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Combining aptamer-modified gold nanoparticles with barcode DNA sequence amplification for indirect analysis of ethanolamine

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ABSTRACT

An indirect analysis strategy for small organic molecules is presented herein, combining aptamer-modified gold nanoparticles (AuNPs) with a designed barcode sequence for amplification. Ethanolamine, which could not be analyzed directly through liquid chromatography coupled with mass spectrometry (LC/MS), was used as a model compound to demonstrate the feasibility of the proposed scheme. A poly-adenosine barcode sequence was part of the designed cDNA A1 (cDNA + barcode sequence) to provide a large number of adenine bases for signal amplification. In the presence of fixed amounts of the aptamer and cDNA A1, the concentration of ethanolamine could be estimated indirectly through measurement of the concentration of the hydrolyzed bases of the unbound cDNA A1. The thiol-terminated aptamer was immobilized on the AuNPs through Au–S self-assembly. The aptamer-immobilized AuNPs were removed, with their bound ethanolamine and cDNA A1, through centrifugation. The unbound cDNA A1 remaining in the supernatant was hydrolyzed and analyzed. Through indirect analysis and amplification of the cDNA A1, the concentration of ethanolamine could be analyzed with a linear range (on a logarithmic scale) between 5 and 5000 nM (detection limit: 1.2 nM). This developed method might be applied as a general platform for indirect detection using LC/MS analysis.

1. Introduction

Aptamers are synthetic single-strand DNA or RNA sequences selected through systematic evolution of ligands by exponential enrichment (SELEX), where a sequence having high binding affinity and specificity is obtained from a random sequence bank [1–3]. Their good selectivity, strong binding affinity, and wide range of binding environments make aptamers alternatives to antibodies. Furthermore, synthesized aptamers are cheap and more stable than antibodies; accordingly, they have been used in various fields and have several applications [1,4,5]. Combined with nanoparticles, aptamers can be used as specific probes [5–8]. Several aptamer-based analytical methods have been developed to detect small molecules [9–11] and proteins [12–14].

Liquid chromatography coupled with mass spectrometry (LC/MS) is used widely in chemistry [15], biochemistry [16], and pharmacology [17] to determine analytes in various samples. Many analytical methods employing LC/MS have been developed in recent years for analyses of specific samples; for example, sample pretreatment or column separation can be used to obtain analytes in solution for ionization and analysis. Nevertheless, there are compounds that cannot be analyzed directly using LC/MS, including those that are hard to ionize [18,19], require a specific column for retention [20], or provide a low signal response [21,22]. Sample derivatization or indirect detection can often be used in these cases, with the latter being the relatively simpler process. With indirect detection, the concentration of the target analyte in the sample is estimated by referencing another compound that can be separated and measured using LC/MS.

Ethanolamine (2-aminoethanol) is a small molecule having a molar mass of 61.08 g mol^{-1}. Because of its structural similarity with choline, ethanolamine is toxic to mammals and is associated with several diseases, including schizophrenia, Alzheimer's, atherosclerosis, and hyperlipemia [23–25]. Ethanolamine is also widely used industrially as a synthetic precursor; it has been marked as an environmental pollutant and is monitored in industrial waste and surface water [26–28]. Nevertheless, the high polarity and low molecular weight of ethanolamine make it hard to retain and separate from other ingredients in a sample matrix. The analytical data for the detection of ethanolamine using various

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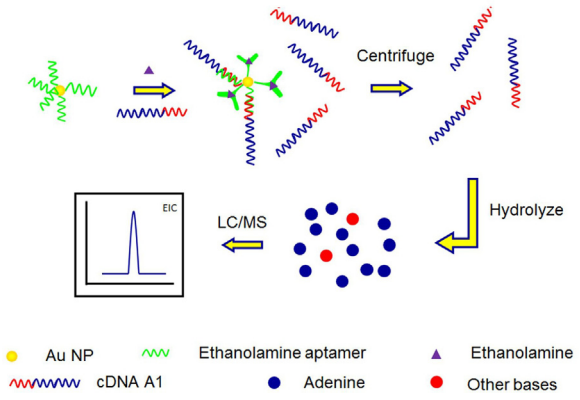

Scheme 1. Indirect detection of ethanolamineName.

methods are summarized in Table S1. Estimating the concentration of ethanolamine with both high accuracy and sensitivity has always been a challenge for the development of LC/MS-based analytical methods.

In this study, we developed an aptamer-based indirect analysis method for the detection of ethanolamine. In the absence of analytes, the ethanolamine aptamer forms a double-stranded structure with its complementary DNA (cDNA). In the presence of the target analyte, however, the aptamer folds to capture the analyte, due to a high binding affinity. With the resulting conformational change, the aptamer is no longer able to bind to the cDNA. Thus, when fixed amounts of the aptamer and cDNA are added into a solution containing the target analyte, the concentration of the unbound cDNA correlates positively with the concentration of ethanolamine. To amplify the signal for MS detection, we added 40 adenosine units to the cDNA of the ethanolamine aptamer to form cDNA A1. By immobilizing aptamers on gold nanoparticles (AuNPs), we could use simple centrifugation to separate the bound and unbound cDNA A1. The unbound cDNA A1 in the supernatant was then hydrolyzed and the concentration of its adenine bases used for indirect quantitation of ethanolamine. The method developed in this study allowed quantification of the target analyte through analysis of hydrolyzed bases, thereby avoiding most of the issues that occur during the direct LC/MS analysis of ethanolamine. This proposed method, using aptamers and cDNA A1, should be adaptable to the detection of other species by using different combinations of aptamers and analytes.

Scheme 1 depicts the indirect detection strategy based on the aptamer-modified AuNPs with cDNA A1. The aptamer was first immobilized on the AuNPs through self-assembly of the 5′-terminus thiol group. The large amount of net negative charge carried by the aptamer stabilized and maintained the dispersion of the AuNPs in 10 mM ammonium formate. If ethanolamine were present in the solution, the aptamer strands would bind with it to form a hairpin structure; in this folded form, the cDNA A1 could not form a double-stranded structure with the aptamer. The aptamer molecules could bind with either ethanolamine or cDNA A1 when both existed in solution. Therefore, when adding fixed amounts of aptamer-modified AuNPs and cDNA A1 into a sample solution containing ethanolamine, the limited number of aptamer molecules on the AuNPs would bind with ethanolamine, due to a strong binding affinity

(K_d = 10 nmol L^{-1}) [23], rather than hybridize with cDNA A1. The AuNPs bearing the bound ethanolamine and cDNA A1 then precipitated through centrifugation. To quantify the unbound cDNA A1, it would be hydrolyzed by heating the supernatant in an acidic environment to release the bases [29,30]. The released bases would then be separated through LC and detected using MS. The content of released bases was related to the concentration of cDNA A1 and, thereby, the concentration of ethanolamine. In the absence of ethanolamine, most of the cDNA A1 bound to the aptamer and the combined complexes precipitated in the solution during centrifugation. Only a small amount of the excess cDNA A1 remained in the supernatant for hydrolysis to provide the adenine signal. In the presence of ethanolamine, however, more cDNA A1 remained in the supernatant—the increase corresponding to the ethanolamine concentration—and the adenine signal increased depending upon the content of cDNA A1. The difference in adenine signals measured in the presence and absence of ethanolamine could, therefore, be used to reflect the concentration of ethanolamine in the sample. We could also observe a large signal even after only a slight change in the concentration of ethanolamine or when an extremely low concentration of ethanolamine was present in the sample, due to the effect of signal amplification. This developed method combines the indirect detection of ethanolamine via cDNA A1 with the enhanced sensitivity provided by the presence of the adenine-rich section in the cDNA A1 sequence.

2. Material and methods

2.1. Chemicals and reagents

All chemicals were of reagent grade or higher. All stock and working solutions were prepared using Milli-Q deionized (DI) water (Millipore, Bedford, MA, USA), which had a measured resistance of greater than 18.2 MΩ·cm. The adenine-appended cDNAs (cDNA A1, cDNA A2) and the aptamer of ethanolamine [23], modified with a thiol group on the 5′-terminus with a carbon chain (6C) and nine bases as a spacer, were purchased from MDBio (Taipei, Taiwan). Their sequences are listed in Table 1. Hydrogen tetrachloroaurate trihydrate was obtained from Alfa Aesar (Ward Hill, MA, USA). Sodium dihydrogen phosphate, disodium hydrogen phosphate, Tris (base), formic acid, tris(2-carboxyethyl)phosphine (TCEP), ethylenediaminetetraacetic acid (EDTA), and sodium chloride were purchased from Sigma (St. Louis, MO, USA). Ammonium formate, ammonium hydroxide, and sodium citrate were obtained from Riedel–de Haën (Morristown, NJ, USA). Acetonitrile was purchased from J. T. Baker (Philipsburg, USA).

2.2. Apparatus

The LC system employed was an Agilent 1100 apparatus (Agilent Technologies, Santa Clara, USA) equipped with a 20-μm sample loop and control software (Chem Station for LC 3D). The Zorbax Eclipse XDB C18 separation column (2.1 × 150 mm, 5 μm) was purchased from Agilent Technologies (Santa Clara, CA, USA). The Security guard cartridge (C18, 4 × 30 mm, 5 μm) was purchased

Table 1
The sequences used in this study.

Name	Sequence (5′–3′)
Ethanolamine aptamer[a]	HS-$(CH_2)_6$—CAA TTG CTG CGA GGT GGG TGG GTG GGA GCA ATT G
cDNA A1	AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA ACA ATT GCT CCC ACC CAC CCA CCT CGC AGC AAT TG
cDNA A2	AAA AAA AAA ACA ATT GCT CCC ACC CAC CCA CCT CGC AGC AAT TG

[a] Ref. [23].