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Efficient vitamin B12-imprinted boronate affinity magnetic nanoparticles for the specific capture of vitamin B12

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ABSTRACT

Vitamin B12 (VB12) has an important function in human physiology. However, analysis of VB12 at natural levels in foods or biological samples is difficult because of its very low concentration level and the presence of high-abundance components which can interfere with the measuring system. Thus, it is essential to develop efficient and selective enrichment approaches for VB12. Molecularly imprinted polymers (MIPs) have important applications from separation and sensing to catalysis. However, there is no report on the preparation of MIPs for VB12. Here, we use boronate affinity-based oriented surface imprinting to prepare MIPs for VB12. A VB12 template was first covalently immobilized onto the surface of boronic acid functionalized magnetic nanoparticles. Subsequently, a thin imprinting coating of poly(2-anilinoethanol) was formed to cover the substrate surface via in-water polymerization. After removing the template, 3D cavities complementary to the molecular size and shape of the template were formed in the imprinting layer. The imprinting coating was highly hydrophilic and presented limited residual boronic acid, thus non-specific binding was avoided. The prepared MIPs exhibited several highly favorable features, including excellent specificity, high binding strength and low binding pH. The prepared MIPs were successfully applied to the analysis of VB12 in human milk.

1. Introduction

Vitamin B12 (VB12, Cyanocobalamine) is an organic macromolecule containing cobalt ion in its structure, which is an important coenzyme for cell development and growth, and its deficiency leads to fatigue, nausea, weakness, weight loss and pernicious anemia [1–3]. This vitamin has to be obtained from natural sources such as dairy products, egg, fish, oysters, meat and poultry and plant products [4]. The determination of VB12 from foods or biological samples is of great significance. Up to now, many analytical methods including chemiluminescent enzyme immunoassay [5], HPLC methods with different detection systems [6,7], fluorescence method [8,9], and riboswitch sensor [10] have been developed for the determination of VB12 in diverse samples. However, chemiluminescent enzyme immunoassay and HPLC methods are very tedious and time consuming. Fluorescence method lacks good selectivity for the determination of VB12 in complex matrices. Although riboswitch sensor is a sensitive and selective method for determination of VB12, it is associated with apparent disadvantages, such as difficulty to prepare and high cost. In addition, direct analysis of VB12 at normal levels in foods or biological samples is difficult because of its very low concentration and the presence of high-abundance components which can interfere with the measuring system. For these reasons it is important to enrich real samples in VB12 prior to analysis.

Molecularly imprinted polymers (MIPs) [11–13] are artificial receptors with antibody-like binding properties or enzyme-like activities, and can exhibit specific capture for template molecules. Up to date, there is no report on the imprinting of VB12. Thus, it is essential to develop facile and efficient imprinting approach for VB12.

As we know, molecular imprinting of macromolecules is challenging due to conformational change of the templates during polymerization and difficulty of template removal. In order to overcome these issues, a large variety of methods have been proposed for the imprinting of macromolecules, such as surface imprinting [14–22], epitope imprinting [23–26], hierarchical imprinting [27], metal coordination [28], Pickering emulsion imprinting [29–31]. Among the methods, surface imprinting is the most popular method, which can solve the problem of diffusion limitation caused by template molecules with large size. In surface imprinting, template immobilization-based oriented surface imprinting is the most efficient imprinting method for macromolecules because it can allow for nearly complete removal of template molecules and provides good accessibility to target molecules [9]. Thus,

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as compared with bulk imprinting, the template immobilization-based oriented surface imprinting can provide better imprinting efficiency for VB12 because VB12 has relatively large size.

Boronic acids [11,32,33] are important functional monomers to bind cis-diol-containing compounds such as nucleosides, saccharides, glycans and glycoproteins in covalent imprinting. A series of boronate affinity-based MIPs for template molecules have been prepared by the strategy of template immobilization-based surface imprinting [34–41]. VB12 contains a cis-diol. Magnetic nanoparticles (MNPs) are the most commonly used as supporting materials in the preparation of enrichment materials owing to their low cost, simplicity to use, and high yield synthesis [42–50]. Thus, boronic acid-functionalized MNPs can be well applied as solid substrate for the immobilization of VB12 in the template immobilization-based surface imprinting. On the other hand, the controllability and hydrophilic nature of the imprinting coating are critical for the binding properties of the prepared MIPs. To date, several monomers have been introduced for the preparation of imprinting coatings, such as acrylamide, dopamine, siloxane, aniline and 2-anilinoethanol. As compared with aniline, more hydrophilic 2-anilinoethanol can be used as a better monomer to prepare imprinting coatings in oriented surface imprinting. Recently, through applying template immobilization-based boronate affinity surface imprinting method using in-water self-polymerization of 2-anilinoethanol as imprinting coatings, our group prepared glycoprotein-imprinted MNPs with excellent binding specificity [39]. In this work, the use of boronic acid is extremely important. As boronic acids can covalently bind cis-diol-containing compounds at relatively high pH values, while the formed complex dissociates when the pH becomes acidic, boronic acid-functionalized MNPs were proved to be highly efficient platforms for the immobilization of cis-diol-containing compounds such as glycoprotein in template immobilization-based surface imprinting. Because VB12 contains a cis-diol, the boronic acid-functionalized MNPs also can be well used to immobilize VB12 in template immobilization-based surface imprinting. As VB12 belongs to the water-soluble vitamins, highly hydrophilic 2-anilinoethanol can be used as a better monomer to prepare imprinting coatings in template immobilization-based surface imprinting, which can reduce or eliminate nonspecific adsorption of VB12. In addition, the large size of VB12 is suitable for the template immobilization-based surface imprinting. Clearly, the developed approach is highly advantageous for capture of VB12. Therefore, it is important to further present a new protocol for the preparation of VB12-imprinted MNPs by the template immobilization-based boronate affinity surface imprinting.

In this work, we present a facile and simple approach for the preparation of VB12-imprinted MNPs for VB12 by the template immobilization-based boronate affinity surface imprinting. The imprinting process involves several steps, as depicted in Fig. 1. First, the boronic acid-functionalized MNPs are prepared and then VB12 is immobilized onto the substrate surface by virtue of boronate affinity interaction. Then, an imprinting coating is deposited onto the substrate surface by in-water polymerization of 2-anilinoethanol. After that, the template is removed by disrupting the interaction with an acidic solution containing sodium dodecyl sulfate (SDS), thus imprinting cavities are well formed in the imprinting layer. In order to achieve better performance of the MIPs, the thickness of the imprinting coating should be adjusted to 1/3 to 2/3 of the molecular size of the template in one of the three dimensions. This imprinting strategy can allow for nearly complete removal of template molecules and provides good accessibility to target molecules, which is more suitable for the imprinting of macromolecule. In addition, excessive binding sites on boronic acid functionalized solid substrate can be nearly completely covered by the imprinting coating, which can effectively suppress or even eliminate non-specific adsorption. The MIPs prepared by the optimal conditions exhibited several highly favorable features, including excellent specificity, high binding strength and low binding pH. The prepared MIPs using this method were successfully applied to determination of VB12 in human milk.

2. Materials and methods

2.1. Materials

2-anilinoethanol, vitamin B12 (VB12), vitamin B6 (VB6), vitamin B2 (VB2), horseradish peroxidase (HRP), adenosine and deoxyadenosine (DA) were purchased from Sigma (St. Louis, MO, USA). Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ammonium persulfate (APS), quinol, ascorbic acid (AsA), cysteine (Cys), uric acid (UA), 2,4-Difluoro-3-formyl-phenylboronic acid (DFFPBA), sodium cyanoborohydride, anhydrous methanol, anhydrous sodium acetate (NaOAc), ethylene glycol, 1,6-hexanediamine, sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), and acetic acid (HAc) were provided by Energy Chemical (Shanghai, China). The ultrapure water (18.0 MΩ cm) was prepared with a Water Pro water system (Axlwater Corporation, TY10AXLC1805-2, China) and used throughout the experiments. All reagents were of at least analytical grade and used without further treatment. Human milk was collected from healthy lactating women. Human samples were treated according to standard ethical protocols.

2.2. Instruments

Transmission electron microscopy (TEM) characterization was performed on a JEM-1010 system (JEOL, Tokyo, Japan). UV absorbance data were acquired from a U-3010 UV spectrophotometer equipped a 1-cm cuvette (Kyoto, Japan). The adsorption isotherm measurements were performed with a U-3010 UV spectrophotometer (Kyoto, Japan). Thermal gravimetric analysis (TGA) curves of silica nanoparticles were obtained using a TG DTA6300 instrument (Seiko, Japan).

2.3. Preparation of DFFPBA-functionalized MNPs (DFFPBA-MNPs)

DFFPBA-functionalized MNPs (DFFPBA-MNPs) were synthesized according to the route shown in Fig. 1A, which consisted of two steps: 1) synthesis of amino-functionalized magnetic nanoparticles (AMNPs), and 2) functionalization of AMNPs with DFFPBA. The AMNPs were synthesized according to a previously reported method with minor modification [51]. Briefly, 2.0 g ferric trichloride hexahydrate was dissolved in ethylene glycol (60 mL) to form a clear orange yellow solution and followed by the addition of anhydrous sodium acetate (4.0 g) and 1,6-hexanediamine (13.0 g). The mixture was stirred vigorously by a mechanical stirring to form a clear red color solution. Finally, the solution was sealed in a PTFE-lined autoclave and reacted at 198 °C for 6 h. The resulting MNPs were rinsed with water and ethanol for three times each, and then dried at 50 °C in a vacuum overnight. To prepare DFFPBA-functionalized MNPs, the mixture of 300 mg AMNPs, 600 mg DFFPBA, 600 mg $NaBH_3CN$ and 60 mL MeOH was mechanically stirred for 24 h at room temperature. The obtained DFFPBA-MNPs were collected by a magnet, washed with water and ethanol for three times each, and then dried at 50 °C overnight. The obtained DFFPBA-MNPs were stored for further use.

2.4. Selectivity of DFFPBA-MNPs

The selectivity of DFFPBA-MNPs was first evaluated using adenosine and deoxyadenosine as test compounds. After DFFPBA-MNPs (3 mg) were dispersed into 200 μL of adenosine, deoxyadenosine or VB12 (1 mg/mL) dissolved in 50 mM sodium phosphate buffer (pH 7.0), the mixture was shaken on a rotator at room temperature for 2 h. The MNPs were then collected at the tube wall by applying a magnet and rinsed with 200 μL of 50 mM sodium phosphate buffer (pH 7.0) for three times. After washing, the MNPs were eluted with 100 μL of acetic acid solution for 1 h on a rotator. Finally, the MNPs were trapped to the tube wall and the eluates were collected. The amounts of adenosine or deoxyadenosine adsorbed by the DFFPBA-MNPs were determined by