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A unique off-on near-infrared cyanine-based probe for imaging of endogenous alkaline phosphatase activity in cells and in vivo

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ABSTRACT

Alkaline phosphatase (ALP) is a class of enzymes that are widely found in various tissues of the human body. It plays important roles in regulating diverse cellular functions. The aberrant levels of serum ALP are implicated in diseases. The development of sensitive and accurate detection tool for evaluating the level changes of ALP in living organisms will be very helpful in the fields of biochemistry, cytology and clinical medicine. Herein, we develop a near-infrared probe (QcyP) for the specific detection of different ALP levels both in different cell lines and in tumor-bearing mice models. The probe is composed of two moieties: a unique heptamethine cyanine fluorophore and a phosphate monoester. ALP can trigger an off-on fluorescence switch via an enzyme-catalyzed cleavage of the phosphate group. The fluorescence regulation mechanism is based on the rearrangement of the conjugated π-electron system. The probe exhibits high selectivity and good sensitivity towards ALP. The results demonstrate that the probe QcyP not only can be applied to discriminate the levels of ALP in different cell lines, but also can distinguish the ALP level changes between hepatic tumor mice model and normal mice.

1. Introduction

Phosphatases are a large and structurally diverse family of signaling enzymes which can remove the phosphate group from protein and nonprotein substrates [1]. Among the various types of phosphatases, alkaline phosphatase (ALP) is the most common and crucial subclass, as it regulates biofunctions in the biogeochemical cycle of phosphorus [2]. ALP not only facilitates the hydrolysis of monoesters of phosphoric acid but also participates in transphosphorylation reaction in the presence of high concentrations of phosphate acceptors [2]. ALP widely exists in organisms from bacteria to eukaryotes [2]. In human, ALP involves in abundant isozymes, including intestinal alkaline phosphatase (IAP), placental alkaline phosphatase (PLAP), germ cell alkaline phosphatase (GCALP) and tissue non-specific alkaline phosphatase (TNAP). Especially, TNAP holds high expression level in liver, bone, kidney, as well as other tissues [3]. ALP behaves important roles in protein cyclic metabolism of phosphorylation and dephosphorylation in cells. It is well documented that the increase of endogenous ALP activity is closely related to the proliferation and differentiation of osteoblasts and bone mineralization [4]. Moreover, accumulating clinical manifestations suggest that the aberrant elevation of serum ALP level and activity is associated with many diseases, such as breast cancer, prostatic cancer, liver dysfunctions, intestinal diseases, bone diseases, and diabetes [2–5]. Therefore, the biological and chemical approaches must be developed to detect the expression level of ALP. And more importantly, the examination of ALP activity can provide invaluable insights into how it works under physiological settings and how it is modulated therapeutically under pathological conditions. Although extensive researches have been began from 1907, there still exist huge challenges for comprehending the complicated and confused cellular phosphoproteome network. The main obstacle is lacking of desirable chemical tools which are capable of in real-time investigating protein dephosphorylation in cells and in vivo [4]. Thence, it is necessary to establish a reliable, simple and sensitive method for ALP detection in biological systems.

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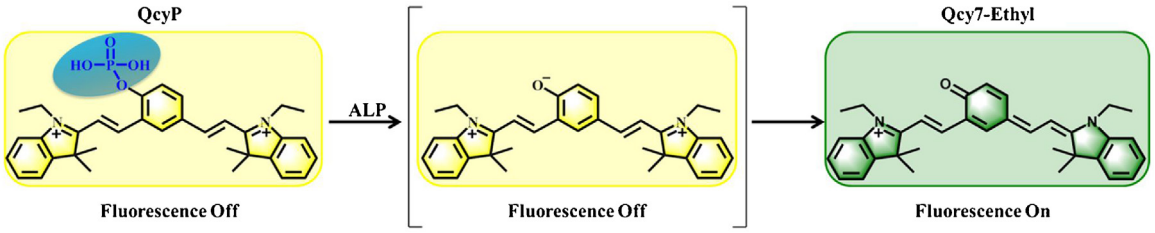

Scheme 1. Illustration of molecular structure and detection mechanism of QcyP towards ALP.

Recently, biological and chemical approaches have been developed for inspecting the expression level and activity of endogenous ALP, such as those based on electrochemistry, chromatography, colorimetric techniques and peptide microarray [6–9]. However, these detection technologies fail to provide an adequate view of ALP dynamic properties by high sensitivity, real-time detection, and high spatial resolution imaging in live samples [10,13]. Fluorescence imaging-based approaches have emerged to address some of these issues via focusing on the detection of ALP activities in cells at the proteome level [10–28]. Among them, small-molecule-based fluorescent probes are highly desirable due to their desirable chemical modification, excellent biocompatibility, and low cost [29]. Despite the promising properties of small-molecule probes offer effective and versatile utilizations to specific detection of ALP in complex proteomes (i.e., cell lysates) and living cells [30–35], but many of them are not suitable for deep imaging in tissues and animals owing to their short emission wavelength at UV–vis region, which hinders their further applications in biological systems. However, near-infrared (NIR) fluorescence bioimaging probes with NIR absorption and emission profiles (650–900 nm) can penetrate tissue more deeply and minimize the interference from background autofluorescence, which greatly facilitates for imaging of physiological processes in vivo [36,37]. Until now, only one hemicyanine-based NIR probe has been employed for the detection of ALP in vivo [10,13]. However, the fluorescent probe that can penetrate deeper tissue for ALP detection is still urgent need. Therefore, it is full of interest and anticipation to further exploit the development of new NIR fluorescent probe for detection of ALP in cells and in vivo.

Herein, we designed and synthesized a NIR fluorescent probe (QcyP) for selective evaluation of ALP enzymatic activity in various different living cells and mice models (Scheme 1). Based on a unique NIR fluorescent fluorophore QCy7-Ethyl, the probe showed an off-on fluorescence signal change with depending on the enzyme-substrate (ES) interaction. The results of kinetic experiments revealed that our probe possessed high binding capacity for ALP. We also evaluated the levels of ALP activities in eight human cell lines using QcyP. Finally, we successfully applied QcyP to compare the real-time process of ALP dynamic dephosphorylation in normal and HepG2 tumor-bearing mice.

2. Experimental

2.1. Synthesis of QcyP

The commercially available compound 4,4-Hydroxyisophthalaldehyde (50 mg, 0.33 mmol) and compound 3 (220.5 mg, 0.70 mmol) were dissolved in 150 mL mixed solution *n*-butyl alcohol/toluene (7:3, v/v). The mixture was refluxed for 3 h, and the reaction was monitored by TLC. Then the solvent was removed in vacuum. The crude product was dissolved into 100 mL CH_2Cl_2, and washed with H_2O (100 mL × 3). The organic layer was separated and concentrated. The silica gel column chromatography was used for the further purification. The eluent was EtOAc/CH_3OH (3:1, v/v). The final product Qcy7-Ethyl was a green solid, yield 70%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.72 (s, 1H), 8.43–8.42 (d, 1H), 8.00–7.99 (d, 1H), 7.91–7.68 (m, 4H), 7.66–7.50 (m, 4H), 6.95–6.93 (d, 1H), 6.80–6.77 (t, 1H), 6.63–6.62 (d, 1H), 6.60–5.98 (d, 1H), 3.35–3.28 (m, 3H), 3.23–3.16 (m, 1H), 1.91–1.83 (m, 12H), 1.79 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 184.85, 183.02, 168.56, 151.39, 143.87, 143.63,143.51, 142.16, 142.07, 138.87, 134.92, 130.35, 130.11, 126.27, 123.62, 123.40, 123.32, 120.31, 115.03, 114.77,112.87, 109.99, 51.54, 49.93, 45.02, 44.96, 26.51, 25.32, 11.53, 10.52. LC–MS (ESI$^+$): $C_{34}H_{37}N_2O^+$ calcd. 489.2900, found [M]$^+$: 489.2906.

Qcy7-Ethyl (25 mg, 5 mmol) was dissolved in 15 mL pyridine and then phosphorus oxychloride (10 μL, 5 mmol) was dropped into the mixed solution at 25 °C. After stirred for 0.5 h, 5 mL H_2O was added into the reaction system for another 0.5 h. Finally, the mixture was removed by rotary evaporator. The crude product was dissolved in the mixture of CH_2Cl_2 (100 mL) and H_2O (100 mL). The water phase was collected and concentrated under reduced pressure. The target product were purified by reversed phase silica C18 chromatographic column (75% CH_3OH/H_2O). The probe QcyP was afforded as a yellow solid, yield 60%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 9.07–9.03 (m, 2H), 8.60–8.45 (m, 2H), 8.27–8.04 (m, 3H), 7.68–7.41 (m, 4H), 7.02 6.82 (m, 3H), 6.26–6.25 (d, 1H), 4.61–4.43 (m, 4H), 1.99–1.78 (m, 18H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 183.99, 183.22, 154.40, 147.32, 145.12, 144.40, 144.03, 142.03, 142.00, 133.40, 133.33, 131.50, 131.25, 130.55, 129.98, 127.24, 127.07, 124.99, 123.28, 115.98, 115.88, 100.06, 54.32, 53.42, 53.36, 26.10, 26.05, 13.96, 13.52. ^{31}P NMR (150 MHz, DMSO-d_6) δ (ppm): −5.22. LC–MS (ESI$^+$): $C_{34}H_{39}N_2O_4P^{2+}$ calcd. 570.2636, found [M−H]$^+$: 569.2564.

2.2. Cell culture and fluorescence imaging

All tested cells were purchased from Chinese Academy of Sciences stem cell bank/stem cell technology platform. All cells were cultured at 37 °C in 5% CO_2. Cell culture medium, such as Dulbecco's modified Eagle's medium (DMEM), RPMI (Roswell Park Memorial Institute) 1640 Medium, Minimum Essential Medium (MEM), and Fetal Bovine Serum (FBS) were purchased from Gibco (Grand Island, USA). A549 cells, HeLa cells, PC9 cells, HepG2 cells, and SMMC-7721 cells were cultured with DMEM supplemented with 10% FBS. SH-SY5Y cells, and HL-7702 cells were cultured with RPMI-1640 supplemented with 10% FBS. HEK293 cells were cultured with MEM supplemented with 10% FBS and 1% L-glutamine. Fluorescent images of cells were acquired on an Olympus FluoView FV1000 laser-scanning microscope with an objective lens (×60). Excitation wavelength at 559 nm and collected wavelengths were in the range of 650–750 nm. Hoechst 33258, MitoTracker® Green FM, CellMask™ Orange Plasma Membrane stain, and ER-Tracker™ Green dye were purchased from Thermo Fisher Scientific Corporation. The co-localization parameters were analyzed by Image-Pro Plus software.