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Enantioanalysis of L-2-hydroxyglutaric acid in urine samples using enantioselective, potentiometric membrane electrodes based on maltodextrins

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Abstract

Quantitative assay of L-2-hydroxyglutaric acid (L-2-HGA) is important for the diagnosis of L-2-hydroxyglutaric aciduria. Three enantioselective, potentiometric membrane electrodes (EPMEs) based on maltodextrins with different dextrose equivalent (DE) (DE: 4.0–7.0 (I), 13.0–17.0 (II), 16.5–19.5 (III)), were designed for the enantioanalysis of L-2-HGA. The enantioselective, potentiometric membrane electrodes can be used reliably for enantiopurity assay of L-2-HGA using the direct potentiometric method in the ranges of 10^{-9}–10^{-5}, 10^{-6}–10^{-3} and 10^{-8}–10^{-5} mol L^{-1} for the enantioselective, potentiometric membrane electrodes based on maltodextrins I, II and III, respectively, with very low detection limits. A high reliability was obtained when the electrodes were used for the assay of L-2-HGA in urine samples.

Keywords: Enantioselective, potentiometric membrane electrodes; Maltodextrins; L-2-hydroxyglutaric acid

1. Introduction

Diagnostics is based on the monitorization of abnormal concentrations of specific substances found in body fluids (e.g., urine, blood). High sensitive and fast methods are required in clinical analysis.

L-2-Hydroxyglutaric acid (L-2-HGA) (Fig. 1) may be found in abnormally higher concentrations in urine as a result of genetic errors or metabolic disorders [1,2] and it is a marker for L-2-hydroxyglutaric aciduria (a rare neurometabolic disorder) [3–5]. L-2-Hydroxyglutaric aciduria was first described by Duran [2]. Its effects are: mental retardation, progressive ataxia combined with subcortical leukoencephalopathy, cerebral atrophy, seizures, pyramidal and extra pyramidal symptoms and severe cerebral dysfunction [4,6–8].

Enantioanalysis is crucial for L-2-HGA because both L- and D-2-hydroxyglutaric acids can be found in urine, and they are markers for different diseases. The best accuracy in enantioselective assay is obtained using direct methods of analysis, characterized through high sensitivity and enantioselectivity. Enantioselective, potentiometric membrane electrodes (EPMEs) are preferred in quantitative chiral discrimination [9]. Carbon paste electrodes are well known for their reliable construction [9]. Different chiral selectors such as cyclodextrins [10,11], crown ether [12,13], antibiotics [14] and maltodextrins [15] were proposed for the design of EPMEs.

Several methods have been reported for the enantioanalysis of L-2-hydroxyglutaric acid in the human body fluids: liquid chromatography/mass spectrometry [16], gas chromatography/mass spectrometry [17], enantio-multidimensional

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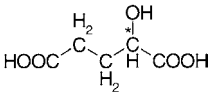

Fig. 1. 2-Hydroxyglutaric acid.

gas chromatography/mass spectrometry [18], capillary gas chromatography [19,20], an enzymatic method [21], NMR [22], and MRI [8,23,24].

In this paper, three EPMEs based on maltodextrins with different dextrose equivalent (DE) values (DE: 4.0–7.0 (I), 13.0–17.0 (II), 16.5–19.5 (III)) are proposed for the enantioanalysis of L-2-hydroxyglutaric acid in urine samples. The maltodextrins are characterized by different values of dextrose equivalent (DE) [25,26], where the DE value is an indication of the extent of starch hydrolysis. Enantioselectivity of these chiral selectors is increasing with the decrease of DE value [27,28].

2. Experimental

2.1. Electrode design

Paraffin oil and graphite powder were mixed in a ratio 1:4 (w/w) to form the plain carbon paste. The modified carbon pastes were prepared by impregnating 100 μl of 10^{-3} mol L^{-1} of each maltodextrin in 100mg of the plain carbon paste. A quantity of carbon paste, free of maltodextrins, was filled in a plastic pipette tip, leaving 3–4 mm in the upper part to be filled with the modified carbon paste containing the maltodextrin. The diameter of all EPMEs was 3 mm. Electric contact was obtained by inserting silver wires into the carbon paste. The internal solution was 0.1 mol L^{-1} KCl. The entire electrode surface was gently rubbed on fine abrasive paper to produce a flat surface. The surface of the electrode was wetted with de-ionized water, refreshed with modified carbon paste and then polished with an alumina paper (polished strips 30144-011, Orion) before use for the analysis. When not in use, each sensor was immersed in 10^{-3} mol L^{-1} of L-2-HGA solution.

2.2. Apparatus

A 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 100 computer-controlled potentiostat (Eco Chemie, Ultrech, The Netherlands) and software 4.9 was used for all potentiometric measurements. An Ag/AgCl (0.1 mol L^{-1} KCl) electrode was used as reference electrode in the cell.

2.3. Reagents and materials

Graphite powder (1–2 μm) and maltodextrins (DE 4.0–7.0 (I), 13.0–17.0 (II), 16.5–19.5 (III)) were purchased from Aldrich (Milwaukee, WI, USA), paraffin oil was purchased from Fluka (Buchs, Switzerland). L- and D-2-hydroxyglutaric acids creatine and creatinine were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Phosphate buffer (pH = 3) was purchased from Merck (Darmstadt, Germany).

De-ionized water from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for all solution preparations. The L-2-hydroxyglutaric acid solutions were prepared by serial dilutions from standard L-2-HGA solution (10^{-1} mol L^{-1}). All diluted and standard solutions of L-2-HGA were buffered at pH = 3.00 using phosphate buffer.

Urine samples were donated from healthy persons. Different aliquots of urine samples were spiked with different amounts of L-2-HGA. All spiked urine samples were buffered at pH = 3 using phosphate buffer.

2.4. Recommended procedure

Direct potentiometric method was employed for potential measurements, E (mV), of each standard L-2-HGA (10^{-10}–10^{-2} mol L^{-1}) solution and urine samples. The electrodes were placed in the standard solutions. Calibration graphs were obtained by plotting E (mV) versus pL-2-HGA. Unknown concentrations of L-2-HGA in urine samples were determined from the calibration graphs.

3. Results and discussion

3.1. EPMEs response

The response characteristics exhibited by the EPMEs based on different types of maltodextrins for the analysis of L-2-hydroxyglutaic acid are summarized in Table 1. All the proposed membrane electrodes exhibited linear and near-Nernestian responses (57–60 mV per decade of concentration) for EPMEs based on maltodextrins I, II and III, respectively, for the determination of L-2-HGA. The response of the proposed EPMEs was non-Nernestian when they were used for the assay of D-2-hydroxyglutaric acid. The detection limits determined for L-2-HGA using statistic methods are low, as shown in Table 1. Enantioselectivity using maltodextrins is based on the formation of inclusion complexes [26–28]. The stability of the complexes formed between the chiral selector and analytes is increasing with the value of DE, because increasing the DE value will result in an increase of the diameter of the helix as shown previously [15]. The response obtained for all three electrodes show good stability and reproducibility for tests performed for more than 2 months (R.S.D. < 1%).

The response times of EPMEs based on maltodextrins I and II are 90 s for concentration of L-2-HGA between 10^{-9} and 10^{-5}, and 10^{-6} and 10^{-3} mol L^{-1}, respectively. For EPME based on maltodextrins III, the response time is 45 s for the concentration of L-2-HGA between 10^{-8} and 10^{-5} mol L^{-1}.

Table 1
Response characteristics of enantioselective, potentiometric membrane electrodes for L-2-HGA[a]

EPME based on	Parameters			
	Slope (mV/decade of concentration)	Intercept E^o (mV)	Linear concentration range (mol L^{-1})	Detection limit (mol L^{-1})
Maltodextrin I	57.3	546.86	10^{-9}–10^{-5}	2.86×10^{-10}
Maltodextrin II	59.7	392.4	10^{-6}–10^{-3}	2.67×10^{-7}
Maltodextrin III	59.3	536.7	10^{-8}–10^{-5}	8.90×10^{-10}

[a] All measurements were made at room temperature; all values are the average of 10 determinations.

3.2. The pH influence on the response of the EPMEs

The effect of the pH variation on the response of the EPMEs based on maltodextrins I, II, and III has been tested by recording the emf of the cell, using direct potentiometric method. All measurements were performed for a concentration of L-2-HGA of 10^{-6} mol L^{-1}, at different pH values selected between 1 and 10. These solutions were prepared by adding small volumes of HCl (0.1 mol L^{-1}) and/or NaOH solution (0.1 mol L^{-1}) to a solution of L-2-HGA. The E (mV) versus pH plots presented in Fig. 2 show that the response of the EPMEs are pH-independent in the pH ranges of 2.0–6.0 (maltodextrins I based EPME), 2.0–5.0 (maltodextrin II based EPME) and 2.0–4.0 (maltodextrin III based EPME), proving the acidic behaviour of the L-2HGA at a pH lower than 2.0 and the basic behaviour of it a pH higher than 6.0.

3.3. Selectivity of the electrodes

The selectivity of all EPMEs was checked using mixed solutions method. The ratio between the concentrations of interfering ion and L-2-HGA was 10:1. The selectivity was investigated against D-2-hydroxglutaric acid (D-2-HGA), creatine, creatinine, Na^+, K^+ and Ca^{2+}. The selectivity coefficients for the enantioselective, potentiometric membrane electrodes, K^{pot}_{sel}, obtained are summarized in Table 2. The values obtained for D-2-HGA, creatine, creatinine, and the inorganic cations (Na^+, K^+ and Ca^{2+}, $K^{pot}_{sel} < 10^{-4}$) demonstrated the enantioselectivity and selectivity properties of the proposed EPMEs for the assay of L-2-HGA.

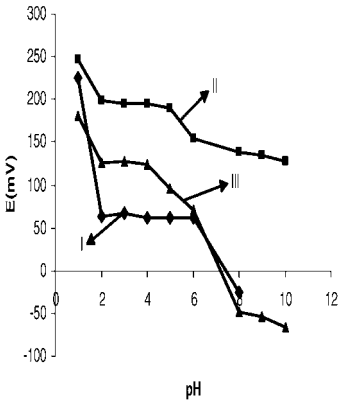

Fig. 2. Effect of pH on the response of the enantioselective, potentiometric membrane electrodes based on maltodextrin I (I), II (II) and III (III), respectively, for the determination of L-2-HGA ($C_{L\text{-}2\text{-}HGA} = 10^{-6}$ mol L^{-1}).

Table 2
Selectivity coefficients for the enantioselective, potentiometric membrane electrodes used for the assay of L-2-HGA[a]

Interference species (J)	pK^{pot}_{sel}		
	EPMEs based on		
	Maltodextrin I	Maltodextrin II	Maltodextrin III
D-2-HGA	2.40	2.42	2.42
Creatine	2.40	2.41	2.40
Creatinine	2.39	2.42	2.40

[a] All measurements were made at room temperature; all values are the average of 10 determinations.

3.4. Analytical applications

The high selectivity and enantioselectvity of proposed EPMEs based on maltodextrins made them suitable for the enantioanalysis of L-2-HGA in urine in order to diagnose 2-hydroxyglutaric aciduria. The analysis of L-2-hydroxyglutaric acid was investigated in the presence of D-2-hydroxyglutaric acid by using different ratios between L- and D-2-HGAs. The results obtained (Table 3) proved again the suitability of the proposed potentiometric membrane electrodes for the enantioanalysis of L-2-HGA. No significant differences in the recovery values were recorded for the different ratios between the enantiomers.

Urine samples (1–6) were donated from healthy persons and spiked with different amounts of L-2-HGA. All spiked urine samples were buffered at pH = 3 using phosphate buffer.

Table 3
The results obtained for the analysis of L-2-HGA in the presence of D-2-HGA[a]

L:D (mol/mol)	% L-2-HGA, Recovery		
	EPMEs based on		
	Maltodextrin I	Maltodextrin II	Maltodextrin III
2:1	99.60 ± 0.03	99.60 ± 0.01	99.78 ± 0.01
1:1	99.91 ± 0.01	99.46 ± 0.02	99.75 ± 0.01
1:2	99.35 ± 0.01	99.54 ± 0.01	99.04 ± 0.01
1:4	99.82 ± 0.01	99.92 ± 0.03	99.80 ± 0.02
1:9	99.76 ± 0.03	99.71 ± 0.02	99.43 ± 0.01

[a] All measurements were made at room temperature; all values are the average of 10 determinations.

Table 4
Recovery of L-2-HGA in urine samples[a]

Sample no.	LC/MS method [16]	% L-2-HGA, Recovery		
		EPMEs based on		
		Maltodextrin I	Maltodextrin II	Maltodextrin III
1	99.84	99.16 ± 0.05	99.92 ± 0.03	99.93 ± 0.02
2	99.67	99.37 ± 0.01	99.93 ± 0.01	98.52 ± 0.01
3	99.43	99.08 ± 0.05	99.27 ± 0.02	99.95 ± 0.01
4	99.65	99.30 ± 0.02	99.66 ± 0.01	99.76 ± 0.04
5	99.76	99.72 ± 0.01	99.80 ± 0.01	99.67 ± 0.02
6	99.80	99.71 ± 0.02	99.68 ± 0.03	99.82 ± 0.03

[a] All measurements were made at room temperature; all values are the average of 10 determinations.

The results recorded for the assay of L-2-HGA in urine samples are shown in Table 4 and they are in good agreement with those obtained using the method proposed by Rashed et al. [16].

4. Conclusions

The proposed EPMEs based on maltodextrins proved to be useful for the enantioanalysis of L-2-HGA in urine samples using direct potentiometric method. Therefore, these electrodes can be reliably used for the diagnosis of L-2-hydroxyglutaric aciduria.

The construction of the electrodes is simple, fast and reproducible. EMPEs have advantages over other techniques used in chiral discrimination (e.g., chromatography), such as higher precision, rapidity, low cost of analysis and no sample pretreatment needed before the analysis itself.

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