Analytica Chimica Acta 784 (2013) 47–52

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

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

Determination of fructose 1,6-bisphosphate using a double-receptor sandwich type fluorescence sensing method based on uranyl–salophen complexes

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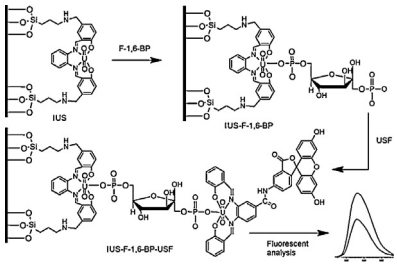
HIGHLIGHTS

- We report a double-receptor sandwich type fluorescence sensing method.
- Using fructose 1,6-bisphosphate as model analyte.
- One receptor is a uranyl–salophen complex immobilized on glass slide surface.
- Another is also a uranyl–salophen complex but labeled with a fluorescence group.
- This method shows high selectivity, high sensitivity and good stability.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 December 2012
Received in revised form 28 April 2013
Accepted 2 May 2013
Available online 13 May 2013

Keywords:
Fructose 1,6-bisphosphate
Fluorescence
Uranyl
Salophen
Double-receptor
Sandwich

ABSTRACT

In this paper, we report a double-receptor sandwich type fluorescence sensing method for the determination of fructose bisphosphates (FBPs) using fructose 1,6-bisphosphate (F-1,6-BP) as a model analyte based on uranyl–salophen complexes. The solid phase receptor is an immobilized uranyl–salophen (IUS) complex which is bound on the surface of glass slides by covalent bonds. The labeled receptor is another uranyl–salophen complex containing a fluorescence group, or uranyl–salophen–fluorescein (USF). In the procedure of determining F-1,6-BP in sample solution, F-1,6-BP is first adsorbed on the surface of the glass slide through the coordination reaction of F-1,6-BP with IUS. It then binds USF through another coordination reaction to form a sandwich-type structure of IUS-F-1,6-BP-USF. The amount of F-1,6-BP is detected by the determination of the fluorescence intensity of IUS-F-1,6-BP-USF bound on the glass slide. Under optimal conditions, the linear range for the detection of F-1,6-BP is 0.05–5.0 nmol mL^{-1} with a detection limit of 0.027 nmol mL^{-1}. The proposed method has been successfully applied for the determination of F-1,6-BP in real samples with satisfactory results.

1. Introduction

Fructose bisphosphates (FBPs), including fructose 1,6-bisphosphate (F-1,6-BP) and fructose 2,6-bisphosphate (F-2,6-BP), are important substrates in living organisms. They lie within the glycolysis metabolic pathway and are produced by phosphorylation of fructose 6-phosphate. They are, in turn, broken down into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, or into fructose 6-phosphate and inorganic phosphate [1–3].

The discovery of FBPs in eukaryotic organisms may be the last milestone of the 20th century in metabolic control research [4]. Intracellular FBPs levels play a key role in controlling cellular glucose homeostasis. F-1,6-BP is an allosteric activator of pyruvate kinase [5]. Fructose 2,6-bisphosphate is the most potent activator

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http://dx.doi.org/10.1016/j.aca.2013.05.002

of phosphofructokinase [6] and also is a potent inhibitor of fructose 1,6-bisphosphatase [7]. FBPs are thus the key regulatory signaling molecules of glycolytic/gluconeogenic flux that provide a switching mechanism between the two opposing pathways of carbohydrate metabolism. FBPs have been implicated in the ability to bind and sequester ferrous ion [8]. FBPs have also been involved in cancers [4,7,9], diabetes [10], liver injury [11] and other diseases for the treatment and diagnosis. Therefore, the detection of FBPs is of importance in pharmaceutical analysis, clinical diagnosis and biochemical study.

FBPs are currently assayed by indirect methods. The most current methods are enzymatic assay methods, including colorimetric aldolase-DNPH method [12–15], spectrophotometrical phosphofructokinase-DNPH method [16,17] and chromatography coupled enzymatic assay method [18]. Although enzymatic methods are sensitive, they are very tedious and difficult because the enzymes are affected by a variety of effecters and pH. Another type of assay methods is radioactive elements labeled competitive binding assay methods [7,19]. Although this type of methods is also sensitive, it is still inconvenient due to the use of enzymes. Therefore, it is desirable to develop new assay method for FBPs.

Double-antibody sandwich immunoassay (DASIA), the most popular immunoassay, is applied extensively for the detection of immunogenic targets [20,21]. In DASIA, the two antibodies are the receptors of the antigen. DASIA has many advantages such as good selectivity, high affinity, high sensitivity, excellent separation and pre-concentrating capability, and simple instruments in use. However, DASIA has also inherent disadvantages, such as the antibodies used are costly and unstable, and it is usually difficult for the determination of weakly immunogenic small molecules. For carrying forward the advantages of DASIA and avoiding its disadvantages, it is important to develop new double-receptor sandwich-type methods for the detection of target analytes.

In a FBP molecule there are two phosphate groups. If there are two receptors of phosphate group, a double-receptor sandwich-type method for the determination of FBPs should be established. Uranyl–salophen complexes are well-studied complexes in terms of structure and property [22]. Previous studies demonstrated that uranyl–salophen can bind phosphate and its derivatives to form stable sandwich type structure with strong affinity and high selectivity [23]. Hence uranyl–salophen has been used as ionophore for the separation and determination of phosphate [24–28]. Therefore, uranyl–salophen should be excellent receptor for establishing sandwich sensing method of FBPs.

This study intended to design a double-receptor sandwich type fluorescence sensing method for the determination of FBPs using F-1,6-BP as a model target based on the combination of F-1,6-BP with uranyl–salophen complexes. For this purpose, an immobilized uranyl–salophen (IUS) complex, which is bound on the surface of glass slides by covalent bonds, was used as the solid phase receptor of F-1,6-BP, and another uranyl–salophen complex labeled with a fluorescent group, or uranyl–salophen–fluorescein (USF), was used as the labeled receptor of F-1,6-BP. In this new method, F-1,6-BP binds first to IUS or the solid phase receptor, and further binds to USF or the labeled receptor to form a sandwich-type structure of IUS-F-1,6-BP-USF. The amount of F-1,6-BP was detected by the determination of the fluorescence intensity of IUS-F-1,6-BP-USF bound on the glass slide. The procedure of preparing IUS or the solid phase receptor and detecting F-1,6-BP is illustrated in Fig. 1. As shown herein, this type of sandwich fluorescence sensing method has the advantages of good selectivity, high affinity, high sensitivity, good stability and low cost for the detection of FBPs.

2. Materials and methods

2.1. Materials

Fructose 1,6-bisphosphate, trisodium salt (F-1,6-BP) and γ-(aminopropyl) triethoxy silane (APTES) were purchased from Sigma (Oakville, ON, USA). 5-Chloromethyl-2-hydroxybenzaldehyde, 3,4-diaminobenzoic acid and o-phenylenediamine were purchased from J & K Technology Co., Ltd. (Beijing, China). 5-Aminofluorescein (5-AF), 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aladding Chemical reagent Co., Ltd. (Shanghai, China). Uranyl nitrate hexahydrate was purchased from Aldrich (Milwaukee, WI, USA). The used chemicals were of analytical grade. Doubly distilled water was used throughout.

2.2. Synthesis of APTES–uranyl–salophen complex

APTES–uranyl–salophen complex was synthesized and characterized according to the procedures described elsewhere [29]. Briefly, 0.36 g of 5-chloromethyl-2-hydroxybenzaldehyde and 0.114 g of o-phenylenediamine were dissolved in 8.0 mL of anhydrous methanol. The mixture was stirred for 1 h for the synthesis of *N*, *N*′-bis (5-chloromethyl salicylidene)-o-phenylenediamine (chlorinated salophen). Then 0.502 g of uranyl nitrate hexahydrate was added and the mixture was stirred for 20 min for the formation of uranyl–salophen complex. The complex was then mixed with 0.378 g of APTES and the mixture was stirred at 40 °C for 24 h. Thus the complex APTES–uranyl–salophen was produced.

2.3. Fabrication of IUS on glass slide

The procedure of cleaning glass slides was operated according to Ref. [30]. The cleaned slides with the size of 30 mm × 20 mm were immersed in the solution containing APTES–uranyl–salophen complex for 1 h. Then the slides were washed three times with anhydrous methanol and three times with water and finally dried at 60 °C.

2.4. Synthesis of carboxylated salophen

Carboxylated salophen was synthesized and characterized according to Ref. [31]. 1.0 mmol of 3,4-diaminobenzoic acid and 2.0 mmol of salicylaldehyde were dissolved in 5.0 mL of anhydrous ethanol. The mixture was stirred for 1 h under reflux, and then cooled to room temperature. The solid mass was filtered, and the product, N,N-bis (salicylidene)-4-carboxy-o-phenylenediamine (carboxylated salophen), was recrystallized from ethanol.

2.5. Synthesis of USF

For the synthesis of USF, 50 μmol of carboxylated salophen was dissolved in 5 mL of anhydrous ethanol containing 100 μmol of NHS and 100 μmol of EDC. The solution was stirred at room temperature for 2 h for the activation of carboxyl. Then 50 μmol of 5-AF was added and the mixture was incubated at room temperature for 24 h for the conjugation of 5-AF with carboxylated salophen. Then 50 μmol of uranyl nitrate was added. A solid mass was produced from the solution through the coordination reaction. The solid mass was filtered, recrystallized in anhydrous ethanol and dried under vacuum to afford USF (24.8 mg, 52% yield). ESI-MS, theoretical m/z 957.2, measured m/z 958.1. ^{1}H NMR (300 MHz, DMSO-d6), δ: 10.08 (s, 2H, OH), 9.48 (s, 1H, amide bond), 9.02 (s, 2H, imino bond),