-1}: 3282.3, 1676.8, 1517.7. *Anal.* Calc. for $C_{12}H_9O_2N_4Cl$: C, 52.09; H, 3.28; N, 20.25. Found: C, 52.09; H, 3.33; N 20.23%. Melting point: 235 °C. ESI-MS: m/z 277.04 (M+H$^+$).

2.4. Screening of metal ion binding with CPCH

Metal ions were screened in a similar manner as described in our previous reports [9–11]. Nineteen metal ions, namely Li(I), Cs(I), Ca(II), Sr(II), Ba(II), Cr(III), Mn(II), Fe(III), Fe(II), Co(II), Ni(II), Cu(II), Ag(I), Zn(II), Cd(II), Al(III), Pb(II), Bi(III), and Ga(III), were dissolved in water to a final concentration of 2.0 mmol L^{-1}. Each metal ion solution (25 μL) was then added to 150 μL of 1.0 mmol L^{-1} CPCH in DMF and 25 μL of 50 mmol L^{-1} HEPES–HCl buffer (pH 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0), and then heated at 60 °C for 5 min. The heated solution was diluted with DMF to 3.0 mL, and subjected to fluorescence intensity measurements.

2.5. Fluorescence properties of CPCH–Al(III) complex

CPCH was dissolved in DMSO to prepare a 1.0 mmol L^{-1} CPCH solution. The CPCH solution (150 μL) was added to 25 μL of 370 μmol L^{-1} Al(III) and 25 μL of 50 mmol L^{-1} HEPES buffer (pH 3.0) containing 100 mmol L^{-1} KCl, and then mixed vigorously. The solution was heated at 60 °C for 5 min, followed by the addition of 4.5 mL of DMF. The excitation and emission spectra of the final solution were measured. In order to examine the effects of organic solvents on the fluorescence intensity, we tested CH_3CN, MeOH, and EtOH in addition to DMF. Different buffers (phosphate and acetate), except HEPES, were also tested, because phosphate and acetate buffers are usually used for weakly acidic pH-buffered solutions. The detection limit of Al(III) was calculated from a 3σ/slope, where σ is the standard deviation of the blank and the slope is the increase in fluorescence intensity against the concentration of Al(III) [14].

Fluorescence quantum yield (φ) of the CPCH–Al(III) complex was measured using quinine sulfate as a standard ($\varphi = 0.54$, λ_{ex} = 350 nm), as reported previously [11].

2.6. UV–Vis spectrum of the CPCH–Al(III) complex

As described above, the CPCH solution (1.0 mmol L^{-1}, 150 μL) was added to 25 μL of Al(III) (0, 0.1, 0.5, 1.0, 1.5, 2.0, 3.0, and 6.0 mmol L^{-1}) and 25 μL of 50 mmol L^{-1} HEPES buffer (pH 3.0), and then mixed vigorously. The mixed solution was heated at 60 °C for 5 min, and was added to DMF to make a 3.0 mL solution. Each solution was subjected to UV–Vis measurements.

2.7. Effects of interfering ions

Twelve different ions, namely Ca(II), Ba(II), Fe(III), Cd(I), Zn(II), Pb(II), Cs(I), Mn(II), Cu(II), Co(II), Cu(I), and Fe(II), were tested for possible interference with the fluorescence of the CPCH–Al(III) complex. Briefly, 12.5 μL of 0.37–7.4 mmol L^{-1} of each test ion dissolved in 50 mmol L^{-1} HEPES buffer (pH 3.0) was added to the tube containing 150 μL of 1.0 mmol L^{-1} CPCH, 25 μL of 370 μmol L^{-1} Al(III), and 12.5 μL of 50 mmol L^{-1} HEPES buffer (pH 3.0), and then mixed vigorously and heated at 60 °C for 5 min. The heated solution was diluted with DMF to 3.0 mL, and subjected to fluorescence intensity measurements.

2.8. Binding ratio of Al(III) to CPCH

Twenty-five microliters of different concentrations of Al(III) (0, 0.5, 0.75, 1.0, 2.0, and 3.0 mmol L^{-1}) were mixed with 150 μL of 1.0 mmol L^{-1} CPCH (in DMSO) and 12.5 μL of 50 mmol L^{-1} HEPES buffer (pH 3.0), and then mixed vigorously and heated at 60 °C for 5 min. The heated solutions were diluted with DMF to 3.0 mL, and subjected to fluorescence intensity measurements.

The fluorescence intensity of each solution was measured at 490 nm with an excitation wavelength of 394 nm, and was plotted against the molar ratio of Al(III) to CPCH. The saturation point of the fluorescence intensity was regarded as the binding ratio of Al(III) to CPCH. The binding constant between CPCH and Al(III) was calculated using a Benesi–Hildebrand plot, according to previous reports [11,15].

3. Results and Discussion

3.1. Fluorescence characteristics of CPCH–Al(III) complex

We first examined whether CPCH could chelate specifically to a metal ion and emit fluorescence using a batch method. In a preliminary experiment, solutions of CPCH with 19 metal ions in acidic, neutral, and basic-buffered media were irradiated with UV light, and the fluorescence emission was comprehensively studied. Among the 19 metal ions tested, CPCH was found to emit an intense fluorescence in the presence of Al(III) under acidic