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Enantioanalysis of glyceric acid using enantioselective, potentiometric membrane electrodes: applications for the diagnosis of PH II and D-glyceric acidemia/acidurias

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Abstract

D- and L-glyceric acids are human metabolites responsible for two different diseases. Excess excretion of D-GA causes D-glyceric academia/acidurias, while excess excretion of L-GA causes hyperoxaluria type 2, PH II. Enantioselective, potentiometric membrane electrodes (EPMEs) based on maltodextrins I (dextrose equivalence (DE) 4.0–7.0), II (DE 13.0–17.0) and III (DE 16.5–19.5) as chiral selectors are proposed for the determination of L-glyceric (EPMEs based on maltodextrins I and III) and D-glyceric (EPME based on maltodextrin II) acid (L- and D-GA). EPMEs based on maltodextrins I and III can be reliably used for the analyses of L-GA using direct potentiometric method, in the concentration range of 10^{-8} to 10^{-6} and 10^{-6} to 10^{-3} mol/L, respectively, with very low detection limits (1.19×10^{-9} and 1.0×10^{-7} mol/L, respectively). The EPME based on maltodextrin II was successfully used for the enantioanalysis of D-GA in the 10^{-5} to 10^{-3} mol/L concentration range with detection limit of 1×10^{-6} mol/L. The enantioselectivity of EPMEs was determined over L-glyceric acid (or D-glyceric acid), and their selectivity over creatine, creatinine and some inorganic cations such as Na^+, K^+ and Ca^{2+}. Simply polishing to obtain a fresh surface ready to be used in a new measurement can regenerate the surface of the electrodes.

Keywords: Enantioselective; Potentiometric membrane electrodes; Maltodextrins; L(D)-Glyceric acid; Hyperoxaluria type 2 (PH II); D-Glyceric academia/aciduria

1. Introduction

Inborn errors of metabolism disorders are rare genetic diseases. They can be diagnosed by assay of organic acids (e.g., L(D)-glyceric acids) in human body fluids. Glyceric acid (2,3-dihydroxypropionic acid, Fig. 1) exists in two configurations, D- and L-enantiomers in mammalian metabolism. The presence of one of these enantiomers in blood or urine in abnormal concentration causes a different type of illness. Enantiomers may originate from different metabolic pathway, due to enzymes deficiencies. D-Glyceric dehydrogenase (D-GDH) catalyzes the interconversion of hydroxypyruvate to D-glycerate in the degradation pathway of serine metabolism [1]. D-GDH also has a glyoxylate-reductase (GR) activity, and it catalyzes the cytoplasmic reduction of glyoxylate to glycolate [2]. The genetic deficiency of D-GDH and GR causes a metabolic disorder named primary hyperoxaluria type 2 (PH II) or L-glyceric acidurias/acidemia [3,4] where hydroxypyruvate is converted to L-glycerate by L-lactate dehydrogenase (LDH) in the presence of NADH [5]. The metabolic disorder leads to excessive urinary oxalate and excretion of L-glycerate. PH II is characterized by urolithiasis or nephrocalcinosis with terminal renal failure [6]. Primary hyperoxaluria type 1 (PH I) is caused by deficiency of hepatic alanine:glyoxylate aminotransferase (AGT), which promotes the transamination of glyoxylate to oxalate. Deficiency of AGT causes oxidation of glyoxylate to oxalate and glycolate by glycolate oxidase and reductase, respectively [7,8]. PH I and II are described by marked increase of oxalate production associated

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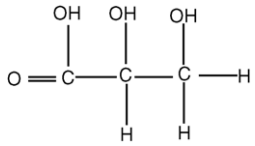

Fig. 1. Glyceric acid.

with hyperglycolate or hyper-L-glycerate in blood and urine [9].

Another disease entity involving glyceric acid is D-glyceric acidemia/aciduira. This disease was first reported by Brandt and coworkers [10,11] showing ketotic hyperglycinemia with abnormal excretion of D-glyceric acid in urine and serum. D-Glyceric acid is an intermediate of one of the pathways of serine degradation and of a minor pathway of fructose metabolism [11,12]. This increase of excretion was explained by deficiency of D-glycerate kinase that involved in the conversion of D-glyceric acid to D-2-phosphoglycerate [13–17]. D-Glyceric acidemia/acidurias is associated with delayed psychomotor growth, mental retardation and seizures [11,12,14,18]. The different syndrome entity of increased excretion of L- and D-glyceric acid in urine and serum is a hallmark of two totally different inborn diseases, hyperoxaluria II and D-glyceric acidamia/aciduira, respectively. Accordingly, enantioanalysis of L- and D-GA is important for the diagnosis of patients with PH II and D-glyceric acidamia/aciduria.

Enantioanalysis of chiral substances of clinical importance is a vital subject for the biomedical applications and early discovery of illnesses. Enantioselective, potentiometric membrane electrodes (EPMEs) based on chiral selectors had been developed for enantiomeric assay. Cyclodextrins [19,20], macrocyclic antibiotics [21], and maltodextrins [22] were used as chiral selectors for enantiomers determination. Maltodextrins have been used as host-complexing agents in enantiomer recognition [23,24]. They consist of D-(+)-glucose units, which are connected through Glu-(1–4)-α-D-Glu linkages [23]. Hydrolysis of starch produces a mixture of amyloses that can be hydrolyzed into shorter oligosaccharides. These complex oligomeric mixtures are distinguished by their dextrose equivalent value, DE (defined as the percent of reducing sugars calculated as glucose on a dry substance basis) [23,24]. Low DE maltodextrin were found to be more efficient in enantiomers recognition [25]. Enantioselective interactions of maltodextrins may refer to its conformational change from the flexible coil to a helix in the presence of complexing enantiomers and buffer salts [26].

Several methods based on capillary electrophoresis [27], colorimetry [28], polarimetry [15] and chromatographic methods [9,29–34] have been reported for the configurational analysis of L- and D-GA.

This work aims to develop EPMEs for the direct enantioanalysis of L- and D-glyceric acids. These EPMEs are based on three maltodextrins varied by different values of DE (maltodextrin I (DE: 4.0–7.0), maltodextrin II (DE: 13.0–17.0) and maltodextrin III (DE: 16.5–19.5)).

2. Experimental

2.1. Materials and reagents

2.1.1. Materials

L- and D-Glyceric acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). Graphite powder (1–2 μm, synthetic) was purchased from Aldrich (Milwaukee, WI, USA). Paraffin oil was purchased from Fluka (Buchs, Switzerland). Maltodextrins [DE 4.0–7.0 (I), 13.0–17.0 (II), and 16.5–19.5 (III)] were purchased from Aldrich (Milwaukee, WI, USA). Phosphate buffer (pH 3.5) was supplied by Merck (Darmstadt, Germany).

2.1.2. Reagents

De-ionized water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for all reagents and solutions preparation. 0.1 mol/L stock solutions of L- and D-glyceric acids were prepared by dissolving 0.4294 g of each separately in 15 mL of phosphate buffer (pH 3.5), stored at 4 °C. Standard solutions of L- and D-glyceric acid were prepared from the stocks solutions of L- and D-glyceric acids and phosphate buffer (pH 3.5) in the concentration range 1×10^{-10} to 1×10^{-2} mol/L. A 10^{-3} mol/L solution of each maltodextrin I–III was prepared.

2.2. Electrode design

Plain carbon paste was prepared by thoroughly mixing 100 mg of graphite powder with 40 μL paraffin oil. Paraffin oil and graphite powder [1:4, (w/w)] were mixed well, followed by the addition of 10^{-3} mol/L aqueous solution of maltodextrin [DE 4.0–7.0 (I), 13.0–17.0 (II), or 16.5–19.5 (III)] (100 μL of each maltodextrin solution to 100 mg graphite powder). The plain carbon paste was filled into a plastic pipette peak leaving a space of 3–4 mm into the top to be filled with the modified carbon paste that contains the maltodextrin. The diameter of enantioselective, potentiometric membrane electrode was 3 mm. Electric contact was obtained by inserting a Ag/AgCl wire into the carbon paste. The internal electrolyte solution of EPMEs was 0.1 mol/L KCl. All the EPMEs tips were gently rubbed on fine abrasive paper to produce a flat surface. The surface of the electrodes was wetted with de-ionized water and then polished with an alumina paper (polished strips 30144-011, Orion) before use for the analysis. When not in use, L- and D-GA electrodes were immersed in 10^{-3} mol/L L- or D-glyceric acid solution, respectively.

2.3. Apparatus

All chronopotentiometric (zero current) measurements were recorded using a Metrohm 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 20 and a software Version 4.9 (Eco Chemie, Utretch, The Netherlands). An Ag/AgCl (0.1 mol/L KCl) electrode was used as reference electrode in the cell.