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Highly water-soluble, pH sensitive and biocompatible PAMAM 'dendrizyme' to maintain catalytic activity in complex medium

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ABSTRACT

The dendrimer based synthetic mimetic enzyme has been drawing great attention. However, this mimetic enzyme is different from the natural enzymes, which are pH sensitive, biocompatible and keep their catalytic activity in biological complex medium. A single zwitterionic layer composed by primary amine and carboxyl groups may be a useful method to obtain these properties. Herein, we report a novel facile method to prepare a mimetic enzyme. The complexes of generation 5 poly(amido amine) dendrimers (G5 PAMAM) with free hemin (G5Hs) were modified by the maleic anhydride and cysteamine. Results showed that the mimetic enzymes (G5HMCs) had pH sensitivity and good stability by varying the pH from 4 to 9, while significant precipitation was observed for free hemin at pH 5 after two days. The G5HMC (3:1) showed optimal catalytic activity at its isoelectric point. Furthermore, G5HMCs displayed excellent biocompatibility. The G5HMCs incubated with fibrinogen were stable for 24 h, while G5Hs immediately formed large aggregates. G5HMC (3:1 2 mg/mL) displayed little cytotoxicity with HeLa cells or A549 cells for 24 h, while G5H (3:1) had serious cytotoxicity, which was also demonstrated by cell morphology observation. At last, G5HMCs fully preserved their catalytic activity in bovine serum albumin (BSA) solution compared with phosphate buffer saline (PBS) solution, while hemin decreased to 73.5–81.5% catalytic activity in BSA solution, which was caused by the less interaction with BSA for G5HMCs than free hemin. The surface functionalization schemes described in this report would represent a versatile method to prepare water-soluble, pH sensitive, biocompatible, and efficient artificial enzymes for biomedical related applications.

1. Introduction

The dendrimer based synthetic enzyme mimic, 'dendrizyme', is considered as one of the best scaffolds to construct artificial enzymes due to their well-defined structures, globular shape and protein-like size [1–4]. Successful artificial enzymes should have the properties of natural enzymes, which have the tremendously high catalytic efficiency, selectivity, pH responsibility and compatibility in biological complex medium [5]. Most 'dendrizymes' have shown good catalytic efficiency and selectivity in non-aqueous solvents [6]. However, it is desirable to construct an enzyme mimic to improve its pH responsibility, biocompatibility and keep its catalytic ability in biological complex medium [7,8].

Most natural enzymes have a hydrophobic pocket region for catalytic reaction and the polar exterior for good solubility, pH responsibility, and biocompatibility in the aqueous solvents [5,9]. The polar exterior mainly consists of primary amine and carboxyl groups in a homogenous distributing state [10]. Constructing a similar surface based on dendrimers as scaffolds may be a feasible method to mimic natural enzymes. In order to enhance the biocompatibility of dendrimers, polyethylene glycol (PEG) is the most frequently used material for the surface functionalization. However, a relatively thick and dense PEG layer is necessary [11], which will significantly reduce the mass transport rate through PEG shell and decrease catalytic rate. These are due to the non-ionic property of PEG shell leading to weak interaction with natural proteins [12], which is obviously different from the zwitterionic property of natural enzymes shell [13]. Natural enzymes modified by PEG have lower bioactivity than native ones, while enzymes functionalized by zwitterionic materials can keep or even increase the bioactivity and stability [14–16]. In addition, the carboxybetaine acrylamide (CBAA) layer fully modified the complexes of G5 PAMAM with hemin has been demonstrated to have good compatibility and maintain the catalytic ability

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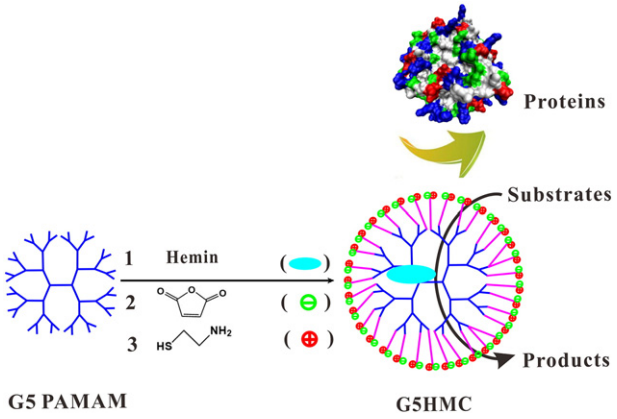
in biological complex medium, where the thickness of CBAA layer is only 3.1 nm [17,18]. However, CBAA contains a permanent positively charged quaternary amine and a negatively charged carboxyl group. The presence of these charged groups leads to be impossible to achieve fully switchable cationic/zwitterionic/anionic states [19]. However; natural enzymes are very sensitive to changes of pH. Each enzyme has its own optimum pH range where it will be most active. A polymer based on amino acid has been achieved reversible charge switching among cationic/zwitterionic/anionic states [20]. G4-PAMAM-OH was functionalized by Boc-Asp-OH to achieve a zwitterionic layer [21]. The biocompatibility was assessed by hemolysis and cell viability assays, while the effects of pH on the charge states and the stability of particles have not been investigated. This reaction also needed harsh conditions. Therefore, a facile method with mild reaction conditions is necessary to construct a highly water-soluble, pH sensitive and biocompatible PAMAM 'dendrizyme' with a single zwitterionic layer composed by primary amine and carboxyl groups, which may maintain catalytic activity in complex medium.

In the present study, a synthetic enzyme mimic with a zwitterionic shell composed by primary amine and carboxyl groups was prepared (Scheme 1). Initially, free hemin molecules were encapsulated in the cavities of G5 PAMAM through supramolecular assembly. Furthermore, maleic acid and cysteamine molecules were conjugated to the dendrimer surface. Thus, a zwitterionic shell with equal amount primary amine groups to carboxyl groups was formed. Hemin is an iron–porphyrin complex that can mimic the catalytic properties of horseradish peroxidase (HRP), which is used for various bio-related detections [22,23]. Porphyrins have been also widely studied in light-harvesting complexes for cancer therapy [24,25]. To the best of our knowledge, it is the first time that highly water-soluble, pH sensitive and biocompatible PAMAM 'dendrizyme' was constructed for catalysis in biological medium. This mimetic enzyme was protected by a zwitterionic shell composed by primary amine and carboxyl groups.

2. Experimental details

2.1. Materials

DMEM medium, Trypsin 0.25% and fetal bovine serum (FBS) were purchased from Sijiqing Biological Engineering Materials Co., Ltd. Dialysis bags (MW Cut-off (MWCO) 14,000) were purchased from Spectrum Laboratories Inc. Vivaspin 500 ultrafiltration tubes with polyethersulfone membrane (MWCO = 10.000) were acquired from Sartorius. Methanol, dimethyl sulfoxide, sodium chloride, sodium hydroxide, acetone, sulphuric acid, and hydrochloric acid were purchased from Sinopharm Chemical Reagent Beijing Co. Ltd. Bovine serum albumin (BSA) was purchased from Sangon Biotech (Shanghai) Co. Ltd. Generation 5 amine-terminated PAMAM dendrimers with ethylenediamine core in 5% methanol solution and fibrinogen from bovine plasma were purchased from Sigma-Aldrich. Cysteamine was purchased from J&K, maleic anhydride, chloro[3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropanoato(2-)]iron(III) (hemin), hydrogen peroxide, 1,2-diaminobenzene (OPD), and 3-[4,5]-dimethylthiahiazo(-z-y1)-3,5-diphenytetrazoliumromide (MTT) were purchased from Aladdin. UV/VIS spectra were recorded with a Shimadzu UV-2550 spectrophotometer (with 10 mm and 5 mm quartz cells). All cell lines were purchased from China Center for Typical Culture Collection.



Scheme 1. Schematic presentation of the construction of highly water-soluble, pH sensitive and biocompatible PAMAM 'dendrizyme' to maintain catalytic activity in complex medium (not to scale).

2.2. Preparation of complexes of G5 PAMAM with free hemin (G5Hs)

G5 PAMAM (8 mg) was dissolved in 400 μL methanol and 400 μL free hemin borate buffer solution to control the final molar ratio of hemin to G5 PAMAM for 1:1, 3:1, or 6:1. The mixed solution was stirred one day, then free hemin was extracted by acetone ($V_{acetone}$:$V_{mixed\ solution}$ = 10:1). The remaining hemin encapsulated in the G5Hs was determined by subtraction of the extracted free hemin from the total hemin. The molar absorption coefficient of hemin at 380 nm in PBS solution (pH 7.4) is 17,460 M^{-1} cm^{-1} [17]. The mixture was dialyzed against water with a cellulose dialysis membrane (MWCO = 14.000).

2.3. Synthesis of maleic modified G5Hs (G5HMs)

Maleic anhydride dissolved in DMSO was added to G5H dissolved in DMSO solution with the molar ratio of maleic anhydride/—NH_2 for 2:1. The mixed solutions were stirred for 24 h and dialyzed against water with a cellulose dialysis membrane (MWCO = 14.000). The leakage of hemin from G5Hs during the maleic modification was measured. Yield: 90%.

2.4. Synthesis of cysteamine modified G5HMs (G5HMCs)

54 mg cysteamine was added into the 27.5 nmol G5HMs aqueous solution. The final molar ratio of sulfhydryl group to double bond of G5FM was 20:1. The mixed solution reacted at room temperature for 24 h. The products of G5HMCs were dialyzed against water with a cellulose dialysis membrane (MWCO = 14.000). The leakage of hemin from G5HMs during the cysteamine modification was measured. Yield: 95%.

2.5. Catalytic reaction with G5HMCs

The catalytic reaction procedures were used according to an already reported procedure [17]. OPD can be oxidized to DAP by hemin in the presence of H_2O_2. Briefly the catalytic reactions at different pH were achieved in citrate-phosphate buffer (0.07 M, pH from 3.0 to 8.0) with the 1500 rpm at 25 °C. Citrate-phosphate buffer (600 μL 0.1 M) was added into 2 mL polyethylene pipe tube, then 200 μL 2×10^{-2} M OPD in water, 50 μL 17.2 μM catalyst in water, 10 μL H_2O_2 was added in turn. The final concentrations of catalytic center (based on hemin) and H_2O_2 were 10^{-6} M and 9.084×10^{-3} M, respectively. The final concentration of OPD was 4.65×10^{-3} M. Then the mixed solutions were incubated in the dark for 4 min and were added to equal volume 2N H_2SO_4 to quench the catalytic reaction. The effects of protein solution on the catalytic rates were measured in PBS solution (0.15 M, pH 7.4) with or without BSA (1 mg/mL) with the 1500 rpm at 25 °C.

Kinetic parameters (K_m, V_{max}, and k_{cat}) were determined by Lineweaver–Burk method. The reciprocal of the initial velocity ($1/v$) was plotted against the reciprocal of the initial substrate concentration ($1/[S]$). k_{cat} was calculated through dividing V_{max} by the catalytic center