Glucosamine hydrochloride functionalized tetraphenylethylene: A novel fluorescent probe for alkaline phosphatase based on the aggregation-induced emission†

Qi Chen,[a] Ning Bian,[ab] Chun Cao,[a] Xi-Long Qiu,[ab] Ai-Di Qi[b] and Bao-Hang Han*[a]

Received 11th February 2010, Accepted 10th April 2010
First published as an Advance Article on the web 10th May 2010
DOI: 10.1039/c002894k

Grafting of glucosamine hydrochloride moieties to tetraphenylethylene (TPE) motif furnished a novel cationic water-soluble tetraphenylethylene derivative (GH-TPE). With aggregation-induced emission properties, GH-TPE was used for fluorometric detection to alkaline phosphatase through enzyme-triggered de-aggregation of the ensemble of GH-TPE and substrate.

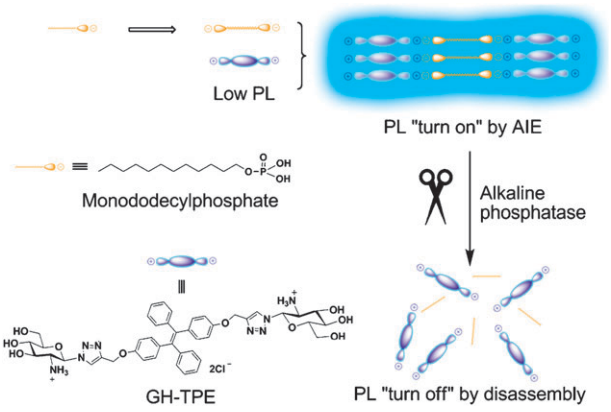
In clinical practice, enzymatic reactions are usually used in the diagnosis of disease. Alkaline phosphatase (ALP, EC 3.1.3.1), as one of the most commonly assayed enzymes, is capable of catalyzing hydrolysis of a wide variety of phosphate compounds and has broad substrate specificity *in vitro*.[1] Several diseases, such as bone disease,[2] liver dysfunction,[3] breast and prostatic cancer,[4] and diabetes,[5] can be preliminarily diagnosed in light of an abnormal level of alkaline phosphatase in serum.[6]

Among various methods for alkaline phosphatase assays, fluorescence-based assays have drawn much attention, because they are high sensitive, convenient, cost-effective, and easy to scale-up to a high-throughput screening format.[7] However, aggregation-caused fluorescence quenching of traditional dyes often takes place when dispersed in aqueous media or interacted with biomacromolecules, resulting in drastically negative effects on efficiencies and sensitivities of biosensors or bioprobes.[8] To overcome this problem, in this work, a novel cationic water-soluble tetraphenylethylene (TPE) derivative, GH-TPE, was prepared as a fluorescent probe for alkaline phosphatase assay based on the aggregation-induced emission effect.

Recently, molecules with aggregation-induced emission (AIE) characteristics provide a unique platform for exploiting novel optical materials [9] and sensors,[10] due to their enhanced emission in aggregate form or solid state.[11] Especially, since Tang's group reported the facile preparation of TPE-based luminophors by a one-step reaction in a one-pot procedure,[9a] TPE-based AIE active materials have already shown practical applications in OLEDs,[9a] chem-sensors,[12] and bio-probes.[8b,13] For example, TPE motif containing charged groups such as a sulfonate group or ammonium group was used in the establishment of a fluorometric assay method for acetylcholinesterase[13c] or studies of label-free DNA assay systems.[13b] To extend the potential applications of the TPE-based AIE active materials in biomacromolecule detection, we designed a glucosamine hydrochloride functionalized TPE, considering introduction of glucosamine hydrochloride moieties into a fluorescent dye molecule can not only improve its water-solubility and bio-compatibility, but also provide many positive-charged ammonium binding sites for the electrostatic interaction between the dyes and other artificial or natural polyanions.[14]

The design rationale for the ALP assay is schematically illustrated in Scheme 1. GH-TPE, possessing two units of glucosamine hydrochloride, is expected to display a weak photoluminescence (PL) in aqueous media. According to previous studies,[13c] monododecylphosphate, as an amphiphilic compound, can form a heteroaggregation complex with positively charged GH-TPEs based on the electrostatic binding when the concentration of monododecylphosphate is much lower than its critical micelle concentration.[15] As a result, the fluorescence of the ensemble would increase significantly, which is induced by the aggregation of GH-TPE. When treated with ALP, the aggregated complex would disassemble after loss of phosphate groups, resulting in a significant emission reduction of the system. Thus, the purpose to use GH-TPE as a fluorescent probe for alkaline phosphatase detection would be achieved.

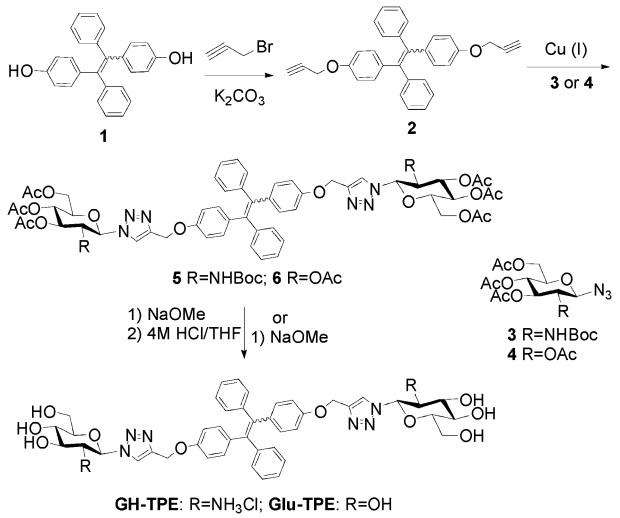
The synthetic route to GH-TPE is shown in Scheme 2. Propargylation of the known TPE derivative **1** [13a] afforded



Scheme 1 Illustration of fluorometric assay for alkaline phosphatase using GH-TPE as a fluorescent probe based on AIE feature.

[a] *National Center for Nanoscience and Technology, Beijing 100190, China. E-mail: hanbh@nanoctr.cn; Fax: +86-10-82545576*
[b] *College of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China*

† Electronic supplementary information (ESI) available: Preparation and characterization details for compounds **2**, **5**, **6**, Glu-TPE, and GH-TPE; absorption and emission spectra of the aggregate suspensions and complexes containing GH-TPE; ^{1}H and ^{13}C NMR spectra of compounds **2**, **5**, **6**, Glu-TPE, and GH-TPE. See DOI: 10.1039/c002894k



Scheme 2 Synthetic route to GH-TPE and Glu-TPE.

propargyl-attached TPE **2** under a basic condition. Cu(I)-catalyzed "click" ligation[16] between **2** and azido-functionalized glucosamine derivative **3**[14] furnished a sugar-bearing TPE **5** smoothly in 96% yield. Formation of the triazole ring is confirmed by the chemical shift at 7.93 ppm (single peak) on the ^{1}H NMR spectrum and two peaks at 122.2 and 144.3 ppm on the ^{13}C NMR spectrum (Fig. S1 in the ESI†). After removing all the acetyl groups with MeONa/MeOH and deprotecting the *N*-Boc groups in aqueous HCl-THF solution (4 M), the desired water-soluble GH-TPE was obtained in an excellent yield (90% in two steps) by a one-pot procedure. Similarly, a glucopyranosyl-bearing TPE (Glu-TPE) was also prepared for the control studies. Compared with the ^{1}H NMR spectrum of protected precursor **5**, we can find that the peak intensities of Boc (δ = 1.24 ppm), Ac (δ = 2.05 ppm), and NH-Boc (δ_{N-H} = 5.98 ppm) groups in **5** disappear after final efficient global deprotection and the peak of NH_3^+ group (δ = 8.61 ppm) in GH-TPE appears clearly (Fig. S1 in the ESI†), which confirms the successful preparation of the hydrochloride salt.

The protected dye **5** is soluble in common organic solvents such as acetonitrile, chloroform, and THF, but insoluble in water. GH-TPE, on the other hand, is soluble in polar solvents including water, DMF, and DMSO. In dilute aqueous solution, GH-TPE, as expected, does show a very limited luminescence. However, when methanol, acetonitrile, THF, or dioxane is added to its aqueous solution, the emission of GH-TPE in the mixture still remains as faint as it is in the aqueous solution, which means these organic solvents can not induce aggregation of the salt. Similar phenomenon was observed for studies of TPE derivatives containing other ammonium groups by Tang's group and was ascribed possibly to the amphiphilic nature of those TPE derivatives.[13b] Further investigation demonstrated that GH-TPE in the DCM/DMSO mixture with high DCM fractions was highly emissive, implying that GH-TPE is AIE-active (Fig. S2 in the ESI†).

With GH-TPE in hand, the investigation into electrostatic interactions between sodium dodecyl sulfonate (SDS) and TPE salt was first performed as model studies for GH-TPE/monododecylphosphate interactions, due to the similar chemical structure and amphiphilic property for SDS and monododecylphosphate. In dilute aqueous solution, GH-TPE is non-luminescent to the aforementioned. After treatment with SDS, the aqueous mixture is highly emmissive, due to formation of the GH-TPE/SDS ensemble. From the free state in aqueous solution to the aggregating state with SDS, the fluorescence intensity of GH-TPE at 469 nm increased by 9 fold (Fig. S3 in the ESI†). The non-emissive nature of free state and emissive nature of the aggregates were clearly indicated in the photographs given in Fig. S3 (ESI†), respectively.

Heteroaggregate complexation of the water-soluble GH-TPE with monododecylphosphate was studied by spectrometric titrations in aqueous phosphate buffer solution (PBS, 10 mM, pH = 8.0) at 25 °C. With addition of the monododecylphosphate [the concentration of monododecylphosphate (0–30 μM) is much lower than its critical micelle concentration, nearly 2.0 mM], the fluorescence intensity of GH-TPE gradually increased by 11 fold (Fig. 1). Meanwhile, a red shift as large as 90 nm (from 377 nm to 467 nm) of its emission maximum was observed. The absorption band around 312 nm also red-shifted by 16 nm in the presence of monododecylphosphate (30 μM) as shown in Fig. S4 (ESI†). These results are in agreement with the formation of the heteroaggregate complex. Photographs of GH-TPE and the ensemble GH-TPE/monododecylphosphate in PBS taken under illumination of a UV lamp are displayed in Fig. 1. To prove the NH_3^+ group of glucosamine is critical for the fluorescence change caused by the electrostatic interaction, Glu-TPE was also treated identically with monododecylphosphate (30 μM) and no significant fluorescence change was observed (Fig. S5 in the ESI†).

As aforementioned, ALP is able to catalyze the hydrolysis of monododecylphosphate into dodecyl alcohol and phosphic acid. Therefore, it is expected that the fluorescence of the ensemble of GH-TPE/monododecylphosphate would reduce after addition of ALP, because the interaction between GH-TPE and monododecylphosphate was weakened after loss of phosphate group, leading to disassembly of GH-TPE/monododecylphosphate heteroaggregate complexes and fluorescence reduction. Fig. 2 shows the fluorescence spectra of the ensemble of GH-TPE [20 μM in PBS (10 mM), pH = 8.0] and

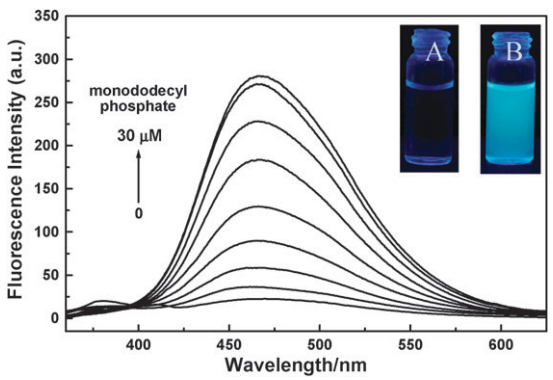


Fig. 1 Fluorescence spectra of GH-TPE [20 μM in PBS (10 mM) buffer solution, pH = 8.0] in the presence of different amounts of monododecylphosphate (from 0 to 30 μM); the inset displays the photos of the corresponding buffer solutions of GH-TPE (20 μM) in the absence (A) and presence (B) of monododecylphosphate (30 μM) under UV light (365 nm) illumination.

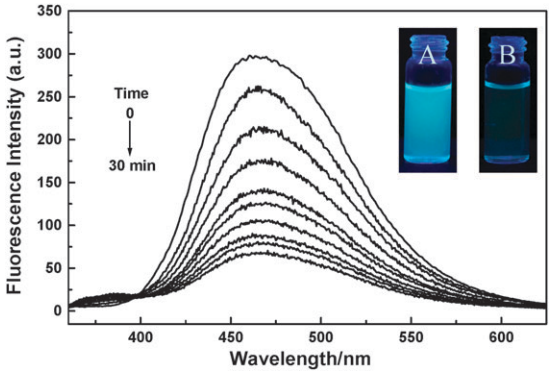


Fig. 2 Fluorescence spectra of the ensemble of GH-TPE [20 μM in PBS (10 mM), pH = 8.0] and monododecylphosphate (30 μM) in the presence of ALP (1.5 U/mL) incubated at 25 °C for different periods; the inset displays the photos of the corresponding solutions of GH-TPE and monododecylphosphate in the absence (A) and presence (B) of ALP (1.5 U/mL) after 30 min of incubation at 25 °C under UV light (365 nm) illumination.

monododecylphosphate (30 μM) in the presence of ALP (1.5 U/mL) after a different reaction time. Apparently, the fluorescence intensity of the ensemble started to decrease gradually upon treatment with ALP. By prolonging the hydrolysis time, significant reduction of the fluorescence intensity was observed. The fluorescence variation of the ensemble solution and the disassemble solution upon addition of ALP can be distinguished with the naked eye under UV illumination as shown in the inset of Fig. 2. Therefore, GH-TPE could be a potentially useful fluorescent probe for detection of ALP.

In summary, a novel cationic water-soluble tetraphenylethylene derivative, GH-TPE, was designed and efficiently synthesized. Grafting of glucosamine hydrochloride moieties into TPE can not only improve its water-solubility and biocompatibility, but also provide positive-charged ammonium binding sites towards other artificial or natural polyanions through electrostatic interaction. By taking advantage of the aggregation-induced enhanced emission feature, a convenient fluorescence-based assay for alkaline phosphatase has been developed. Heteroaggregate complexation of the GH-TPE with monododecylphosphate can "turn on" the photoluminescence. After addition of alkaline phosphatase, the fluorescence gradually reduced through enzyme-triggered de-aggregation of the ensemble of GH-TPE/monododecylphosphate. These results reveal that GH-TPE possesses potential applications in biomacromolecule detections and related inhibitor screening.

This work was supported by the Ministry of Science and Technology of China (National Basic Research Program, Grant 2007CB808000) and the National Science Foundation of China (Grant 20972035).

Notes and references

1 H. N. Fernley, *Enzyme*, 1971, **4**, 417.
2 D. Goltzman and D. Miao, *Encyclopedia Endocr. Dis.*, 2004, **1**, 164.
3 K. Ooi, K. Shiraki, Y. Morishita and T. Nobori, *J. Clin. Lab. Anal.*, 2007, **21**, 133.
4 G. Ramaswamy, V. R. Rao, L. Krishnamoorthy, G. Ramesh, R. Gomathy and D. Renukadevi, *Indian J. Clin. Biochem.*, 2000, **15**, 110.
5 G. M. M. Rao and L. O. Morghom, *Enzyme*, 1986, **35**, 57.
6 Y. Liu and K. S. Schanze, *Anal. Chem.*, 2008, **80**, 8605.
7 H. Sahoo, A. Hennig, M. Florea, D. Roth, T. Enderle and W. M. Nau, *J. Am. Chem. Soc.*, 2007, **129**, 15927.
8 (*a*) J. B. Birks, *Photophysics of Aromatic Molecules*, Wiley, London, 1970; (*b*) H. Tong, Y. N. Hong, Y. Q. Dong, M. Haussler, J. W. Y. Lam, Z. Li, Z. F. Guo, Z. H. Guo and B. Z. Tang, *Chem. Commun.*, 2006, 3705.
9 (*a*) Y. Dong, J. W. Y. Lam, A. Qin, J. Liu, Z. Li, B. Z. Tang, J. Sun and H. S. Kwok, *Appl. Phys. Lett.*, 2007, **91**, 11111; (*b*) K. H. Cheng, Y. Zhong, B. Y. Xie, Y. Q. Dong, Y. Hong, J. Z. Sun, B. Z. Tang and K. S. Wong, *J. Phys. Chem. C*, 2008, **112**, 17507; (*c*) S.-J. Lim, B.-K. An and S. Y. Park, *Macromolecules*, 2005, **38**, 6236; (*d*) Y. Liu, X. Tao, F. Wang, X. Dang, D. Zou, Y. Ren and M. Jiang, *J. Phys. Chem. C*, 2008, **112**, 3975.
10 (*a*) T. Sanji, K. Shiraishi and M. Tanaka, *ACS Appl. Mater. Interfaces*, 2009, **1**, 270; (*b*) M. C. Zhao, M. Wang, H. J. Liu, D. S. Liu, G. X. Zhang, D. Q. Zhang and D. B. Zhu, *Langmuir*, 2009, **25**, 676; (*c*) M. Wang, D. Q. Zhang, G. X. Zhang, Y. L. Tang, S. Wang and D. B. Zhu, *Anal. Chem.*, 2008, **80**, 6443; (*d*) M. Wang, D. Q. Zhang, G. X. Zhang and D. B. Zhu, *Chem. Commun.*, 2008, 4469.
11 (*a*) J. Luo, Z. Xie, J. W. Y. Lam, L. Cheng, H. Chen, C. Qiu, H. S. Kwok, X. Zhan, Y. Liu, D. Zhu and B. Z. Tang, *Chem. Commun.*, 2001, 1740; (*b*) J. Chen, C. C. W. Law, J. W. Y. Lam, Y. Dong, S. M. F. Lo, I. D. Williams, D. Zhu and B. Z. Tang, *Chem. Mater.*, 2003, **15**, 1535; (*c*) J. Xu, L. Wen, W. Zhou, J. Lv, Y. Guo, M. Zhu, H. Liu, Y. Li and L. Jiang, *J. Phys. Chem. C*, 2009, **113**, 5924; (*d*) J. He, B. Xu, F. Chen, H. Xia, K. Li, L. Ye and W. Tian, *J. Phys. Chem. C*, 2009, **113**, 9892; (*e*) S. Dong, Z. Li and J. Qin, *J. Phys. Chem. B*, 2009, **113**, 434.
12 (*a*) L. Liu, G. Zhang, J. Xiang, D. Zhang and D. Zhu, *Org. Lett.*, 2008, **10**, 4581; (*b*) A. Qin, J. W. Y. Lam, L. Tang, C. K. W. Jim, H. Zhao, J. Sun and B. Z. Tang, *Macromolecules*, 2009, **42**, 1412.
13 (*a*) H. Tong, Y. N. Hong, Y. Q. Dong, M. Haeussler, Z. Li, J. W. Y. Lam, Y. P. Dong, H. H. Y. Sung, I. D. Williams and B. Z. Tang, *J. Phys. Chem. B*, 2007, **111**, 11817; (*b*) Y. N. Hong, M. Haussler, J. W. Y. Lam, Z. Li, K. K. Sin, Y. Q. Dong, H. Tong, J. Z. Liu, A. J. Qin, R. Renneberg and B. Z. Tang, *Chem.–Eur. J.*, 2008, **14**, 6428; (*c*) M. Wang, X. Gu, G. Z. D. Zhang and D. Zhu, *Anal. Chem.*, 2009, **81**, 4444.
14 Q. Chen, Q.-Y. Cheng, Y.-C. Zhao and B.-H. Han, *Macromol. Rapid Commun.*, 2009, **30**, 1651.
15 (*a*) N. C. Maiti, S. Mazumdar and N. Periasamy, *J. Phys. Chem. B*, 1998, **102**, 1528; (*b*) Z. EI-Hachemi, G. Mancini, J. M. Ribó and A. Sorrenti, *J. Am. Chem. Soc.*, 2008, **130**, 15176.
16 (*a*) H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004; (*b*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596.