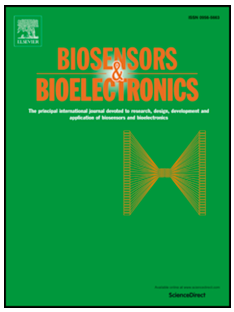


An amperometric biosensor of L-fucose in urine for the first screening test of cancer

Kouta Takeda, Ryo Kusuoka, Misaki Inukai, Kiyohiko Igarashi, Hiroyuki Ohno, Nobuhumi Nakamura

PII: S0956-5663(20)30817-4

DOI: https://doi.org/10.1016/j.bios.2020.112831

Reference: BIOS 112831

To appear in: *Biosensors and Bioelectronics*

Received Date: 15 September 2020

Revised Date: 15 November 2020

Accepted Date: 17 November 2020

Please cite this article as: Takeda, K., Kusuoka, R., Inukai, M., Igarashi, K., Ohno, H., Nakamura, N., An amperometric biosensor of L-fucose in urine for the first screening test of cancer, *Biosensors and Bioelectronics* (2020), doi: https://doi.org/10.1016/j.bios.2020.112831.

CRediT author statement

Kouta Takeda: Methodology, Validation, Investigation, Writing - Original Draft, Visualization. **Ryo Kusuoka**: Validation, Investigation. **Misaki Inukai**: Validation, Investigation. **Kiyohiko Igarashi**: Conceptualization, Resources. **Hiroyuki Ohno**: Resources. **Nobuhumi Nakamura**: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration.

An amperometric biosensor of L-fucose in urine for the first screening test of cancer

Kouta Takeda[1†], Ryo Kusuoka[1], Misaki Inukai[1], Kiyohiko Igarashi[2, 3], Hiroyuki Ohno[1], and Nobuhumi Nakamura[1, *]

[1]*Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan*

[2]*Department of Biomaterial Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*

[3]*Protein Discovery and Engineering Team, VTT Technical Research Center of Finland Ltd., FI-02044 VTT, Espoo, Finland*

*Corresponding author. Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan, Tel.: +81-42-3887482; Fax: +81-42-3887482; E-mail address: nobu1@cc.tuat.ac.jp (N. Nakamura).

Present Addresses

[†]Institute for Molecular Science, National Institutes of Natural Sciences, Okazaki, 444-8787, Aichi, Japan

Abstract

Quantitative routine detection of fucose, which is a cancer marker, in urine is effective for the preliminary screening of cancer. Amperometric biosensing methods have the advantage of being simple, rapid, and precise for urinalysis. However, coexisting electroactive interferences such as ascorbic acid (AA), dopamine (DA), and uric acid (UA) prevent accurate measurements. In this work, an amperometric L-fucose biosensor unaffected by interferences was developed and utilizes direct electron transfer type bioelectrocatalysis of pyrroloquinoline quinone (PQQ)-dependent pyranose dehydrogenase from *Coprinopsis cinerea* (*Cc*PDH). The isolated PQQ domain from *Cc*PDH was immobilized on gold nanoparticle (AuNP)-modified electrodes, which obtained a catalytic current at a lower potential than the oxidation potential of the interfering compounds. Applying an operating potential of -0.1 V vs. Ag|AgCl (3 M NaCl) enabled the detection of L-fucose while completely eliminating the oxidation of AA, DA, and UA on the electrodes. The increase in the specific area of the electrodes by increasing the AuNP drop-casting time resulted in an improvement in the sensor performance. The biosensor exhibited a linear range for L-fucose detection between 0.1 mM and 1 mM (R^2 = 0.9996), including a cut-off value, the sensitivity was 3.12 ± 0.05 μA mM^{-1} cm^{-2}, and the detection limit was 13.6 μM at a signal-to-noise ratio of three. The biosensor can be used to quantify the concentration of L-fucose at physiological levels and does not require urine preprocessing, making it applicable to practical use for point-of-care testing with urine.

Keywords:

enzyme biosensor; direct electron transfer; bioelectrocatalysis; gold nanoparticle; PQQ; ascorbic acid interference

1. Introduction

L-Fucose (6-deoxy-L-galactose, Fig. 1A) is present in a wide variety of different organisms (Vanhooren and Vandamme 1999). In mammals, fucose is frequently located at the ends of *N*-, *O*-, or lipid-linked glycans or linked directly to some proteins, playing an important role in a variety of biological systems, including immunity and cancer (Becker and Lowe 2003; Schneider et al. 2017). Cancer cells have numerous changed fucosylated glycans compared to normal tissue due to a change in the activity of enzymes involved in L-fucose metabolism, such as fucosidase and fucosyltransferase (Adamczyk et al. 2012; Keeley et al. 2019; Scott and Munkley 2019; Taniguchi and Kizuka 2015). The L-fucose concentration in serum increases with increasing α-L-fucosidase activity in liver cancer (Deugnier et al. 1984; Manchil et al. 2016; Tatsumura et al. 1977). L-Fucose is also excreted in its free form in the urine of patients with liver cancer and cirrhosis (Endo et al. 1980). Sakai et al. reported that its level also increases in patients with a wide variety of malignancies (Sakai et al. 1990). Urinary free L-fucose, therefore, clinically serves as a marker of digestive organ cancer and cirrhosis. It may also be useful as a marker for various hematologic malignancies (Takubo et al. 2002).

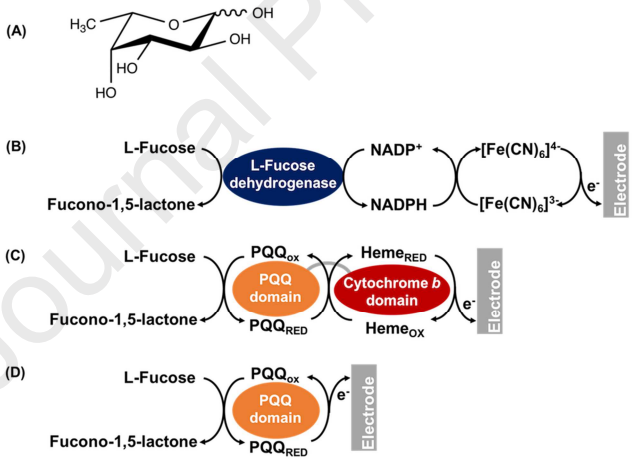


Figure 1. Chair structure of L-fucose (6-deoxy- L-galactose) (A). Reaction scheme of amperometric L-fucose biosensors. MET of bioelectrocatalysis for L-fucose oxidation by NAD(P)-dependent L-fucose dehydrogenase (B). DET of bioelectrocatalysis for L-fucose oxidation by full-length PQQ-dependent pyranose dehydrogenase (*Cc*PDH) (C) or the isolated PQQ domain of *Cc*PDH in this work (D). Electron pathways are shown as arrows.

Traditional approaches for detecting free L-fucose include enzymatic-spectrophotometric methods using nicotinamide adenine dinucleotide (NAD)-dependent fucose dehydrogenase (EC 1.1.1.122) (Endo et al. 1980; Sakai et al. 1990; Tsay and Dawson 1977), anion-exchange high-performance liquid chromatography (HPLC) employing fluorescence detection (Suzuki et al. 1992) or pulsed amperometric detection (PAD) (Fleming et al. 1995), and reverse-phase HPLC monitored by UV absorbance (Fu and O'Neill 1995) or fluorescence emission (Anumula 1994).

Different analytical methods have proposed quartz crystal microbalance (QCM) sensors that combine Au nanoparticles (AuNPs) for signal amplification (Wang et al. 2010). All these techniques, however, are considered to be time-consuming and laborious and require expensive equipment. Conversely, an amperometric sensor has advantages such as quick response times, simplicity of instrumentation, low cost, and the possibility of miniaturization. The following two amperometric sensors have been reported for free L-fucose monitoring: a sensor with a diamond paste electrode (Stefan-van Staden et al. 2012) and an enzymatic biosensor (Tsiafoulis et al. 2004). Enzymatic biosensors are of particular interest due to their operational simplicity and high selectivity. Tsiafoulis et al. demonstrated an amperometric L-fucose biosensor based on mediated electron transfer (MET)-type bioelectrocatalysis employed nicotinamide adenine dinucleotide phosphate (NADP)-dependent fucose dehydrogenase (Tsiafoulis et al. 2004). In this bioelectrocatalysis, the enzymatically regenerated NADPH is oxidized on the electrode through ferricyanide mediation (Fig. 1B). Direct electron transfer (DET)-type bioelectrocatalysis, in which enzymes can directly communicate with electrodes without redox mediators in the catalytic reaction, enables minimization of the overpotentials and the construction of simple bioelectrochemical devices. The fungal pyrroloquinoline quinone (PQQ)-dependent pyranose dehydrogenase from the basidiomycete *Coprinopsis cinerea* (*Cc*PDH) is one of the redox enzymes with DET ability (Fig. 1C), which consists of the catalytic PQQ domain, an N-terminal cytochrome *b* domain, and a C-terminal family 1 carbohydrate-binding module (Matsumura et al. 2014; Takeda et al. 2016; Takeda et al. 2019). This enzyme specifically catalyzes the oxidation of L-fucose and some rare sugars, such as D-arabinose and L-galactose, but not D-glucose, D-galactose, and D-fructose (Sakuta et al. 2018; Takeda et al. 2015). Besides, PQQ-dependent enzymes have advantages to designing bioelectrochemical devices over enzymes that require a free-cofactor like NAD(P) because PQQ is tightly bound to the active site of the enzyme. Therefore, we have recently developed a DET-type amperometric L-fucose biosensor by using *Cc*PDH (Takeda et al. 2020a).

However, ascorbic acid (AA), dopamine (DA), and uric acid (UA) are considered the most common coexisting electroactive species in urine samples due to their low oxidation potentials. These compounds are easily and directly oxidized at the electrode, and their electrochemical signals cause high noise in detecting the oxidation current of L-fucose. Eliminating the influence of those interferences is required for practical use. It is also desirable to directly measure urine without the pretreatment of samples to remove interferences for rapid and simple detection. Coating the sensor with polymeric films such as Nafion reduces the permeability of negatively charged substances (Dalmasso et al. 2013; Matuszewski et al. 1990; Miyashita et al. 2009). However, coating methods lower the signal of the analyte and increase the response time due to the prevention of diffusion, and it is difficult to eliminate this influence completely. In principle, this problem can be solved by determining an operating potential that is lower than the oxidation potentials of interferences. Here, to overcome this issue, an amperometric L-fucose biosensor that operates at a potential that is not affected by AA, DA, and UA was developed for direct testing in urine.

We also found DET-based bioelectrocatalysis of the PQQ domain only without the

cytochrome *b* domain (Takeda et al. 2020b). When electrons are directly transferred from PQQ to the electrode without heme *b*, an onset potential of the catalytic current drop to lower. This is because the redox potential of PQQ is lower than that of heme *b*. Thus, the PQQ domain-immobilized electrodes can be expected to obtain the catalytic current of L-fucose at a low potential where the oxidation of AA, DA, and UA does not occur. In the present study, an amperometric L-fucose biosensor unaffected by electroactive interferences was demonstrated and utilized DET-type bioelectrocatalysis of the PQQ domain separated from full-length *Cc*PDH (Fig. 1D).

2. Material and methods

2.1. Materials

The recombinant dehydrogenase domain (PQQ domain: residues 240-649) of *Coprinopsis cinerea* pyranose dehydrogenase (*Cc*PDH) was expressed in *Pichia pastoris* and purified as described in ref (Matsumura et al. 2014). The apo-form of the recombinant PQQ domain was reconstituted using the same procedure as for PQQ-dependent alcohol dehydrogenase (Takeda et al. 2013). PQQ, sodium citrate, ascorbic acid, uric acid, and dopamine hydrochloride were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). L-Fucose was purchased from Sigma-Aldrich (USA). 2-Mercaptoethanol was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Ja-pan). Hydrogen tetrachloroaurate (III) tetrahydrate (H_4AuCl_4) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). The multi-purpose artificial urine was prepared with reference to Sarigul et al. (Sarigul et al. 2019).

2.2. Preparation of enzyme electrodes

A polycrystalline gold electrode (a diameter of 1.6 mm, AuE) and gold rotating disk electrode (a diameter of 3 mm, RDE) modified with AuNPs (AuNPs/AuEs) were used for the direct bioelectrocatalysis of the PQQ domain. AuNPs with a particle diameter of 14-15 nm were prepared by citric reduction of H_4AuCl_4 with reference to Frens (Frens 1973). AuNPs were fabricated following a procedure described in ref (Murata et al. 2010). The obtained AuNP solution was centrifuged (10,000 g, 30 min) in 1.5 mL Eppendorf tubes, and then 98% of the remaining supernatant volume was discarded. The precipitated AuNPs were suspended by ultrasonication as a 50-fold concentrated AuNP dispersion. One microliter and 3.5 μL of the concentrated AuNPs were added dropwise onto the surface of the AuE and RDE, respectively, and then air-dried. Three-time and 15-time drop-casted AuNP electrodes were obtained by repeating this process 3 and 15 times, respectively. The PQQ domain was immobilized on the electrodes following previous work (Takeda et al. 2020b). Briefly, AuNP-modified electrodes were cleaned in 0.5 M H_2SO_4 by cyclic voltammetry between -0.2 and +1.5 V vs Ag|AgCl (3 M NaCl) for 20 cycles at a scan rate of 0.1 V/s before modification with a self-assembled monolayer (SAM). SAM-modified electrodes were prepared by immersion in 20 mM 2-mercaptoethanol for 1 h at room temperature. The surface was rinsed with Milli-Q water to remove excess thiol molecules. The PQQ domain was adsorbed on the

SAM-functionalized gold electrodes using 10 μL of 1 μM holo-PQQ domain in a 20 mM sodium acetate buffer solution (pH 6.0) at 4 °C for over 10 h. Before each measurement, the electrodes were gently rinsed with the same buffer to remove weakly adsorbed enzyme.

2.3. Electrochemical experiments

All electrochemical measurements were conducted using an ALS Model 702B electrochemical analyzer (BAS Inc., Tokyo, Japan) with a three-electrode cell system. A conventional three-electrode cell was used in which a platinum wire served as the counter electrode, and Ag|AgCl (3 M NaCl) served as the reference electrode. Before the measurements, N_2 bubbling was used to remove oxygen from the solutions in the electrochemical cell. All the potentials cited in this paper are with respect to the Ag|AgCl (3 M NaCl) electrode, with a potential of +209 mV vs. the normal hydrogen electrode (NHE). Cyclic voltammetry (CV) measurements were performed in 100 mM acetate buffer (pH 6.0) at ambient temperature with the PQQ domain-immobilized 3-time drop-casted AuNPs/AuE as used a working electrode. Amperometric i-t curve experiments were carried out with the RDE secured to an RRDE-2 analytical rotator (BAS Inc., Tokyo, Japan). The PQQ domain-immobilized AuNPs/RDEs were stirred at 500 rpm during the measurements. To prepare samples at designated L-fucose concentrations, a stock solution of L-fucose was successively added to 100 mM acetate buffer (pH 6.0) or the multi-purpose artificial urine at ambient temperature. An enzyme electrode was used for an independent measurement, and the data were obtained results from triplicate independent experiments.

3. Results and Discussion

3.1. Interference effects on the enzyme electrodes

Nanostructured electrodes were prepared by drop-casting citrate-reduced AuNPs three times on the surface and fabricated with a 2-mercaptoethanol self-assembled monolayer (SAM), followed by immobilization of the PQQ domain according to a previous study (Takeda et al. 2020b). We examined the amperometric responses of three relevant electroactive species and L-fucose in this enzyme electrode. Fig. S1 shows cyclic voltammograms (CVs) of AA, DA, and UA and catalytic currents of L-fucose oxidation in 100 mM acetate buffer at pH 6.0. The appearance of oxidation waves in AA and UA started at approximately -0.05 V and +0.2 V, respectively, with no reduction waves, implying an irreversible oxidation process of both AA and UA at the PQQ domain-modified AuNP electrode. The CV of DA showed a quasi-reversible electron transfer with a midpoint potential of +0.219 V. Details of these electrochemical process is in the references (Bacil et al. 2020; Huang et al. 2006; Kern 1954; Retna Raj and Ohsaka 2003; Struck and Elving 1965; Zhao et al. 2006). The bioelectrocatalytic current of L-fucose yielded an ideal sigmoidal wave shape with an onset potential of approximately -0.2 V and a steady-state current density of 120 $\mu A/cm^2$ in the potential range from +0.05 to +0.4 V (Fig. S1) (Takeda et al. 2020b).

Electrochemical oxidation of interferences would be suppressed by decreasing the operating potential; however, bioelectrocatalysis of the PQQ domain becomes suspended when the

potential is too low. Thus, analyses of interferences from three relevant electroactive species to L-fuocose oxidation on the PQQ domain-modified AuNP electrodes were performed by stepwise decreases in the applied potential. We considered that the amounts of AA, DA, and UA in urine samples were 50 mg, 1 mg, and 480 mg per 24 h urine, respectively, which are taken from the high ends of the standard ranges in the literature (Shier et al. 1982; Tsiafoulis et al. 2004). Assuming 1.5 L of urine excretion per 24 h, concentrations of 0.19 mM AA, 0.0046 mM DA, and 1.9 mM UA were used to measure the amperometric response. 0.1 mM L-fucose, which is lower than the cut-off value (0.17 mM) estimated from a 60 kg healthy adult male, was used in the experiments (Takeda et al. 2020a). Fig. 2A compares amperometric responses of AA, DA, UA and L-fuocose at an applied potential of +0.3 V. The addition of 0.1 mM L-fucose results in a well-defined response; however, the successive addition of each interfering compound, particularly AA, gave recognizable current responses. At an applied potential of 0 V, the interfering signals from DA and UA were excluded, and AA was reduced to double digits but still discernible (Fig. 2B). Applying a potential of -0.1 V completely diminished the amperometric responses of AA, while the response of L-fucose oxidation remained (Fig. 2C). Further injection of L-fucose to the electrode still showed a clear oxidation signal in the presence of interferences.

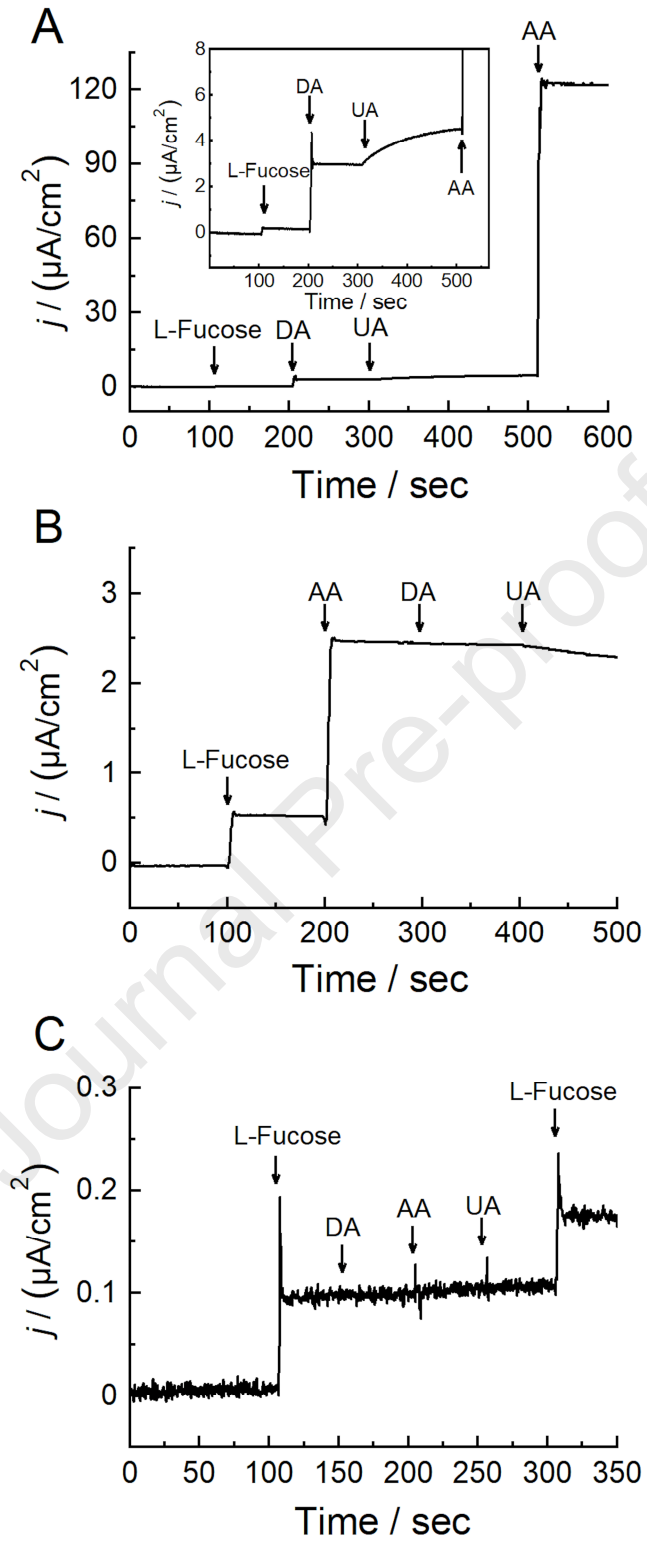


Figure 2. Amperometric responses of the PQQ domain modified AuNP rotated electrode upon additions of 0.1 mM L-fucose, 1.9 mM uric acid (UA), 0.0046 mM dopamine (DA), and 0.19 mM ascorbic acid (AA) in 100 mM acetate buffer, pH 6.0, at the applied potential of (A) +0.3 V, (B) 0 V, and (C) -0.1 V vs. Ag|AgCl (3 M NaCl). The electrodes rotation rate was 500 rpm. The inset shows magnification of amperometric responses at +0.3 V.

3.2 Amperometric response of the PQQ domain-modified AuNP-electrodes

The operating potential of -0.1 V on the PQQ domain immobilized electrodes can detect L-fucose without the oxidation reaction of AA, DA, and UA. Thus, the amperometric responses of L-fucose were studied at an applied potential of -0.1 V for evaluating sensor properties. Fig. 3A, inset, shows current-time plots of the rotated PQQ domain-modified AuNPs/AuE with successive additions of 0.1 mM and 1 mM L-fucose in buffer solution at pH 6.0. In the stepwise addition of 0.1 mM L-fucose, the catalytic current density increased rapidly and approached the steady-state current within 5 s. The linear relationship between the catalytic current and the concentration is shown in Fig. 3A. The three-time drop-casted AuNP electrode obtained a current density with a linear response in the L-fucose concentration range from 0.1 mM to 1 mM (R^2 = 0.998). This range includes a cutoff value of 0.17 mM of L-fucose, as it has been assumed that a 60 kg adult male excretes 20 mg creatinine/kg and 1.5 L urine/day (Takeda et al. 2020a). The sensor sensitivity was 0.91 ± 0.02 μA mM^{-1} cm^{-2} (n = 3), and the detection limit was calculated to be 20.8 μM at a signal-to-noise ratio of three (S/N = 3).

Other than the abovementioned AuNP electrode, which was prepared by drop-casting AuNPs on the AuE surface 3 times, electrodes with 15 times of drop-castings were used (Fig. 3B). The specific surface area increased with an increasing number of AuNP drop-castings on the electrode, which increased the catalytic current (i.e., signal) due to increasing the number of immobilized proteins on the electrode (Takeda et al. 2020b). Previous research reported roughness factors (the electroactive surface area/geometrical surface area, R_f) of AuNP electrodes. The electroactive surface area of the 15-time drop-casted AuNP electrode (R_f = 323 ± 10) increased 5.8-fold compared to that of the 3-time drop-casted AuNP electrode (R_f = 56 ± 1). It should be noted that the units of current density (μA/cm2, j) are represented by dividing the geometrical surface area of the electrodes. Both electrodes display a linear response range of 0.1 mM to 1 mM and improvement in the sensor sensitivity and the detection limit (Fig. 3B). The sensor sensitivity of the 15-time drop-casted AuNP electrode was 3.12 ± 0.05 μA mM^{-1} cm^{-2} (R^2 = 0.999; n = 3), and the detection limit (S/N = 3) was calculated to be 13.6 μM, which were comparable values to those of the full-length *Cc*PDH-immobilized electrode at an applied potential of +0.1 V (sensor sensitivity of 4.54 μA mM^{-1} cm^{-2} with an extended linear range from 0.04 mM to 2.1 mM) (Takeda et al. 2020a). Thus, the PQQ domain-modified AuNP electrode can be used as an amperometric biosensor for the direct detection of L-fucose in urine.

Furthermore, as the first step in research toward practical use, we attempted to quantify L-fucose in artificial urine using the proposed sensor. The multi-purpose artificial urine proposed by Sarigul et al., which is a significantly close similarity with human urine (Sarigul et al. 2019), was used as artificial urine for evaluating sensor properties. Figure 3C depicts amperometric response resulting from the addition of 0.1 mM L-fucose at an applied potential of -0.1 V in undiluted multi-purpose artificial urine and the corresponding calibration plots, where a linear response was observed (R^2 = 0.998). The sensor sensitivity of the PQQ domain-modified AuNP electrode which has R_f of 78 ± 4 was 1.69 ± 0.02 μA mM^{-1} cm^{-2} (n = 3), and the limit of detection (S/N = 3) was found to be 39.5

μM. After the successive addition of L-fucose, 0.0046 mM DA, 0.19 mM AA and 1.9 mM uric UA, no significant interference changes in the catalytic current were also observed. The PQQ domain modified AuNP electrode showed reproducible results with the standard error of all sensor sensitivities in Fig. 3 within 2%. These results demonstrated the biosensor capable of measuring L-fucose in at least artificial urine with high accuracy.

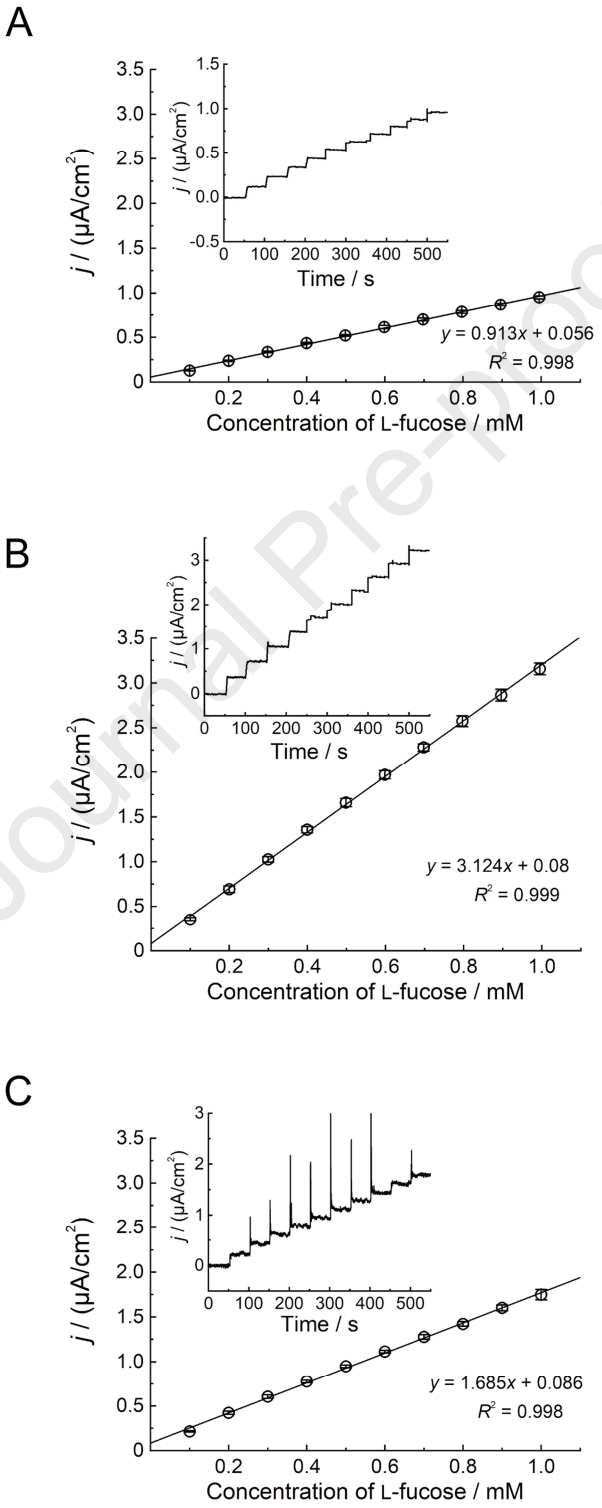


Figure 3. Calibration plots for L-fucose of the PQQ domain-modified AuNP rotated electrodes with

(A) 3-time drop-casting AuNPs (R_f = 56 ± 1) and (B) 15 time drop-casting AuNPs (R_f = 323 ± 10) in 100 mM acetate buffer (p H 6.0). (C) Calibration plots for L-fucose of the PQQ domain-modified AuNP rotated electrodes (R_f = 78 ± 4) measured in artificial urine. Insets shows corresponding amperometric responses to the sequential addition of 0.1 mM L-fucose. All measurements were performed an applied potential of -0.1 V vs. Ag|AgCl (3 M NaCl) and rotation rate of 500 rpm.

4. Conclusion

In summary, an amperometric L-fucose biosensor that utilizes DET-type bioelectrocatalysis of the PQQ domain from *Cc*PDH has been reported for the first screening test of cancer using urine. With an applied potential of -0.1 V, the oxidation of AA, DA, and UA was avoided, and the L-fucose oxidation still provided a clear catalytic current. The PQQ domain-modified AuNP electrode exhibited high sensitivity with a useful linear range and a low detection limit. Due to the lack of influence of interfering substrates, the sensor can directly detect the concentration of L-fucose in urine. As a result, pretreatment of urine samples to remove interferences or modifications to prevent contamination to the sensor are not required, which leads to cost reduction and simple measurements. This sensor is expected to be utilized in point-of-care testing for the first screening of cancer.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT author statement

Kouta Takeda: Methodology, Validation, Investigation, Writing - Original Draft, Visualization. **Ryo Kusuoka**: Validation, Investigation. **Misaki Inukai**: Validation, Investigation. **Kiyohiko Igarashi**: Conceptualization, Resources. **Hiroyuki Ohno**: Resources. **Nobuhumi Nakamura**: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration.

Acknowledgements

Funding was provided by a Grant-in-Aid for Young Scientific Research (B) (17 K17703 to K.T.) and a Grant-in-Aid for Scientific Research (B) (19H03013 to K.I.) from Japan Society for the Promotion of Science (JSPS). This research was also supported by a Grant-in-Aid for Innovative Areas from the Japanese Ministry of Education, Culture, Sports, and Technology (MEXT) (No. 18H05494 to K.I.). K.I. thanks to the Business Finland (BF, Previously the Finnish Funding Agency for Innovation (TEKES)) for the support of the Finland Distinguished Professor (FiDiPro) Program "Advanced approaches for enzymatic biomass utilization and modification (BioAD)."

304 **References**

305 Adamczyk, B., Tharmalingam, T., Rudd, P.M., 2012. Biochim. Biophys. Acta 1820(9), 1347-1353.
306 Anumula, K.R., 1994. Anal. Biochem. 220(2), 275-283.
307 Bacil, R.P., Chen, L., Serrano, S.H.P., Compton, R.G., 2020. Phys. Chem. Chem. Phys. 22(2),
308 607-614.
309 Becker, D.J., Lowe, J.B., 2003. Glycobiology 13(7), 41R-53R.
310 Dalmasso, P.R., Pedano, M.L., Rivas, G.A., 2013. Biosens. Bioelectron. 39(1), 76-81.
311 Deugnier, Y., David, V., Brissot, P., Mabo, P., Delamaire, D., Messner, M., Bourel, M., Legall, J.Y.,
312 1984. Hepatology 4(5), 889-892.
313 Endo, M., Matsue, H., Sato, S., Majima, M., Hiyama, N., 1980. Clin. Chim. Acta 103(3), 269-275.
314 Fleming, S.C., Gill, M., Laker, M.F., 1995. J. Liq. Chromatogr 18(1), 173-180.
315 Frens, G., 1973. Nat. Phys. Sci. 241(105), 20-22.
316 Fu, D., O'Neill, R.A., 1995. Anal. Biochem. 227(2), 377-384.
317 Huang, X.J., Im, H.S., Yarimaga, O., Kim, J.H., Lee, D.H., Kim, H.S., Choi, Y.K., 2006. J. Phys.
318 Chem. B 110(43), 21850-21856.
319 Keeley, T.S., Yang, S.Y., Lau, E., 2019. Cancers 11(9).
320 Kern, D.M.H., 1954. J. Am. Chem. Soc. 76(4), 1011-1015.
321 Manchil, P., Joy, E., Kiran, M., Sherubin, J., Khan, M., Aravind, B., 2016. J. Pharm. Bioallied Sci.
322 8(5), 147-150.
323 Matsumura, H., Umezawa, K., Takeda, K., Sugimoto, N., Ishida, T., Samejima, M., Ohno, H.,
324 Yoshida, M., Igarashi, K., Nakamura, N., 2014. PLoS One 9(8), e104851.
325 Matuszewski, W., Trojanowicz, M., Lewenstam, A., 1990. Electroanal. 2(8), 607-615.
326 Miyashita, M., Ito, N., Ikeda, S., Murayama, T., Oguma, K., Kimura, J., 2009. Biosens. Bioelectron.
327 24(5), 1336-1340.
328 Murata, K., Kajiya, K., Nukaga, M., Suga, Y., Watanabe, T., Nakamura, N., Ohno, H., 2010.
329 Electroanal. 22(2), 185-190.
330 Retna Raj, C., Ohsaka, T., 2003. J. Electroanal. Chem. 540(2), 69-77.
331 Sakai, T., Yamamoto, K., Yokota, H., Hakozaki-Usui, K., Hino, F., Kato, I., 1990. Clin. Chem. 36(3),
332 474-476.
333 Sakuta, R., Takeda, K., Igarashi, K., Ohno, H., Nakamura, N., 2018. Energy Technol. 6(2), 273-279.
334 Sarigul, N., Korkmaz, F., Kurultak, İ., 2019. Sci. Rep. 9(1), 20159.
335 Schneider, M., Al-Shareffi, E., Haltiwanger, R.S., 2017. Glycobiology 27(7), 601-618.
336 Scott, E., Munkley, J., 2019. Int. J. Mol. Sci. 20(6). 1389.
337 Shier, N.W., Heinrichs, T.F., Hart, W., 1982. J. Food Sci. 47(1), 334-335.
338 Stefan-van Staden, R.I., Nejem, R.M., van Staden, J.F., Aboul-Enein, H.Y., 2012. Analyst 137(4),
339 903-909.
340 Struck, W.A., Elving, P.J., 1965. Biochemistry 4(7), 1343-1353.
341 Suzuki, J., Kondo, A., Kato, I., Hase, S., Ikenaka, T., 1992. Clin. Chem. 38(5), 752-755.
342 Takeda, K., Igarashi, K., Yoshida, M., Nakamura, N., 2020a. Bioelectrochemistry 131, 107372.

Takeda, K., Kusuoka, R. Birrell, J.A., Yoshida, M., Igarashi, K., Nakamura, N., 2020b. Electrochemica acta 539(1), 136982.

Takeda, K., Matsumura, H., Ishida, T., Samejima, M., Igarashi, K., Nakamura, N., Ohno, H., 2013. Bioelectrochemistry 94, 75-78.

Takeda, K., Matsumura, H., Ishida, T., Samejima, M., Ohno, H., Yoshida, M., Igarashi, K., Nakamura, N., 2015. PLoS One 10(2), e0115722.

Takeda, K., Matsumura, H., Ishida, T., Yoshida, M., Igarashi, K., Samejima, M., Ohno, H., Nakamura, N., 2016. Biochem. Biophys. Res. Commun. 477(3), 369-373.

Takeda, K., Umezawa, K., Varnai, A., Eijsink, V.G., Igarashi, K., Yoshida, M., Nakamura, N., 2019. Curr. Opin. Chem. Biol. 49, 113-121.

Takubo, T., Aoyama, Y., Nakamae, H., Tanaka, K., Ohta, K., Yamane, T., Hino, M., 2002. Leuk. Lymphoma 43(11), 2241-2243.

Taniguchi, N., Kizuka, Y., 2015. Adv. Cancer Res. 126, 11-51.

Tatsumura, T., Sato, H., Mori, A., Komori, Y., Yamamoto, K., Fukatani, G., Kuno, S., 1977. Cancer Res. 37(11), 4101-4103.

Tsay, G.C., Dawson, G., 1977. Anal. Biochem. 78(2), 423-427.

Tsiafoulis, C.G., Prodromidis, M.I., Karayannis, M.I., 2004. Biosens. Bioelectron. 20(3), 620-627.

Vanhooren, P.T., Vandamme, E.J., 1999. J. Chem. Technol. Biotechnol. 74(6), 479-497.

Wang, Y., Chalagalla, S., Li, T., Sun, X.L., Zhao, W., Wang, P.G., Zeng, X., 2010. Biosens. Bioelectron. 26(3), 996-1001.

Zhao, Y., Bai, J., Wang, L., Hong, X., Huang, P., Wang, H., Zhang, L., 2006. Int. J. Electrochem. Sci. 1(7), 363-371

366 **Figure legends**

367 **Figure 1.** Chair structure of L-fucose (6-deoxy- L-galactose) (A). Reaction scheme of amperometric
368 L-fucose biosensors. MET of bioelectrocatalysis for L-fucose oxidation by NAD(P)-dependent
369 L-fucose dehydrogenase (B). DET of bioelectrocatalysis for L-fucose oxidation by full-length
370 PQQ-dependent pyranose dehydrogenase (*Cc*PDH) (C) or the isolated PQQ domain of *Cc*PDH (D).
371 Electron pathways are shown as arrows.

372

373 **Figure 2.** Amperometric responses of the PQQ domain modified AuNP rotated electrode upon
374 additions of 0.1 mM L-fucose, 1.9 mM uric acid (UA), 0.0046 mM dopamine (DA), and 0.19 mM
375 ascorbic acid (AA) in 100 mM acetate buffer, pH 6.0, at the applied potential of (A) +0.3 V, (B) 0 V,
376 and (C) -0.1 V vs. Ag|AgCl (3 M NaCl). The electrodes rotation rate was 500 rpm. The inset shows
377 magnification of amperometric responses at +0.3 V.

378

379 **Figure 3.** Calibration plots for L-fucose of the PQQ domain-modified AuNP rotated electrodes with
380 (A) 3-time drop-casting AuNPs (R_f = 56 ± 1) and (B) 15 time drop-casting AuNPs (R_f = 323 ± 10)
381 in 100 mM acetate buffer (p H 6.0). (C) Calibration plots for L-fucose of the PQQ domain-modified
382 AuNP rotated electrodes (R_f = 78 ± 4) measured in artificial urine. Insets shows corresponding
383 amperometric responses to the sequential addition of 0.1 mM L-fucose. All measurements were
384 performed an applied potential of -0.1 V vs. Ag|AgCl (3 M NaCl) and rotation rate of 500 rpm.

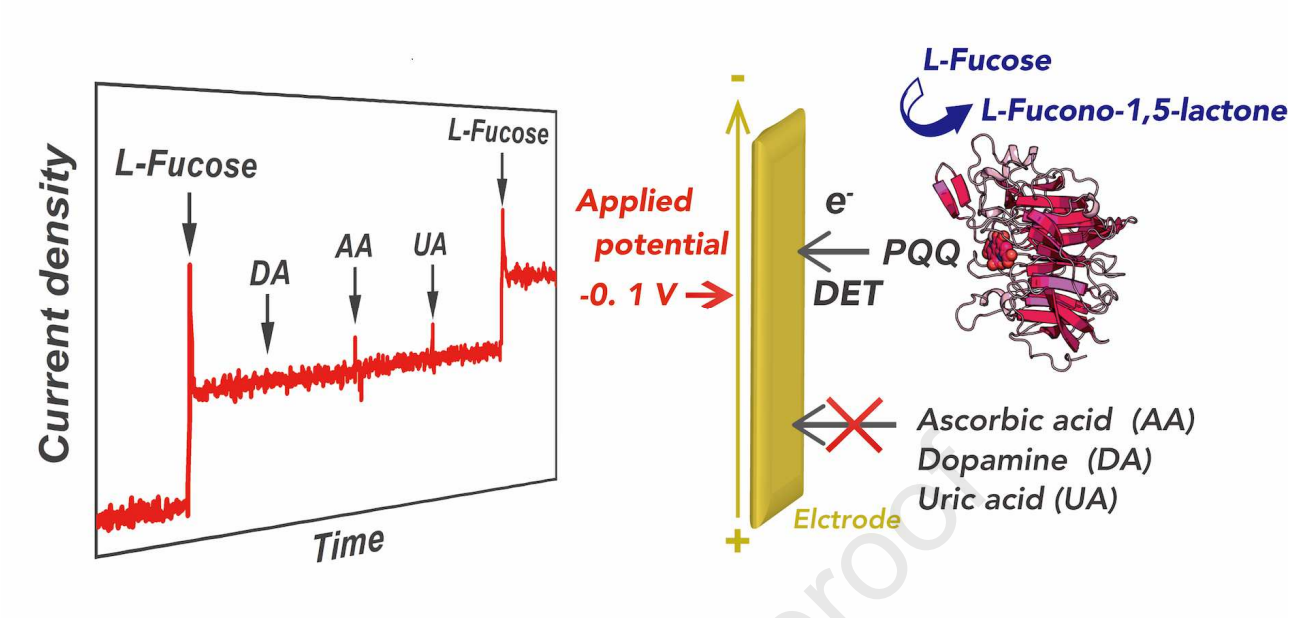
385

386

387

388

389 **Graphical Abstracts**



390

Highlights

・An amperometric L-fucose biosensor based on direct electron transfer was developed.

・An isolated PQQ domain was immobilized on gold nanoparticle-modified electrodes.

・Enzyme electrodes obtained catalytic currents depending on the substrate level at a low potential.

・Applying the potential of -0.1 V completely eliminated the oxidation of AA, DA, and UA on electrodes.

・The sensor can be used to measure L-fucose without being affected by urinary interferences.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: