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論文名	Development of Electrochemical Platforms Devised for Bacterial Activity Monitoring （バクテリアの活性度評価のために考案された電気化学的プラットフォームの開発）
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論　文　要　旨

Microorganisms have been receiving great attention not only for a number of useful applications to the bio-related engineering fields, but also for the infectious diseases threatening the human activities. Regarding the latter, the detection of pathogenic bacteria has been an urgent issue for decades. Although some established techniques, such as culturing and colony counting, and polymerase-chain-reaction methods, are currently available for this purpose, these techniques suffer from time-consuming operations or poor cost-effectiveness. In this connection, the development of an accurate, robust and cost-effective detection technique making real-time quantification possible is still an important goal to be achieved.

In this study, I have developed two different types of electrochemical platforms for the observation of bacterial activity, by using conducting polymers (CPs) as the inclusion matrices and by using redox shuttle molecules transferred from bacteria to an electrode surface. It was found that these devices work not only for the detection of bacteria, but also work as platforms for monitoring the bacterial activity.

Concerning the first model, CP-film based devices doped with several different bacterial species have been developed. It was found that the bacterial presence and their activity were detected with the devices due to the excellent electrical characteristics and structural flexibility of CPs. In this study, several Gram-positive and negative bacteria were immobilized in polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT) films. Thanks to the negative charges located on their outer membranes, the bacterial species were automatically inserted into the polymer films in order to compensate for the positive charges resulting on the polymer backbone during electrochemical polymerization. It was found in this study that the insertion did not impair the living status of the bacteria and made the electrochemical communication possible with the electrodes. Based on these results, I have developed the CP films, which could be utilized as the platforms for studying the bacterial activity.

The second model devices were fabricated by transferring isoprenoid quinones, such as ubiquinone and menaquinone, from the cytoplasmic membrane of bacteria to an electrode surface by damaging their outer cell walls during *in-situ* desiccation. This process, resulting from the bacterial presence, produced the current responses, which were also utilized to acquire the internal information on the redox activity of the bacteria. In this case, adoption of an indium-tin-oxide (ITO) electrode, rather than a commonly used carbon electrode, resulted in a dramatic improvement in the resolution of the electrochemistry of the quinones. This novel and straightforward technique was applied to profiling the isoprenoid quinones, the concentrations of which depended on oxygen availability during culture.

The purpose of this study is therefore to investigate the effectiveness of these model devices by applying to bacterial activity monitoring. More specific discussions are outlined below:

Chapter 1 provides a brief introduction of bacteria, conducting polymers, isoprenoid quinones along with a review of related literature.

Chapter 2 discusses the electrochemical insertion of bacterial species into a PPy film prepared on an ITO electrode. *Pseudomonas aeruginosa*, *Escherichia coli, Acinetobacter calcoaceticus, Serratia marcescens, Shewanella oneidensis* and *Bacillus subtilis* were inserted either vertically or horizontally in the polymer films, depending on polymerization conditions. It was found that the pH values of the monomer solutions strongly affected the film growth rates. The viability of the inserted bacteria was higher than that for the bacteria directly applied on bare electrode surfaces. The oxygen consumption rate of a PPy/*E. coli* film in contact with a glucose solution was clearly higher than that for the PPy film without the bacteria, demonstrating that the PPy/bacteria film could be used as a platform for observing the bacterial activity.

Chapter 3 focuses on the electrochemical characteristics of PEDOT films doped with bacteria. The voltammograms of the PEDOT/bacteria films exhibited sharp cathodic and broad anodic peaks, which suggested the redox reactions of secretions from bacteria, such as ubiquinone. The excellent stability of the PEDOT/bacteria films was indicated by the fact that this polymer could be electrochemically cycled without losing its redox activity. Other results demonstrated that the polymerization potential and time were the main factors that determined the viability of bacteria. In fact, the viability was strongly dependent on the thickness of the polymer film; more than 90% of *P. aeruginosa* were found viable after the polymerization, when the film thickness was above 0.5 μm. Further investigation on the viability of the inserted bacteria against the voltage stimulus revealed that *P. aeruginosa* and *S. oneidensis* were alive for anodic scans (between 0.0 V to +0.5 V *vs*. Ag|AgCl|sat. KCl), while the cathodic scans (between 0.0 V and -0.7 V) killed about 80% of bacteria after 30 scan cycles. One possible reason for this high fatality is that the cathodic voltage impaired certain components of the cell membrane, such as lipid bilayers and membrane bound proteins. However, no such deterioration was observed for the voltammetric response of PPy/*E. coli*, and the bacteria remained viable even after 30 scan cycles between -0.7 V and +0.5 V.

Chapter 4 discusses a rapid detection technique of bacteria by damaging their cell walls. By applying an *E. coli* suspension on an ITO electrode followed by evaporation in a desiccator, two pairs of well-defined redox peaks based on the surface-confined events were observed in a

pH-7.0 phosphate buffer. The excellent agreements in the mid-peak potential values between the dried bacteria and authentic compounds made possible the assignment of these two pairs to isoprenoid ubiquinone (UQn) and menaquinone (MKn), which were both present in the bacterial cell envelopes (“n” denotes the number of the prenyl-unit). The damage caused by the dehydration to the outer cell membrane led to the hydrophobic transfer of these quinones from the cytoplasmic membrane onto the ITO surface. Identical redox responses were observed by desiccating the suspension by heat, in a vacuum, and by microwave irradiation. The several different Gram-positive and negative bacterial species resulted in the redox peaks assigned to the adsorbed isoprenoid quinones, demonstrating that this procedure made the detection of the quinones possible without laborious solvent extraction procedures. The oxygen-dependent production of UQn and MKn by *E. coli* and *P. aeruginosa* was also studied to demonstrate that this technique could be used not only for bacteria detection, but also for profiling of the isoprenoid quinones, which play important roles in electron and proton transfer in bacteria. The present technique provides a much more straightforward way than the solvent extraction, in that one sample can be prepared in 1 min by heat evaporation of the bacterial suspension.

Chapter 5 concludes this thesis by summarizing the previous chapters.

審 査 結 果 の 要 旨

本論文は細菌の活性度を評価するための電気化学デバイスの設計と応用について研究成果をまとめたものであり，次のような成果を得ている。

(1) 導電性ポリマであるポリピロールは合成時に陰電荷を有する物質を取り込む。このことを利用し，電極上に作製したポリピロール薄膜に細菌を生存状態で挿入する方法を開発した。また，検討した細菌全てについて膜への挿入が観測されたため，挿入は負の表面電荷を持つ細菌に対して起こる一般的な現象であると結論した。さらに，膜作製時の pH により細菌の挿入方向が変わることも見出した。すなわち，酸性条件下では細菌は膜に対して垂直方向に，中性条件下では水平方向に挿入されることを観測し，これがポリマ膜の成長速度と関連していることを明らかにした。

(2) 膜に挿入固定された大腸菌の活性度を電気化学的に評価した。薄相電気化学セルを用い，大腸菌による酸素消費量がグルコース濃度に依存することを示し，固定化電極が細菌の活性度評価デバイスとして機能することを示した。

(3) ポリピロールよりも高い pH において重合可能なポリ(3,4−エチレンジオキシチオフェン)膜について同様の検討を行った。この結果，細菌生存率は膜厚の増大に伴い高くなることを示し，さらに電極電位が生存率に及ぼす影響についても議論した。

(4) 細菌を乾燥することにより，内膜に存在するイソプレノイドキノンを電極に吸着させる方法を開発した。この方法では，主にユビキノンとメナキノンが抽出され，酸化インジウムスズ電極を用いたとき，酸化還元ピークの分解能が飛躍的に向上することを示した。さらに，この電極で観測されるユビキノン，メナキノンのピーク強度からキノンプロファイルが可能であり，対象細菌の呼吸様式が動的に評価できることを示した。

以上の成果は，開発した電気化学デバイスが細菌の活性度を評価するために有用である

ことを明らかにしており，細菌の電気化学計測の発展に貢献するところ大である。さらに，申請者が自立して研究を行うに十分な能力と学識を有することを証したものである。学位論文審査委員会は，本論文の審査ならびに最終試験の結果から，博士（工学）の学位を授与することを適当と認める。