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Real-Time Monitoring of Mitochondrial ATP Synthesis and Hydrolysis by Surface Infrared Spectroscopy

Ryo-taro Yamaguchi[1], Ayumi Hirano-Iwata[2], Yuki Aonuma[1], Yuya Yoshimura[3], Yasuo Shinohara[3], Yasuo Kimura[1], Michio Niwano[1]

[1]Laboratory for Nanoelectronics and Spintronics, Research Institute of Electrical Communication, Tohoku University, Sendai 980-8577, [2]Graduate School of Biomedical Engineering, Tohoku University, Sendai 980-8579, [3]Institute for Genome Research, The University of Tokushima, Tokushima 770-8503, Japan

Mitochondria play key roles in the production of cell's energy. Their dominant function is the synthesis of adenosine 5'-triphosphate (ATP) from adenosine diphosphate (ADP) and phosphate (Pi) through the oxidative phosphorylation. Evaluation of drug-induced mitochondrial toxicity has become increasingly important since mitochondrial dysfunction has recently been implicated in numerous diseases including cancer and diabetes mellitus. Mitochondrial functions have been monitored via oxygen consumption, mitochondrial membrane potential, and more importantly via ATP synthesis since ATP synthesis is the most essential function of mitochondria. Various analytical methods have been employed to investigate ATP synthesis in mitochondria, including high performance liquid chromatography (HPLC), bioluminescence technique, and pH measurement. However, most of these methods are based on destructive analysis or indirect monitoring through the enzymatic reaction.

Infrared absorption spectroscopy (IRAS) is one of the useful techniques for real-time, label-free, and direct monitoring of biological reactions [1,2]. However, the strong water absorption requires very short path length in the order of several micrometers. Transmission measurements with thin path length are not suitable for mitochondrial assays because solution handlings necessary for evaluating mitochondrial toxicity, such as rapid mixing of drugs and oxygen supply, are difficult in such a narrow space. On the other hand, IRAS in the multiple internal reflection (MIR) geometry provides an ideal optical configuration to combine solution handling and aqueous-phase measurement. We have recently reportedon a real-time monitoring of drug-induced necrotic and apoptotic cell death using MIR-IRAS [3,4]. Clear discrimination between viable and damaged cells has been demonstrated, showing a promise as a label-free and real-time detection for cell-based assays.

In the present study, we have applied our MIR-IRAS system to mitochondria-based assays by monitoring ATP synthesis in isolated mitochondria from rat livers. Mitochondrial ATP synthesis and hydrolysis were *in situ* monitored with MIR-IRAS, while dissolved oxygen level and solution pH were simultaneously monitored with O2 and pH electrodes, respectively. It is demonstrated that ATP synthesis and hydrolysis can be monitored by the IR spectral changes in phosphate groups in adenine nucleotides and MIR-IRAS is useful for evaluating time-dependent drug effects of mitochondrial toxicants.

References

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