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Letter to the Editor

Development of a galactose biosensor with galactose oxidase-immobilized epidermis of *Solanum lycopersicum*: Potential point-of-care testing for citrin deficiency in high-prevalence areas

Dear Editor,

The determination of galactose is important in clinical chemistry when a number of inherited metabolic diseases are signified by an increase of galactose in blood and urine, including defects of enzymes in galactose metabolism, namely, galactose-1-phosphate uridyltransferase (MIM#230400), galactokinase (MIM#230200) and galactose epimerase (MIM #230350) [1], causing galactosemia and being more common in Caucasians [2]. On the other hand, the hyperexcretion of galactose, often accompanied by galactitol, is highly suggestive of the relatively prevalent citrin deficiency (MIM#605814) in Asian infants with prolonged neonatal jaundice and conjugated hyperbilirubinemia [3,4]. With the causative gene *SLC25A13* (MIM*603859) being first described in 1999 [5], the disease is a relatively new one and diagnosis can be difficult. A prompt diagnosis or exclusion of citrin deficiency definitely aids management of the condition. As such, a rapid, simple and reliable method of the determination of galactose in plasma or urine will be of great value in aiding with the diagnosis, allowing prompt management and close monitoring of the disease progress and response towards treatment. Currently, galactose is measured in the clinical laboratory by polarimetry, fluorometry, spectrophotometry and chromatography, which are sophisticated, costly and time-consuming [6]. The determination of galactose by making use of the oxidation reaction of the analyte by galactose oxidase (E.C. 1.1.3.9) was first described in 1962 [7]. Since then, several attempts have been made to construct biosensors for determination of galactose, with the enzyme galactose oxidase immobilized on different membranes/surfaces [6,8–12]. A glucose biosensor has recently been developed with glucose oxidase immobilized on epidermis of common tomato (*Solanum lycopersicum*) and proven to be a success [13]. In this report, we used a simple and inexpensive biosensor method for fast and accurate determination of galactose in urine samples, which is anticipated to be valuable in quick diagnosis of citrin deficiency. The detection mechanism of our galactose biosensor is based on the enzymatic oxidation of galactose to galactohexodialdose with a concomitant depletion in dissolved O_2 in the sample solution and the decrease in O_2 was readily monitored by the O_2 sensor.

The epidermis of common tomato fruit was taken out, with excess flesh carefully removed, cleansed with copious amounts of deionized water and trimmed into 2-cm disks. One hundred microliters of 0.50 % w/v galactose oxidase (specific activity of 54 U/mg, Sigma-Aldrich, St. Louis, MO) solution was added to inner surface of each disk of epidermis evenly and left to almost dryness. This step was repeated for another 3 times. One hundred microliters of 0.30 % w/v medium molecular weight chitosan (Sigma-Aldrich) solution (pH 5.4) was added to the disks evenly and left dry for 2 h, followed by rinsing with 0.10-mol/l pH 6.5 phosphate buffer for 10 min. The prepared epidermis disks were then protected from light and stored in 0.10-mol/l pH 6.5 phosphate buffer with 2.0-mmol/l copper sulfate at 4 °C prior to use. A galactose oxidase-immobilized disk of tomato epidermis was positioned on the surface of a Pasco CI-6542 O_2 sensor and covered with a 2-cm disk of pre-soaked dialysis membrane (MW cutoff 13000, Sigma-Aldrich). The two layers were kept in a steady position on the O_2 sensor with an *O*-ring. The O_2 sensor was then immersed into an aliquot of stirred 0.10 mol/l phosphate buffer solution, pH 7.0 at 22 ± 1 °C, into which the D-galactose standards were injected, to make up to 10.0 ml. Standards of different galactose concentrations were prepared by spiking various amounts of D-galactose solution into 2.5-ml aliquots of pooled residual urine samples from normal subjects in a hospital laboratory. The dissolved O_2 signal was captured at a sampling rate of 10/s and processed by a datalogger system. In this work, the optimal biosensor was constructed with a galactose oxidase-immobilized tomato epidermis linked by 0.30% w/v medium molecular weight chitosan solution operating in 0.50-mol/l pH 7.0 phosphate buffer, with a sensitivity of 0.325-mg/l change in dissolved O_2 per mmol/l galactose, i.e., 44% more sensitive than a galactose biosensor similarly constructed using eggshell membrane [14].

Fig. 1 displays a typical response curve of the galactose biosensor. The linearity range is up to 7.0-mmol/l urinary galactose as tested on three biosensors. The CVs (obtained from 5 replicate measurements on each of 4 biosensors) were 9.4% and 3.2% at 2.0 and 4.0 mmol/l of galactose, respectively. The limit of detection based on a signal-to-noise ratio of 3 (determined from 20 replicate blank measurements on a single biosensor) was 0.44 mmol/l and thus the clinical cutoff for normal urinary galactose in infants (<1.1 mmol/l) [15] is covered. The recoveries obtained from three replicate measurements on a single biosensor at 1.0–5.0 mmol/l ranged from 88% to 110%. The results were reproducible (concentrations >90% retained) after a period of 30 days, demonstrating that our developed biosensor is relatively stable and suitable for routine clinical testing. The interference tests were carried out with some potential interferents in urine samples by exposing the biosensor to interferents in 0.50-mol/l phosphate buffer at pH 7.0. The decrease in the O_2 level was calculated as galactose

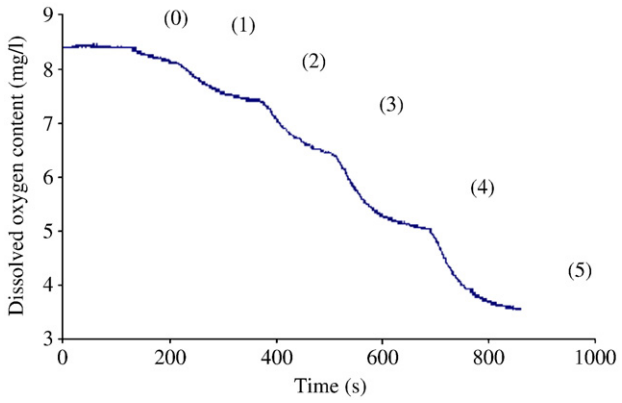

Fig. 1. A typical response curve of the optimized galactose biosensor on exposure to various concentrations of standard galactose solutions: (0) 0.0, (1) 1.0, (2) 2.0, (3) 3.0, (4) 4.0, and (5) 5.0 mmol/l.

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Table 1
Effect of common potential interferents on the galactose biosensor. Apart from L-ascorbic acid, no tested agents caused significant interference in the biosensor at 2.0 mmol/l galactose.

Potential interferent	Concentration (mmol/l)	Mean decrease in O_2 level equivalent to galactose (mmol/l)[a]
L-Ascorbic acid	2.0	2.6
β-D-Fructose	16	−0.15
β-D-Glucose	40	−0.040
D-Lactic acid	16	−0.040
Sodium chloride	3200	0.17
D-Xylose	16	−0.050
α-Lactose	20	0.070

[a] 5 replicates were measured.

equivalence, i.e., the concentration of urinary galactose that can produce the same decrease as the interferents. All these interferents tested did not affect the biosensor system, apart from a high urinary concentration of L-ascorbic acid, which caused a positive interference as depicted in Table 1. The threshold used for a significant change was ± 10% at level tested, i.e., 0.20 mmol/l at 2.0-mmol/l galactose. Fortunately, L-ascorbic acid, an unstable analyte, is rarely elevated to this extent in the age group in which citrin deficiency commonly presents, i.e., <6 months [16].

Our study was limited by the difficulty in collecting urine samples during acute manifestation from sufficient patients with genetically confirmed citrin deficiency for validation. Citrin deficiency is a relatively prevalent condition among Asians, especially in southern China, where the allele frequency was estimated to be up to 1/40 and thus an estimated prevalence of 1 in 1600. The similarly estimated minimum prevalences of the condition were 1 in 4225 in Chinese overall, 1 in 4761 in Japanese and 1 in 12544 in Koreans [17]. Galactosuria (>10 mmol/l) is one of the biochemical features of citrin deficiency and often detected in patients presenting with neonatal intrahepatic cholestasis [18]. In the cohort described by Song et al., 8 out of 9 (89%) of patients were detected with galactose in their urine samples [19]. Galactosuria might alternatively be reflected by the presence of reducing sugar in a clinical laboratory which is a more commonly used test yet also less specific. However, this abnormality might be absent after the acute period [20] and the sensitivity of this assay might be the best during acute manifestation of the disease prior to treatment and resolving of the jaundice. When a biosensor is usually a reagent-less self-contained integrated device with a compact size, the invention and development of biosensors give an alternative to the traditional time-consuming, expensive and highly sophisticated chromatographic and spectroscopic methods for quantitative analysis of given analytes in complex biological and environmental samples [21]. In this study, we have successfully developed and optimized a tomato epidermis-immobilized galactose oxidase-based amperometric biosensor for determination of galactose in urine matrix, which displayed improved sensitivity, fast response, satisfactory dynamic range, linearity and reproducibility, with minimal clinically significant interferents identified. These favorable characteristics would allow rapid, near-patient and inexpensive determination of galactose in urine samples of patients and aid in diagnosing galactossemia or citrin deficiency. In particular, the low cost of this biosensor would make low-threshold screening of citrin deficiency in high-prevalence area, such as the remote mainland China, possible.

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References

[1] Brunetti-Pierri N, Opekun AR, Craigen WJ. Two familial cases of high blood galactose of unknown aetiology. J Inherit Metab Dis 2006;29:762.
[2] Cheung KL, Tang NL, Hsiao KJ, et al. Classical galactosaemia in Chinese: A case report and review of disease incidence. J Paediatr Child Health 1999;35:399–400.
[3] Ohura T, Kobayashi K, Abukawa D, et al. A novel inborn error of metabolism detected by elevated methionine and/or galactose in newborn screening: Neonatal intrahepatic cholestasis caused by citrin deficiency. Eur J Pediatr 2003;162:317–22.
[4] Tamamori A, Fujimoto A, Okano Y, et al. Effects of citrin deficiency in the perinatal period: Feasibility of newborn mass screening for citrin deficiency. Pediatr Res 2004;56:608–14.
[5] Sinasac DS, Crackower MA, Lee JR, et al. Genomic structure of the adult-onset type II citrullinemia gene, SLC25A13, and cloning and expression of its mouse homologue. Genomics 1999;62:289–92.
[6] Gulce H, Ataman I, Gulce A, Yildiz A. Enzyme Microb Tech 2002;30:41–4.
[7] de Verdier C, Hjelm M. A galactose-oxidase method for the determination of galactose in blood plasma. Clin Chim Acta 1962;7:742–4.
[8] Amiss TJ, Sherman DB, Nycz CM, Andaluz SA, Pitner JB. Engineering and rapid selection of a low-affinity glucose/galactose-binding protein for a glucose biosensor. Protein Sci 2007;16:2350–9.
[9] Dahodwala SK, Weibel MK, Humphrey AE. Galactose oxidase: applications of the covalently immobilized enzyme in a packed bed configuration. Biotechnol Bioeng 1976;18:1679–94.
[10] Taylor PJ, Kmetec E, Johnson JM. Design, construction, and applications of a galactose selective electrode. Anal Chem 1977;49:789–94.
[11] Rajendran V, Lrudayaraj J. Detection of glucose, galactose, and lactose in milk with a microdialysis-coupled flow injection amperometric sensor. J Dairy Sci 2002;85:1357–61.
[12] Kondakova L, Yanishpolskii V, Tertykh V, Buglova T. Galactose oxidase immobilized on silica in an analytical determination of galactose-containing carbohydrates. Anal Sci 2007;23:97–101.
[13] Han H, Li Y, Yue H, Zhou Z, Xiao D, Choi MM. Clinical determination of glucose in human serum by a tomato skin biosensor. Clin Chim Acta 2008;395:155–8.
[14] Wen G, Zhang Y, Zhou Y, Shuang S, Dong C, Choi MMF. Biosensors for determination of galactose with galactose oxidase immobilized on eggshell membrane. Anal Lett 2005;38:1519–29.
[15] Hommes FA, editor. Techniques in diagnostic human biochemical genetics: A laboratory manual. Wiley-Liss; 1991.
[16] Chalmers AH, Cowley DM, McWhinney BC. Stability of ascorbate in urine: Relevance to analyses for ascorbate and oxalate. Clin Chem 1985;31:1703–5.
[17] Lu YB, Kobayashi K, Ushikai M, et al. Frequency and distribution in East Asia of 12 mutations identified in the SLC25A13 gene of Japanese patients with citrin deficiency. J Hum Genet 2005;50:338–46.
[18] Hutchin T, Preece MA, Hendriksz C, et al. Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) as a cause of liver disease in infants in the UK. J Inherit Metab 2009. doi:10.1007/s10545-009-1116-x.
[19] Song YZ, Li BX, Chen FP, et al. Neonatal intrahepatic cholestasis caused by citrin deficiency: clinical and laboratory investigation of 13 subjects in mainland of China. Dig Liver Dis 2009;41:683–9.
[20] Nagasaka H, Okano Y, Tsukahara H, et al. Sustaining hypercitrullinemia, hypercholesterolemia and augmented oxidative stress in Japanese children with aspartate/glutamate carrier isoform 2-citrin-deficiency even during the silent period. Mol Genet Metab 2009;97:21–6.
[21] Davis J, Vaughan DH, Cardosi MF. Elements of biosensor construction. Enzyme Microb Tech 1995;17:1030–5.

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