Chinese Chemical Letters 27 (2016) 847–851

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Original article

A selective, sensitive and label-free visual assay of fructose using anti-aggregation of gold nanoparticles as a colorimetric probe

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ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form 23 December 2015
Accepted 11 January 2016
Available online 23 January 2016

Keywords:
Colorimetric sensor
Gold nanoparticles
Anti-aggregation
Fructose

ABSTRACT

A new convenient colorimetric sensor for fructose based on anti-aggregation of citrate-capped gold nanoparticles (AuNPs) is presented. 4-Mercaptophenylboronic acid (MPBA) induces the aggregation of AuNPs, leading to a color change from red to blue. Fructose as a potent competitor has strong affinity for MPBA and a borate ester is formed between MPBA and fructose. There is an obvious color change from blue to red with increasing the concentration of fructose. The anti-aggregation effect of fructose on AuNPs was seen by the naked eye and monitored by UV–vis spectra. Our results showed that the absorbance ratio (A_{519}/A_{640}) was linear with fructose concentration in the range of 0.032–0.96 mmol/L (R^2 = 0.996), with a low detection limit of 0.01 mmol/L (S/N = 3). Notably, a highly selective recognition of fructose was shown against other monosaccharide and disaccharide (glucose, mannose, galactose, lactose and saccharose). With anti-aggregation assays higher selectivity is achievable. The results of this work provide a rapid method for evaluating the quantitative analysis of fructose in human plasma at physiologically meaningful concentrations and at neutral pH. The proposed procedure can be used as an efficient method for the precise and accurate determination of fructose.

1. Introduction

Fructose is an important dietary source of carbohydrates, and is a simple sugar found in many fruits. In equal amount, it is sweeter than glucose or sucrose and is therefore commonly used as a bulk sweetener. An increase in high fructose consumption results in obesity and metabolic disorders. Owing to its unique metabolic properties, fructose promotes adverse metabolic changes, including glucose intolerance, hyperlipidemia, hypertension, and hyperuricemia [1–5]. It is obvious that fructose is poorly absorbed from the digestive tract when it is consumed alone. Absorption improves when fructose is consumed in combination with glucose and amino acids [6]. Conventional methods such as high performance liquid chromatography [7], fluorometry [8], chemiluminescence [9] and electrochemical analysis [10] have been used to measure fructose. However, these analytical methods demand bulky and expensive equipments, and complicated sample treatment, which limit their applications for rapid and on-field analysis. D-Fructose dehydrogenase (FDH) is used as a biosensor to detect the presence of D-Fructose. The enzymes usually suffer from several disadvantages. For example, they are instable and susceptible to denaturation and inactivation under inappropriate experimental conditions such as high temperature and very acidic/basic pH and their action is easily blocked by chemicals [11–13]. The costly enzyme makes the procedure for fructose determination too expensive to perform.

Several studies are still being carried out to obtain faster and more selective methods of fructose analysis. To this end, we see considerable research interest in developing colorimetric methods based on synthetic ligands as the fructose-recognition moieties. The effectiveness of boronic acids as receptors and ligands in chemosensors for saccharides is more evident in the related publications. The strength of boronic acid binding to saccharides is determined by the orientation and relative position of the hydroxy groups, thus boronic acids can differentiate structurally similar saccharide molecules. It is now known that monoboronic acids exhibit inherent fructose selectivity among monosaccharides and have greater binding affinity for fructose at neutral pH [14]. The generally observed binding affinity of phenylboronic acids with monosaccharides follows the order of fructose > galactose > mannose > glucose [15,16]. The fructose sensor using boronic acids as a recognition ligand based on photonic crystals, microcantilevers, gold electrodes and surface-enhanced Raman scattering have been developed [17–20]. In a

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http://dx.doi.org/10.1016/j.cclet.2016.01.022

study recently published, a colorimetric sensor based on AuNPs for the detection of fructose by modification of AuNPs by 3-aminophenyl boronic acid (APB) and L-glutamic acid-(2,2,2)-trichloroethyl ester (GTE) moieties is presented [21]. However, the sophisticated instrumentation, relatively complex sample pretreatment procedures, long detection time and high limit of detection involved in these methods limited their practical applications.

Gold nanoparticles (AuNPs)-based colorimetric sensing methods provided an alternative for fructose detecting. Due to the unique chemical and physical properties, AuNPs have been used for developing the colorimetric sensors, which can be easily monitored by the naked eye or an ultraviolet–visible (UV–vis) spectrophotometer [22,23]. In colorimetric methods based on Au NPs, the modification is the critical step. The analyte-specific ligand is applied as a modifier. The coordination between the ligand and the analyte induces the aggregation and thus color change of AuNPs, which provides the basis for qualitative and quantitative analysis of the analyte. In comparison, a modification-free AuNPs assay is not only cost-effective, but also avoids complicated surface modifications and tedious separation processes.

Herein, a facile, highly sensitive colorimetric strategy for fructose detection is proposed based on the anti-aggregation of AuNPs through the competition reaction between the self-dehydration condensation of the aggregation agent 4-mercaptophenylboronic acid (MPBA) and the esterification of MPBA with fructose. With the addition of fructose, MPBA would prefer reacting with fructose to form stable boronic ester *via* boronic acid–diol binding. An increase in the concentration of fructose decreases the amount of free MPBA thiol group, resulting in less AuNPs aggregation and the solution color change from blue to red. Due to the extraordinarily high extinction coefficient of AuNPs and the specific affinity of MPBA for fructose, the proposed assay method shows excellent sensitivity and selectivity. The anti-aggregation effect of fructose on AuNPs was seen by the naked eye and monitored by UV–vis spectra.

2. Experimental

2.1. Chemicals and materials

All chemicals used in the experiments were of analytical grade and were used without further purifications. Tetrachloroauric(III) acid trihydrate, trisodium citrate dihydrate, methanol, 4-mercaptophenyl boronic acid (MPBA) and fructose were obtained from Merck (Darmstadt, Germany).

2.2. Apparatus

Absorption spectra were recorded on an Agilent 8453 UV-visible Spectrophotometer. The size and monodispersity of Au NPs were determined by TEM using transmission electron microscope (Philips EM 208).

2.3. Synthesis of gold nanoparticles

The Au seeds were synthesized according to the Frens method. Briefly, an aqueous solution of 100 mL of 1 mmol/L $HAuCl_4$ was heated to boiling with stirring; then 10 mL of 1% (wt/v) aqueous sodium citrate was added all at once. The color of the mixed solution changed from yellow to wine red in several minutes, indicating the formation of Au NPs. The boiling and stirring were continued for 15 min. The seed solution was cooled to the room temperature and was stored in a dark bottle at 4 °C.

Colorimetric assay of fructose based on anti-aggregation of AuNPs: 1 mL of fructose with different concentrations was added to a tube containing MPBA (final concentration was 1.34 μmol/L). The reaction was incubated at room temperature for 10 min and then the fructose-MPBA solution was mixed with the AuNPs solution (2 mL). The absorption spectrum was recorded.

3. Results and discussion

3.1. Sensing mechanism of fructose based on anti-aggregation of AuNPs

Fig. 1 illustrates the method for the colorimetric detection of fructose based on anti-aggregation on AuNPs. The AuNPs in aqueous solution remain dispersed and the solution appears ruby red because the AuNPs are stabilized against aggregation due to the negative capping agent's electrostatic repulsion [24]. Meanwhile, the aggregation agent, MPBA, has strong binding affinity for AuNPs due to the specific Au–S interaction, which is responsible for the significant aggregation of AuNPs and a visible color change of AuNPs from wine red to blue due to the inter-particle crosslinking. With addition of fructose, while the boronic acid moiety preferentially reacts with *cis*-2,3-ribose diol of fructose to form stable borate ester *via* boronic acid–diol binding dependent on the pH, preventing the AuNPs from aggregation *via* MPBA. Accordingly, with the increase of fructose concentration, the color changes from blue to purple, and finally to wine-red, which corresponds to AuNPs changing from an aggregation to a dispersion state.

3.2. Feasibility of fructose detection

The feasibility of using this assay for the colorimetric visualization of fructose is displayed in Fig. 2. The as-prepared AuNPs showed a distinctive wine-red color with the adsorption peak at 519 nm. These AuNPs were very stable owing to the electrostatic repulsion invoked by citrate ligands adsorbed on the particle surfaces. Upon the addition of MPBA into the AuNPs solution, a new strong absorbance peak appeared at 640 nm and the solution color clearly changed from ruby red to blue, indicating the aggregation of the AuNPs. The anti-aggregation effect of fructose on AuNPs is further supported by TEM investigation. The TEM image of AuNPs (Fig. 3a) containing MPBA in the presence of fructose revealed uniform monodisperse particles (Fig. 3b), while obvious aggregation of AuNPs occurred in the absence of fructose (Fig. 3c).

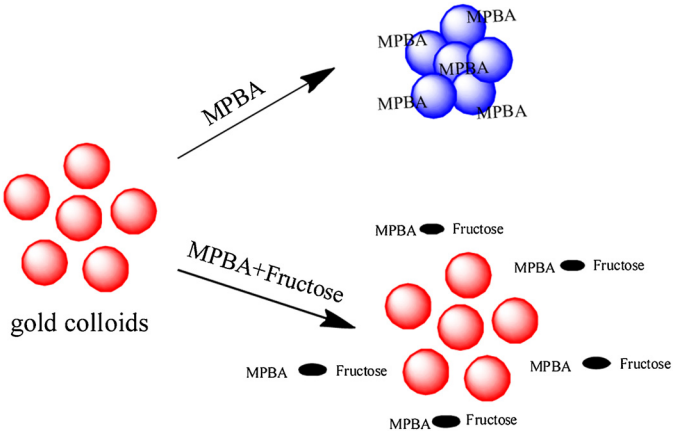

Fig. 1. Schematic illustration of the analytical process for detecting fructose based on the anti-aggregation of unmodified Au NPs.