Supplementary materials

Employment of 1-Methoxy-5-Ethyl Phenazinium Ethyl Sulfate as a Stable Electron Mediator in Flavin Oxidoreductases-Based Sensors

Maya Fitriana ^{1,2}, Noya Loew ^{3,4}, Arief Budi Witarto ², Kazunori Ikebukuro ¹, Koji Sode ^{3,*} and Wakako Tsugawa ^{1,*}

- ¹ Department of Biotechnology and Life Science, Graduate School of Engineering, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan; s206376w@st.go.tuat.ac.jp (M.F.); ikebu@cc.tuat.ac.jp (K.I.); tsugawa@cc.tuat.ac.jp (W.T.)
- ² Faculty of Biotechnology, Sumbawa University of Technology, Jl. Raya Olat Maras, Batu Alang, 84371 Sumbawa Besar, Indonesia; witarto@gmail.com (A.B.W.)
- ³ Joint Department of Biomedical Engineering, The University of North Carolina at Chapel Hill and North Carolina State University, Chapel Hill, NC 27599, USA; ksode@email.unc.edu (K.S.)
- ⁴ Department of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan; noya-loew@rs.tus.ac.jp (N.L)
- * Correspondence: ksode@email.unc.edu (K.S.); tsugawa@cc.tuat.ac.jp (W.T.); Tel.: +1-919-966-3550 (K.S.); +81- 42-388-7027 (W.T.)

Cyclic voltammograms of mediators



Cyclic voltammetry (CV) measurements were carried out for 1 mM mPMS (black dashed line) or mPES (red solid line) in 100 mM PPB (pH 7.0). Potential range: 0.4 to -0.4 V vs Ag/AgCl; scan rate: 0.1 V/s. Working electrode (WE): glassy carbon (GC); reference electrode (RE): Ag/AgCl; counter electrode (CE): platinum wire.

Oxidation and reduction peaks were observed at -0.11 V and -0.17 V vs Ag/AgCl, respectively, for mPMS and at -0.11 V and -0.18 V vs Ag/AgCl, respectively, for mPES. Thus, the half wave potential of both mediators was -0.14 V vs Ag/AgCl.



Response currents of lactate sensors toward common redox substances

(a) Response currents to blank sample and 10 mM ascorbic acid (AA), uric acid (UA), acetaminophen (Ac), and lactate (Lac). Applied potential: +0.2 V vs Ag/AgCl. Gray area: background current. (b) Response currents to 10 mM ascorbic acid at various applied potentials.

Sensor composition: 1 U *Av*LOx A96L, 100 nmol mPES, 40 μ g sucrose, and 20 μ g Tween 20. Waiting time: 60 s. N = 3. Sampling point: 10 s.



Storage stability of lactate sensors with 1 U of AvLOx A96L

Calibration curves of lactate sensors stored at 25°C in the dark, evaluated after storage for 0, 90, 100 or 120 days. Linear regression was determined for up to 30 mM lactate; sensitivities were 0.68 - 0.77 μ A/mM (R² > 0.98) for all calibration curves. Sensor composition: 1 U *Av*LOx A96L, 100 nmol mPES, 40 μ g sucrose, and 20 μ g Tween 20. Waiting time: 60 s. Applied potential: +0.2 V vs Ag/AgCl. N = 3. Sampling point: 10 s.

Table S1

The limit of detection (LOD) of the lactate sensor with 5 U AvLOx A96L during storage

Day storage ¹	0	12	28	48	
LOD (mM)	1.1	1.1	0.5	1.0	

¹ with 2 days prestorage



Stability of mPES electrode strips

Sensor composition: 100 mmol mPES. Sample composition: 0-50 mM lactate, 5 U/ μ L *Av*LOx A96L, 4% sucrose, and 2% Tween 20 in 100 mM PPB (pH 7.0). Waiting time: 60 s. Applied potential: +0.2 V vs Ag/AgCl. N = 3. Sampling point: 10 s. Sensor strips were stored for 0, 14, or 30 days at (a) 25°C or (b) 45°C in the dark. Linear regression: 0-30 mM lactate; sensitivities: 0.65-0.78 μ A/mM; R² > 0.95.