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A design for fast and effective screening of hyaluronidase inhibitor using gold nanoparticles

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ABSTRACT

A hyaluronic acid-coated gold nanoparticle (HA-AuNPs) was synthesized and further employed for hyaluronidase (Hyal) assay and for screening effective inhibitors. The negatively charged hyaluronate on the surface of AuNP enhanced the solubility and stability of colloidal HA-AuNPs in aqueous solution. The HA-AuNPs in the solution possess a characteristic absorption band at 518 nm, whereas at the aggregation form, the band gradually red-shifts to 630 nm in various concentration of NaCl solution. The NaCl-induced red-shift absorption became steady when NaCl concentration reached 375 mM or higher concentration. In an enzymatic assay, Hyal promotes the hydrolysis of hyaluronate on AuNPs to produce more negatively charged HA oligomers, which can increase the surface shielding of AuNPs and to be released into solution to bind with sodium ion from NaCl. Both effects can largely prevent the aggregation of AuNPs. With this feature, inhibitors of Hyal can be effectively screened by monitoring the absorption change at 630 nm. Several effective inhibitors were found from a chemical library containing 2000 compounds. Thioguanine, harmine, and phenylbiguanide were selected for IC_{50} measurement using this newly designed method with values of 4.5, 13.3, and 28.7 μM, respectively. The same concept can be easily applied to the case of other polysaccharide degrading enzyme, such as chitosanase, condroitinase, haparinase, etc.

1. Introduction

With the advances in combinatorial chemistry and molecular biology in the past few decades, there has been a significant increase in the number of compounds synthesized and isolated from natural products. Therefore, high-throughput screening (HTS), a technique that involves testing large libraries of chemicals for their ability to combat a chosen target for a particular disease, has been widely used in drug discovery. The appropriate selection of target correlated to disease and effective methods for detection are the keys for the successful drug screening system. The ability of a chemical to affect the activity of the selected target may be reflected on the changes of viscosity [1], turbidity [2–4] and/or color [5]. However, most of these methods are tedious and difficult to handle for large amount of samples in a regular laboratory, particularly for those assays with no color change.

Due to the easy preparation [6–8] of high biocompatibility and colorimetric characterization, the gold nanoparticle (AuNP) solution has attracted tremendous attention in the past few years. The unique physical and chemical properties associated with particle size and interactions between particle-to-particle [9–11] have made colloidal gold a good candidate for biosensing applications. The colorimetric property of AuNPs varies noticeably with the distance between particles [12,13] which is due to the surface plasmon resonance (SPR) shift. Several biosensors based on the aggregation/dispersion of AuNPs have been proposed to detect proteins [14,15], nucleic acids [16,17], small molecules, and metal ions [18–21]. However, the application of AuNPs in enzymatic study and inhibitor screening is to a great extent less reported.

Hyaluronidase (Hyal) is an enzyme that catalyzes the hydrolysis of hyaluronic acid (HA). The biological function of Hyal has been implicated in allergic reaction, inflammation, and cancer development [22–24]. Several methods, such as the conventional Morgan–Elson method [25], immune luminescence and ELISA [26,27] have been developed for Hyal assay. However, those methods often suffered from low efficiency, high cost, and/or time-consuming procedures. Taking the advantages of the ease of HA-AuNPs preparation and the detectable colorimetric shift from chemical-induced colloidal aggregation/dispersion of HA-AuNPs under the existence of Hyal, a fast and convenient method is developed for monitoring the catalytic efficiency of Hyal. Screened

Abbreviations: AuNPs, gold nanoparticles; HA-AuNPs, hyaluronic acid-coated gold nanoparticles; SPR, surface plasmon resonance; Hyal, hyaluronidase; BTHyal, bovine testicular hyaluronidase.

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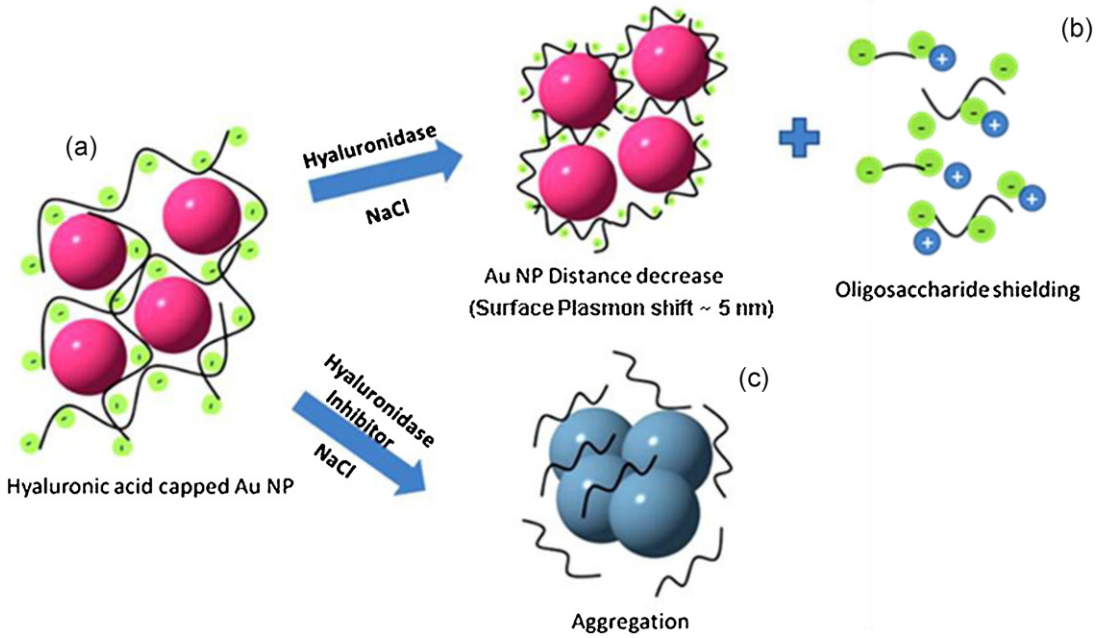

Fig. 1. Schematic illustration of the colorimetric detection of Hyal inhibitors based on the aggregation/dispersion of the HA-AuNPs. (a) The colloidal HA-AuNPs are stabilized by negatively charged HA. (b) The hydrolysis of HA catalyzed by Hyal can produce more negatively charged oligosaccharides which prevent the aggregation of AuNPs in the presence of electrolyte (e.g. NaCl). (c) When Hyal is inhibited, HA oligomers are not sufficiently produced. As a consequence, the presence of electrolyte induces the aggregation of AuNPs.

potential inhibitors from a chemical library were also evaluated. The principle of this proposed method is illustrated in Fig. 1. The negatively charged hyaluronate on the surface of AuNPs enhances the stability and dispersion of colloidal HA-AuNPs (Fig. 1a). Yet, the dispersion can be destroyed by adding excessive electrolyte such as NaCl. The existence of Hyal can hydrolyze HA to produce more negatively charged HA oligomers. These oligomeric fragments may stay on AuNPs and increase the surface shielding of AuNPs and/or may be released into the solution to bind with sodium ions. Consequently, the presence of Hyal can increase electrolyte-holding capacity of HA-AuNPs and prevent them from aggregation (Fig. 1b). When Hyal activity is inhibited, the HA-AuNPs will aggregate at the presence of electrolyte (Fig. 1c). Therefore, the inhibitor of Hyal can be screened by monitoring the change of color. The study successfully demonstrates that HA-AuNPs method can be employed for screening Hyal inhibitor.

2. Materials and methods

Tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate, hyaluronic acid, bovine testicular hyaluronidase (BTHyal) were purchased from Sigma. All chemicals were of analytical grade or the highest purity available. A chemical library (Microsource Discovery Systems Inc.) containing 2000 biological active compounds was used for screening the potential candidate inhibitor. All compounds were prepared and stocked in DMSO with concentration of 10 mM.

2.1. Preparation of HA-AuNPs

A 10-mL aqueous solution containing hyaluronic acid (0.01%) and $HAuCl_4$ (0.5 mM) was prepared and stirred at room temperature for 1 h. The solution was kept at 4 °C before $NaBH_4$ solution (2 mL, 0.1 M) was added. The color of solution changed from black to dark red immediately after the addition of $NaBH_4$. The HA-AuNPs (that is, the black suspended particles) were separated by centrifugation and further washed with deionized water several times. The nanoparticles were kept at 4 °C before use.

2.2. Characterization of HA-AuNPs

The FT-IR spectra of HA-AuNPs were collected by Nicolet™ Avatar™ spectrometer (ranging from 500 to 4000 cm^{-1}) and analyzed by OMNIC software. UV–vis spectrometer (HP Diode Array 8453A) was utilized to measure the spectra of the HA-AuNPs in various conditions. The images of HA-AuNPs were taken by scanning electron microscope (JEOL JSM 6700F).

2.3. Colorimetric screening of BTHyal inhibitors

Two thousand compounds from the chemical library (MicroSource Discovery Systems, Inc.) were used for screening the potential candidate of BTHyal inhibitor. Each compound solution (20 μL, 0.5 mM in 5% DMSO) was mixed with BTHyal (5 μL, 0.1 mg/mL) and injected into a well of a 96-well plate containing HA-AuNPs (125 μL, 0.25 mM). After reaction for 2 min at room temperature, NaCl solutions (90 μL, 1 M) were added, and the resulting mixture was analyzed using an ELISA reader (Multi scan GO, Thermo Scientific) at a wavelength of 630 nm or simply inspected by naked eye. Same procedures and conditions were used to measure the inhibitory ability of the selected compounds except the final concentration of the compound which varied at a range from 0.2 to 250 μM.

3. Results and discussion

3.1. Characterizations of HA-AuNPs

Gold nanoparticles coated with hyaluronic acid were prepared using sodium borohydride reduction method as described in Section 2. The formation of network-like structure of hyaluronic acid on the surface increased the stability of nanoparticles, which possess a shelf life, under ambient temperature, for more than 6 months. Based on scanning electron microscopy (SEM) analysis (Fig. 2A) and dynamic light scattering spectroscopy analysis (90Plus Particle Size Analyze, Brookhaven Instrumt., US) (Fig. 2B),