（様式 5）

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学位（博士）論文要旨

(Doctoral thesis abstract)

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論 文 題 目 (Title)	Engineering of lactate oxidase for wearable sensor applications

論文要旨（2000 字程度）
(Abstract(400 words))
※欧文・和文どちらでもよい。但し、和文の場合は英訳を付すこと。
(in English or in Japanese)

L-lactate is an essential biomarker for clinical diagnostics, sports medicine, and food quality control. Almost L-lactate measurement system employs lactate oxidase (LOx)-based amperometric lactate biosensor. LOx-based electrochemical lactate sensors have already been commercialized due to high enzyme stability and specificity. However, there are still some problems on enzyme reaction to detect lactate level accurately and to achieve the development of wearable lactate sensing system.

In Chapter 2 “Turning lactate oxidase into dehydrogenase to minimize the oxygen interference effect on lactate sensor”, LOx from *Aerococcus viridans* (AvLOx) was engineered for decreasing of the reactivity for oxygen to minimize oxygen interference effect on sensor application. To decrease the reactivity for oxygen, the oxygen accessible pathway from enzyme surface to cofactor was predicted by computational analyzation using AvLOx structure and blocked by mutagenesis study. Then, oxygen insensitive AvLOx mutant was acquired without loss of the activity for artificial electron acceptor. Moreover, another LOx from *Enterococcus hirae* (EhLOx) was analyzed its structure and also predicted its oxygen accessible pathway using EhLOx structure. Using same strategy, an oxygen insensitive EhLOx mutant was obtained without loss of the activity for artificial electron acceptor.

In Chapter 3 “Engineering of lactate oxidase to develop a quasi-direct electron transfer type lactate sensor”, the oxygen insensitive AvLOx mutant was introduced further mutation for

development of a quasi-direct electron transfer (quasi-DET) type lactate sensor. In this system, a rationally engineered AvLOx was modified with amine-reactive phenazine ethosulfate (PES) and was expected to show the DET reaction via modified PES. Since the modification of wild type AvLOx by PES did not result quasi-DET, engineered AvLOx with additional Lys residue was designed. The additional Lys residue was introduced by site-directed mutagenesis on the substrate entrance. Then, PES-modified AvLOx mutant could measure L-lactate concentration without interferents effect, therefore, quasi-DET type lactate sensing element was developed in this study.

In Chapter 4 “Design of fusion enzyme with lactate oxidase and flavocytochrome b_2 heme domain for development of direct electron transfer type lactate sensor”, Fcb2 from *Pichia pastoris* (PpFcb2) was acquired as a high productivity lactate sensing element. Because PpFcb2 catalytic domain was low thermal stability, PpFcb2 heme domain was fused into oxygen insensitive AvLOx mutant. Additionally, to expand the detection range, another mutation was introduced into AvLOx to relieve the substrate inhibition. The designed enzyme“b2LOxS” showed response current against lactate with direct electron transfer (DET) reaction on electrode without substrate inhibition. Moreover, b2LOxS and DET type glucose dehydrogenase immobilized thin-film electrode achieved simultaneous detection of lactate and glucose based on DET reaction without any artificial electron mediator.

In Chapter 5 “Conclusion”, the Chapter 2-4 were summarized and described future perspectives of this study.

（英訳）※和文要旨の場合(400 words)