Chinese Chemical Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

Glycine-conjugated porphyrin fluorescent probe with iRGD for live cell imaging

Qian Zhang[a,b], Xue Dong[a,b], Kun-Peng Wang[a,b], Ting-Ting Zhu[a,b], Feng-Nan Sun[a,b], Shu-Xian Meng[a,b,*], Ya-Qing Feng[a,b]

[a] School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
[b] Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, China

ARTICLE INFO

Article history:
Received 3 January 2017
Received in revised form 13 January 2017
Accepted 2 March 2017
Available online xxx

Keywords:
Fluorescence probe
iRGD
Glycine
Porphyrin
Near-infrared

ABSTRACT

A porphyrin modified by glycine has been synthesized and developed as a near-infrared (NIR) fluorescence probe to detect tumor. Porphyrins' longwavelength emission at ~650 nm can efficiently avoid the spectral crosstalk with Spontaneous fluorescence in the visible light region. A disulfide-based cyclic RGD peptide named iRGD c (CRGDKGPDC), a tumor homing peptide, harbors a cryptic C-end Rule (CendR) motif that is responsible for neuropilin-1 (NRP-1) binding and for triggering extravasation and tumor penetration of the peptide to improve the imaging sensitivity and therapeutic efficacy. We used *N* – hydroxy succinimide as an activator to introduce the glycine methyl ester to detect tumor. We got a porphyrin modified by glycine. The affinity between probe and tumor cell entered GLC-82 cells (human glandular lung cancer cell line) can be observed by Confocal Microscope. The toxicity of probe has been identified by MTT Assay. The summary has been gotten that the porphyrins were nontoxic to GLC-82 cells and glycine modified porphyrin has a good affinity with GLC-82 cells under the iRGD function by our experiment.

1. Introduction

Tumor is a great life threat to human. The past decade we have witnessed the burgeoning of optical imaging and its wide applications for biomedicines such as genomics, proteomics, cell biology, and drug discovery [1].

As we all know, early-stage tumor has been detected by the invention of kinds of probes, especially the invention of radiationless fluorescence probes [2,3]. Fluorescence probe which can be used to improve tumor early detection is still a popular topic among related experts and researchers. Recently, the near-infrared (NIR) fluorescence probes are increasingly popular in biological imaging and sensing for the reason that longwavelength (650–900 nm) excitation and emission have the advantages of minimum photodamage to biological samples, deep tissue penetration, and minimum interference from background to fluorescence by biomolecules in the living systems [4–6]. However, the development of NIR fluorescence probes has some troubles, such as photostabilities and fluorescence quantum yields of probes, targeting of tumors. Up to now, the common NIR fluorophores only have cyanine dyes, such as Cy5 and Cy7 [7,8].

Porphyrins [9], due to the unique properties of photophysical and photochemical, have a special relevance to photodiagnosis (PD) and in photodynamic therapy (PDT) of oncological and non oncological diseases [10]. Moreover, we used porphyrins as fluorescence probes because they are relatively stable and have good photostability [11].

Tumor-penetrating internalizing RGD peptide (iRGD) is known to have a relatively high and specific affinity for $\alpha v\beta 3$ – integrin receptor [12–14], which is increased vascular and tissue permeability in a tumor-specific and neuropilin-1-depenendent manner, allowing co-administered drugs to penetrate into extravascular tumor tissue. The CendR motif, a class of cell and tissue-penetrating peptides with a specific R-x-x-R carboxyl-terminal motif, is not active unless it occupies a C-terminal position in the peptide after the cleavage, recognizing neuropillin-1(the receptor for the CendR peptides) and facilitating the penetration into the tumor of iRGD and co-administered drugs [15].

In this study, the iRGD [16], which having tumor-penetrating ability and good cell membrane permeability, was used to improve

* Corresponding author.
E-mail address: msxmail@tju.edu.cn (S.-X. Meng).

http://dx.doi.org/10.1016/j.cclet.2017.03.001

Please cite this article in press as: Q. Zhang, et al., Glycine-conjugated porphyrin fluorescent probe with iRGD for live cell imaging, Chin. Chem. Lett. (2017), http://dx.doi.org/10.1016/j.cclet.2017.03.001

the permeability of cell membrane firstly, and then the fluorescent probe TPP-glycine was apt to enter into cells.

2. Results and discussion

2.1. The synthesis of TPP-glycine

The synthetic process of TPP-glycine was shown in Scheme 1. The TPP and TPP-glycine were identified by analyzing the absorption of hydrogen in Fig. 1.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.91 (6H), 8.83 (d, 2H, J = 4.7 Hz), 8.48 (d, 2H, J = 8.0 Hz), 8.35 (d, 2H, J = 8.0 Hz), 8.26 (3H), 8.25 (3H), 7.85–7.80 (m, 6H), 7.80–7.76 (m, 4H), 4.15 (s, 3H), −2.73 (s, 2H). MALDI-TOF MS $C_{46}H_{32}N4O_2$ calcd. for 672.25, found 672.21.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.91–8.74 (m, 8H), 8.34–8.17 (m, 10H, ArH), 7.83–7.70 (m, 9H, ArH), 4.44 (d, 2H, CH_2), 3.89 (s, 3H, CH_3), −2.78 (s, 2H). MALDI-TOF MS $C_{48}H_{35}N_5O_3$ calcd. for 729.27, found 730.26.

2.2. The optical properties of TPP, TPP-COOH, TPP-glycine

The absorption, emission intensity and fluorescence quantum yield results were displayed in Fig. 2 and Table 1, respectively.

These results indicate that introduced carboxyl group and further glycine methyl ester has no significant influence on the optical properties of the porphyrin core.

2.3. In vitro imaging

As shown in Fig. 3, the aggregation degree of TPP and TPP-glycine had no obvious change. However, aggregation degree of TPP-glycine D4 is much stronger than TPP D2 when interacted with iRGD [17]. Therefore, iRGD has the function which promotes probes to penetrate GLC-82 cytomembrane easily. The results were identical with those reported in literature [18].

2.4. MTT assay

MTT assay [19] can be used to detect the toxicity of cells. The results in Fig. 4a–c showed that the cell viability decreased with the increase of concentration of probe. Compared with TPP-glycine and TPP, the toxicity of TPP-COOH is obviously bigger than the others when the concentration of probes is higher than 5 μmol/L. The date has showed that added iRGD has no significant influence on cells. In a practical application, the safe concentration of probes for cells are lesser than 1 μmol/L. From Fig. 4, the values of cell viability are greater than 90% when the concentration of probes is lesser than 1 μmol/L. Therefore, the probes can be applied to detect tumor cells.

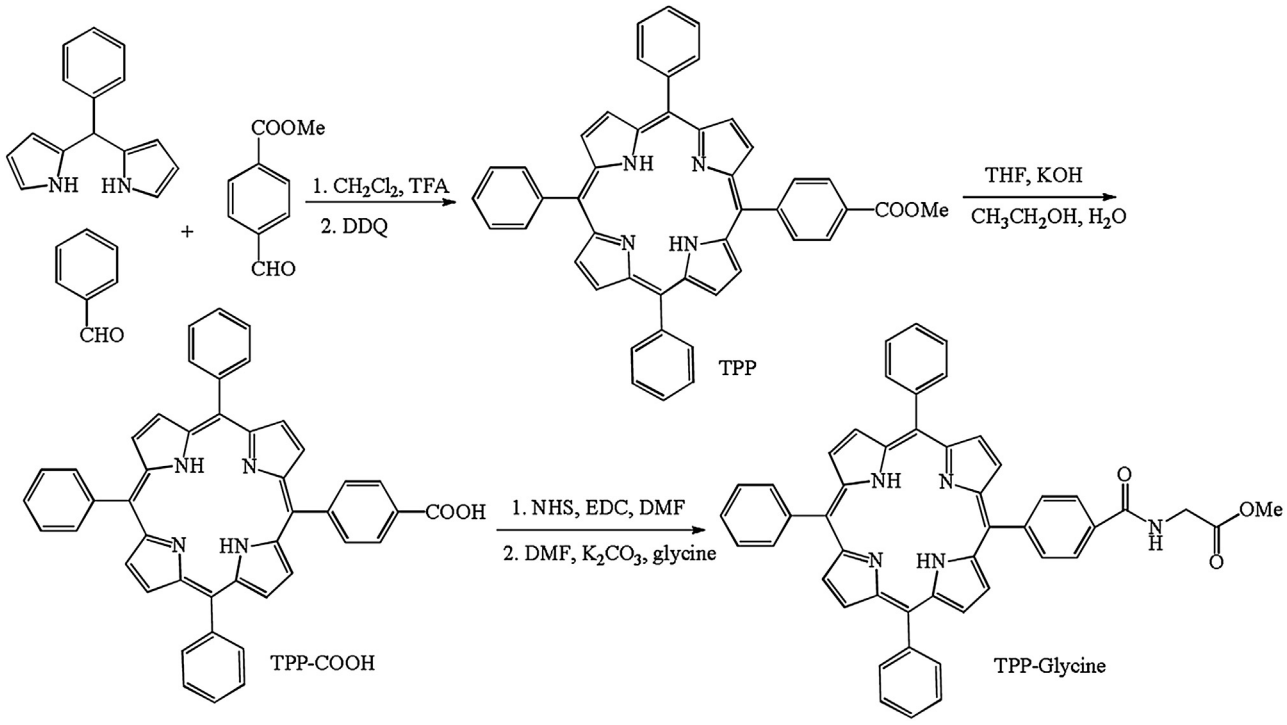
3. Conclusion

In this paper, TPP-glycine was synthesized and characterized by UV–spectrophotometer, fluorescence spectrophotometer, fluorescence microscope, and MTT assay. Experimental results showed that the porphyrins modified with glycine methyl ester had a good affinity with GLC-82 cells than other porphyrins when interacted with iRGD. And it also indicated that iRGD can penetrate GLC-82 cell membranes contribute to enter into cells of fluorescent probe TPP-glycine.

4. Experimental

4.1. Materials

Silica gel (200–300 mesh, 300–400 mesh), Liquid NMR (Bruker AVANCE III 400 M), MALDI-TOF-MS (Bruker Autoflex tof/tof III), UV–vis spectrophotometer (Shimadzu UV-1800), Fluorescence spectrophotometer (Varian Cary Eclipse), Transient Fluorescence (Horiba JY Fluorolog-3), Flow Cytometry (FACScalibur).



Scheme 1. Synthesis of the probe.

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