Biosensors and Bioelectronics 54 (2014) 7–14

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Direct detection of hyaluronidase in urine using cationic gold nanoparticles: A potential diagnostic test for bladder cancer

Ahmed Ibrahim Nossier [a], Sanaa Eissa [b], Manal Fouad Ismail [c], Mohamed Ahmed Hamdy [c], Hassan Mohamed El-Said Azzazy [d,*]

[a] Biochemistry Department, Faculty of Pharmacy, Misr University for Science and Technology (MUST), 6th October City, Egypt
[b] Oncology Diagnostic Unit, Medical Biochemistry & Molecular Biology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[c] Biochemistry Department, Faculty of Pharmacy, Cairo University, Giza, Egypt
[d] Department of Chemistry & Yousef Jameel Science & Technology Research Center, The American University in Cairo, New Cairo, Egypt

ARTICLE INFO

Article history:
Received 13 August 2013
Received in revised form
10 October 2013
Accepted 11 October 2013
Available online 31 October 2013

Keywords:
Hyaluronidase
Hyaluronic acid
Bladder Cancer
Gold Nanoparticles
Chitosan

ABSTRACT

Hyaluronidase (HAase) was reported as a urinary marker of bladder cancer. In this study, a simple colorimetric gold nanoparticle (AuNP) assay was developed for rapid and sensitive detection of urinary HAase activity. Charge interaction between polyanionic hyaluronic acid (HA) and cationic AuNPs stabilized with cetyl trimethyl ammonium bromide (CTAB) led to formation of gold aggregates and a red to blue color shift. HAase digests HA into small fragments preventing the aggregation of cationic AuNPs. The nonspecific aggregation of AuNPs in urine samples was overcome by pre-treatment of samples with the polycationic chitosan that was able to agglomerate all negatively charged interfering moieties before performing the assay. The developed AuNP assay was compared with zymography for qualitative detection of urinary HAase activity in 40 bladder carcinoma patients, 11 benign bladder lesions patients and 15 normal individuals, the assay sensitivity was 82.5% vs. 65% for zymography, while the specificity for both assays was 96.1%. The absorption ratio, A_{530}/A_{620} of the reacted AuNP solution was used to quantify the HAase activity. The best cut off value was 93.5 μU/ng protein, at which the sensitivity was 90% and the specificity was 80.8%.The developed colorimetric AuNP HAase assay is simple, inexpensive, and can aid noninvasive diagnosis of bladder cancer.

1. Introduction

Hyaluronidases (HAases) are a family of extracellular matrix-digesting endoglycosidases that digest hyaluronic acid (HA); a nonsulfated linear glycosaminoglycan consisting of repeating units of D-glucuronic acid and N-acetyl-D-glucosamine (Laurent and Fraser, 1992). HA is one of the most versatile macromolecules of the extracellular matrix of connective, growing and tumor tissues (Catterall, 1995; Delpech et al., 1997; Tammi et al., 2002). It actively regulates several physiological processes such as cell adhesion, migration and proliferation (Turley et al., 2002). HAases are present in normal tissues (e.g., liver, kidney, and testis) and the expression of these enzymes appears to be tissue specific (Zhu et al., 1994). Interestingly, HAase is termed as a spreading factor as it is essential for the spread of bacterial infections, toxins and venoms (Girish et al., 2004). Elevated plasma levels of HAase and HA have been reported to be associated with the presence of tumors. Angiogenic HA fragments generated by the enzymatic action of HAase have been detected in high levels in numerous human tumors, such as bladder cancers (Lokeshwar et al., 1997; Eissa et al., 2005) and prostate cancers (Lokeshwar et al., 2001). High urinary levels of HAase have been used for detection of high-grade bladder carcinoma (Eissa et al., 2010; Pham et al., 1997).

Bladder carcinoma is among the five most common malignancies worldwide. It is the second most common tumor of the genitourinary tract and the second most common cause of death in patients with genitourinary tract malignancies (Howlader et al., 2011). Classical cytology and cystoscopy are the main methods for the diagnosis of patients with bladder cancer. Voided urine cytology has a lower sensitivity for detecting low-grade tumor making the frequent use of invasive cystoscopy necessary. The development of a sensitive noninvasive diagnostic test that may specifically detect bladder carcinoma in the early stages would improve the clinical outcomes as it would allow early treatment (Kaufman et al., 2009).

Several methods have been reported for assaying HAase activity most of which depend on determining the amount of degraded HA generated by the action of HAase. Physicochemical methods such as turbidometry and viscometry (Bachtold and Gebhardt,

* Correspondence to: Department of Chemistry, The American University in Cairo, SSE, Rm # 1184, AUC Avenue, P.O. Box 74, New Cairo 11835, Egypt. Tel.: +20 226 152 559; fax+20 227 957 565.
E-mail address: hazzazy@aucegypt.edu (H.M.E. Azzazy).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.10.024

1952; Balazs and Euler, 1952) require large amounts of enzymes and are relatively inaccurate. While, spectrophotometric methods such as fluorometric (Nakamura et al., 1990; Rich et al., 2012; Fudala et al., 2012) and colorimetric approaches (Bonner and Cantey, 1966; Gregory and Robert, 1997) are appropriate for high-throughput platforms for assaying enzymatic activity and screening inhibitors. However, these methods require substrate modifications with fluorescein or biotin at the free carboxyl groups of HA that may affect the activity of HAases. HA substrate gel zymography was also developed (Guntenhoner et al., 1992), but this method involves time-consuming prerequisite steps such as overnight soaking.

Gold nanoparticles (AuNPs) display a distinctive phenomenon known as surface plasmon resonance (SPR) that depends on the AuNP size and the inter-particle distance (Rechberger et al., 2003). In colloidal solution this SPR is responsible for the red coloration of dispersed AuNPs with interparticle distance larger than the average particle diameter, and the blue coloration of aggregated AuNPs with interparticle distance smaller than the average particle diameter (Jain et al., 2006).These exclusive optical properties have allowed the use of AuNPs in developing simple and rapid colorimetric assays for detection of biomarkers as proteins, peptides and nucleic acids with higher sensitivity and specificity than the existing detection methods (Radwan and Azzazy, 2009; Choi et al., 2010).

Kim et al. (2009) developed a fast and simple method to assay HAase activity using cationic gold nanoparticles (cysteamine stabilized AuNPs). The assay is based on charge interaction between polyanionic HA and cationic AuNPs leading to formation of gold aggregates and a simultaneous red to blue color shift. After the enzymatic reaction of HAase, polymeric HA is degraded into small fragments which are unable to aggregate AuNPs. In comparison with other HAase assays, this method showed comparable or even better performance with no need for substrate modifications with fluorescin as in the fluorometric method (Nakamura et al., 1990) or any additional steps to provoke color changes of fragmented HA as in the colorimetric approach (Bonner and Cantey, 1966). However, this assay was not investigated for detection of HAase activity in clinical specimens probably due to non-specific

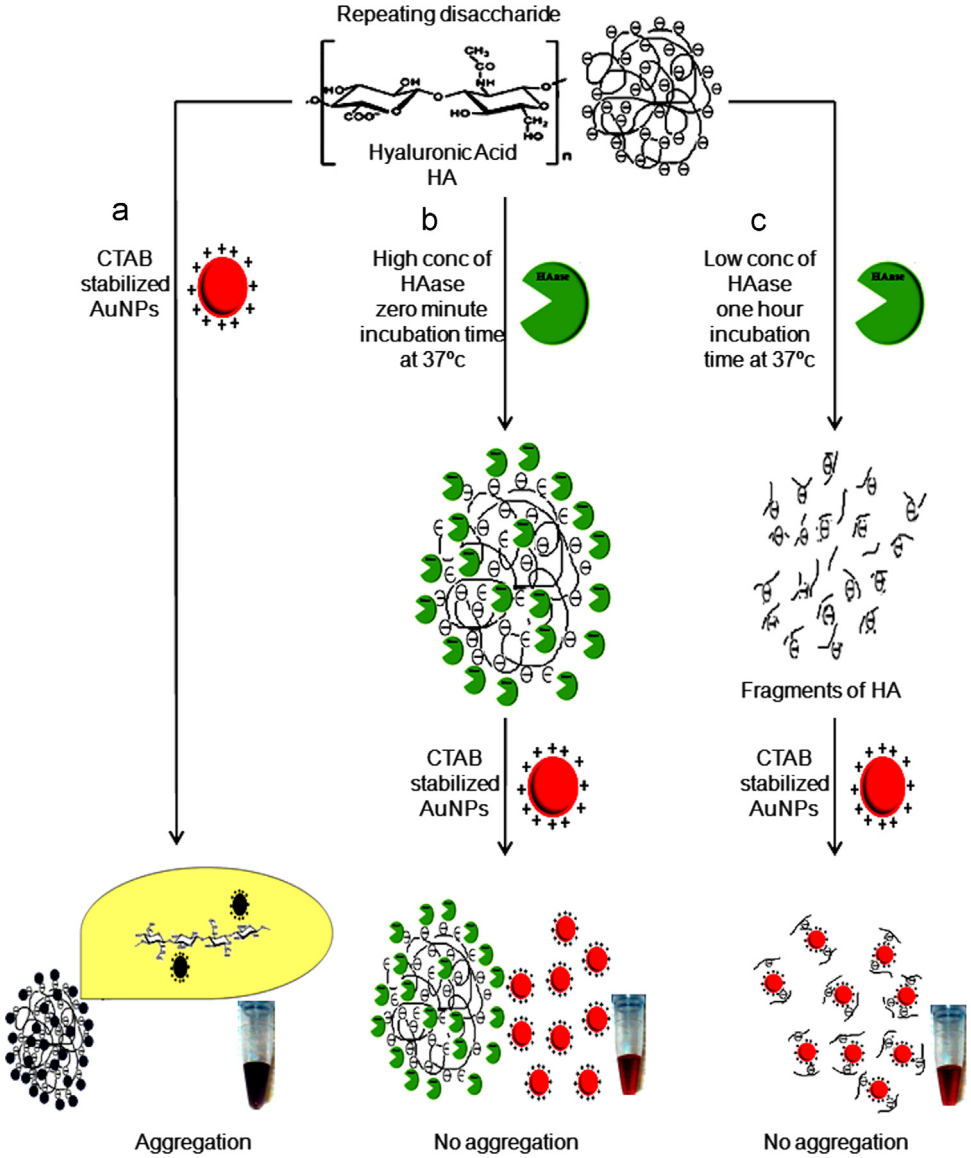

Fig. 1. The principle of cationic AuNP based colorimetric assay for direct detection of HAase activity. (a) Polyanionic polymer (HA) causes the aggregation of CTAB stabilized AuNPs with red to blue color shift. (b) At high concentration of HAase, enzyme molecules bind all HA molecules electrostatically preventing aggregation of AuNPs and the solution remains red. (c) At low concentration of HAase, it needs an enzymatic reaction time at 37 °C to allow the enzyme molecules to digest HA into small fragments and upon addition of AuNPs the solution remains red.