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A simple and sensitive method for lactose detection based on direct electron transfer between immobilised cellobiose dehydrogenase and screen-printed carbon electrodes

Gulnara Safina[a,1], Roland Ludwig[a,b], Lo Gorton[a,*]

[a] Department of Analytical Chemistry/Biochemistry, Lund University, Box 124, 221 00 Lund, Sweden
[b] Research Centre Applied Biocatalysis, Petersgasse 18, 8010 Graz, Austria

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ABSTRACT

A rapid and simple approach of lactose analysis is proposed based on 3rd generation amperometric biosensors employing cellobiose dehydrogenase (CDH) from *Trametes villosa* or *Phanerochaete sordida* immobilised on screen-printed carbon electrodes (SPCEs). After optimisation of the working conditions of the biosensors – pH of the carrier buffer, flow rate and applied potential – the sensors were able to detect lactose in a concentration range between 0.5–200 μM and 0.5–100 μM employing *T. villosa* and *P. sordida* CDH, respectively. The limit of detection is 250 nM (90 μg/L) for both. Biosensors based on SPCEs modified with multiwalled carbon nanotubes showed a higher sensitivity than unmodified SPCEs. Cross-linking with glutaraldehyde or poly(ethyleneglycol)diglycidyl ether improved not only the stability but also the analytical response. The developed sensor has been successfully applied for the determination of lactose in dairy (milk with different percentages of fat, lactose-free milk and yogurt) with a good reproducibility (RSD = 1.5–2.2%). No sample preparation except a simple dilution process is needed. The biosensor is easy to make and operate, is inexpensive and reveals a high sensitivity and reliability.

1. Introduction

The control of food quality requires robust, sensitive, selective and, if possible, high-throughput detection methods. The development of such methods for the industrial practitioner is an important task of analytical chemistry. For food analysis, chromatographic methods [1,2] and mass spectrometry [3] are the most commonly used methods due to their high sensitivity and reliability, however, those techniques require the application of expensive equipment and well trained users. Also time and labour consuming sample preparation steps might be needed in order to carry out reliable analyses.

Biosensors have been intensively developed during the last decades and are analytical devices consisting of a biosensing part (enzymes, antibodies, DNA, receptors, cells, etc.) in close contact with a transducer, which converts the biological response into a physicochemical signal [4]. Enzyme biosensors are especially promising tools for bioanalysis, because they combine a high specificity of biorecognition (substrate specificity) with an extremely sensitive detection due to the enzymatic amplification of the analytical signal. The relatively cheap and simple setup makes it a good alternative to the conventional expensive and more complex analytical techniques used in food quality control. Recent developments in the field of biosensors and their application in food analysis are reviewed by Mello and Kubota [5]. According to this review, amperometric enzyme based biosensors deserve special attention due to their simplicity, quick generation of analytical responses, relatively low cost and the possibility to be operated by unskilled personnel. Examples of biosensors are known to be developed or successfully applied for the control of wine quality [6–8], detection of ethanol, lactate, glutamate [9], hazardous compounds (e.g., pesticides) in milk, fruit juice and rice [10–16].

One of the most significant needs in the dairy industry is lactose monitoring, which is required for maintaining specified lactose levels during the production steps and in the final milk products. Lactose control in food products is, besides economic reasons, especially important in lactose reduced or lactose-free products for the 75% of adults worldwide suffering from lactose intolerance [17]. All previously described electrochemical biosensors for lactose detection and quantification in milk, foodstuff and urine described in [18–21] are bi-enzymatic and based on a cascade of enzymatic reactions combining either β-galactosidase and glucose oxidase,

* Corresponding author at: Department of Biochemistry, Lund University, Box 124, 221 00 Lund, Sweden. Tel.: +46 46 222 7582; fax: +46 46 222 45 34.
E-mail addresses: Gulnara.Safina@chem.gu.se (G. Safina), Lo.Gorton@biochemistry.lu.se (L. Gorton).
[1] Present address: Department of Analytical and Marine Chemistry, University of Gothenburg, 41296 Gothenburg, Sweden.

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or β-galactosidase and galactose oxidase. The coupling of the oxidation reaction of the sugar oxidising enzyme (glucose oxidase or galactose oxidase) is based on either measuring the formation of the produced H_2O_2 (1st generation biosensor) or the use of an artificial mediator facilitating the electron transfer between the oxidising enzyme and the electrode (2nd generation biosensor). However, the application of such multi-enzyme biosensors raises certain difficulties such as interferences from galactose and glucose present in β-galactosidase treated lactose reduced milk.

A lactose biosensor was previously proposed by Stoica et al. [22] based on the direct electron transfer between one single redox enzyme—cellobiose dehydrogenase (CDH) (either from *Trametes villosa* and *Phanerochaete sordida* both white-rot basidiomycete fungi), and solid spectrographic graphite electrodes, i.e., a 3rd generation biosensor. CDHs obtained from basidiomycete white-rot fungi are only able to convert rapidly cellodextrins and lactose but strictly discriminate against other oligosaccharides and monosaccharides (e.g., galactose, glucose). CDH consists of one FAD containing and one heme containing domain. The heme *b* cofactor is located close to the surface of the protein that makes it possible to deliver the electrons to a polarised electrode surface via a direct electron transfer mechanism [22]. Lactose is oxidised in a $2e^-$, $2H^+$ reaction at the FAD domain to form lactobionic acid and the fully reduced FAD. The FAD domain sequentially delivers the electrons to the heme domain, which acts as "a built in electron transfer mediator" shuttling the electrons to the electrode [23].

Our approach to improve the given design was to use commercially available screen-printed electrodes instead of the previously used graphite rod electrodes [22], which makes it possible to miniaturise the assay format, makes it more convenient and suitable for use in on-line mode, improves the sensitivity by generating a higher surface area with multiwalled carbon nanotubes [24–26] and increases the stability by cross-linking. Although biosensors for food analysis have been developed based on this design [27] there are only few approaches towards screen-printed biosensors for lactose determination [28]. We believe that this technique provides an easy-to-use and robust platform for reliable and fast lactose quantification.

2. Experimental

2.1. Reagents

Cellobiose dehydrogenase (CDH; cellobiose:(acceptor) 1-oxidoreductase; EC 1.1.99.18) from *T. villosa* (volumetric activity 276.5 U mL^{-1}, specific activity 45.6 U mg^{-1}) and *P. sordida* (volumetric activity 140 U mL^{-1}, specific activity 33 U mg^{-1}) were used for electrode modification. A fresh stock solution of β-lactose (Sigma–Aldrich, Stockholm, Sweden) was prepared and stored over night to reach mutarotational equilibrium. The working buffer was made from 0.02 M citric acid (Sigma–Aldrich), 0.1 M potassium chloride and titrated with sodium hydroxide solution (both from Merck/VWR International AB, Stockholm, Sweden) to obtain pH values from 3.0 to 6.5. Glutaraldehyde solution (50%, Sigma–Aldrich) and poly(ethyleneglycol)diglycidyl ether (PEGDGE) (Merck) were used as cross-linkers for electrode modification. Potassium ferrocyanide was purchased from Sigma–Aldrich. All other reagents used were of analytical grade.

Diary products as milk (Skånemejerier, Sweden) with different fat contents (1.5 and 3%), lactose-free milk (Valio, Finland), yogurt ("Turkish", Lindahls Mejeri AB, Sweden) were purchased from local stores.

All the solutions were prepared using Milli-Q water (Millipore 18.2 MΩ cm).

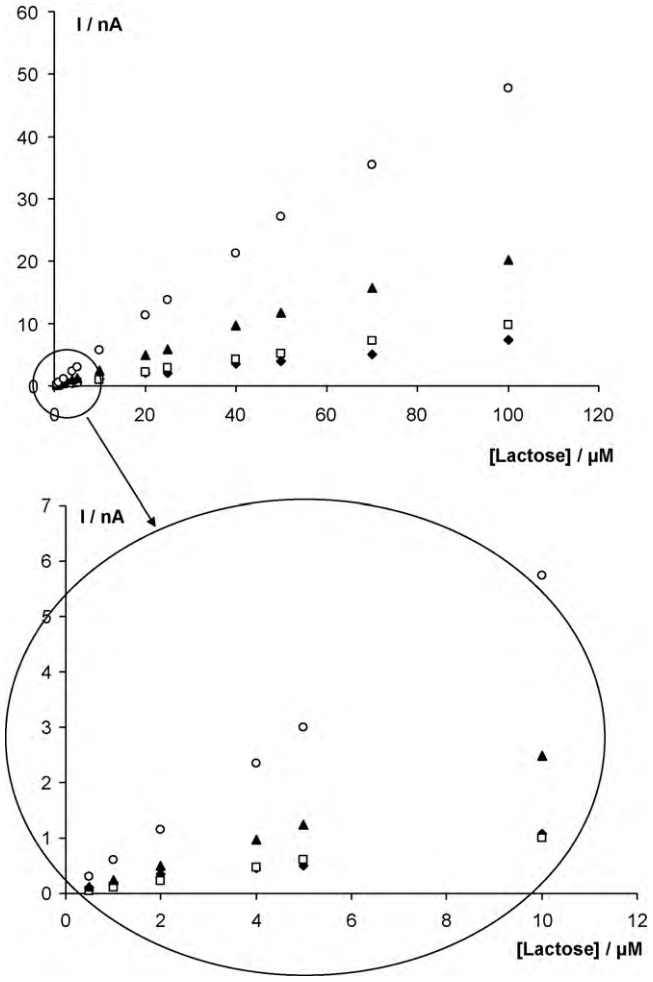

Fig. 1. Calibration curves for lactose obtained using biosensors based on (♦) *T. villosa* CDH modified SPCE, (□) *T. villosa* CDH modified SPCE-MWCNT, (▲) *P. sordida* CDH modified SPCE, and (○) *P. sordida* CDH modified SPCE-MWCNT.

2.2. Electrochemical measurements

Screen-printed electrodes were purchased from DropSens (Oviedo, Spain) and used for the preparation of the biosensors. The screen-printed electrodes used in this work consisted of a working electrode made of either carbon (SPCE) or carboxyl functionalised multiwalled carbon nanotubes (SPCE-MWCNT), a silver reference electrode and a carbon counter electrode deposited by printing technology on the ceramic support. The electrodes modified with enzyme were placed into a methacrylate wall-jet flow through electrochemical cell (DropSens) for measurements. The electrode was connected to a three-electrode potentiostat (Zäta Elektronik, Höör, Sweden). The electrochemical cell was connected to a flow-injection system consisting of a peristaltic pump (Gilson, Villier-le-Bel, France) and a six-port valve electrical injector (Rheodyne, Cotati, CA, USA). An aliquot of 50 μL of the diluted sample was applied by a loop mounted to the injector. The resulting current was recorded on a strip chart recorder (Kipp&Zonen, Utrecht, The Netherlands). All measurements were performed at room temperature.

2.3. Biosensor preparation (modification of the electrode with enzyme)

In order to immobilise CDH on the screen-printed electrodes, 5 μL of enzyme solution was deposited on the top of the working