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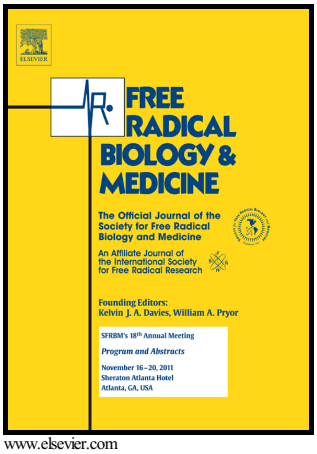

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Extracellular Hydrogen Peroxide Measurements Using a Flow Injection System in Combination with Microdialysis Probes – Potential and Challenges

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Abstract

There is a strong need for techniques that can quantify the important reactive oxygen species hydrogen peroxide (H_2O_2) in complex media and *in vivo*. We combined chemiluminescence-based H_2O_2 measurements on a commercially available flow injection analysis (FIA) system with sampling of the analyte using microdialysis probes (MDPs), typically used for measurements in tissue. This allows minimally invasive, quantitative measurements of extracellular H_2O_2 concentration and dynamics utilizing the chemiluminescent reaction of H_2O_2 with acridinium ester. By coupling MDPs to the FIA system, measurements are no longer limited to filtered, liquid samples with low viscosity, as sampling via a MDP is based on a dynamic exchange through a permeable membrane with a specific cut-off. This allows continuous monitoring of dynamic changes in H_2O_2 concentrations, alleviates potential pH effects on the measurements, and allows for flexible application in different media and systems. We give a detailed description of the novel experimental setup and its measuring characteristics along with examples of application in different media and organisms to highlight its broad applicability, but also to discuss current limitations and challenges. The combined FIA-MDP approach for H_2O_2 quantification was used in different biological systems ranging from marine biology, using the model organism *Exaiptasia pallida* (light stress induced H_2O_2 release up to ~2.7 µM), over biomedical applications quantifying enzyme dynamics (glucose oxidase in a glucose solution producing up to ~60 µM H_2O_2 and the subsequent addition of catalase to monitor the H_2O_2 degradation process) and the ability of bacteria to modify their direct environment by regulating H_2O_2 concentrations in their surrounding media. This was shown by the bacteria *Pseudomonas aeruginosa* degrading ~18 µM background H_2O_2 in LB-broth). We also discuss advantages and current limitations of the FIA-MDP system, including a discussion of potential cross-sensitivity and interfering chemical species.

Keywords:
Hydrogen peroxide, H_2O_2, ROS, Flow injection analysis, FIA, Microdialysis probe, Extracellular measurement, Optical sensor, Chemiluminescence

1. Introduction

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) [1] and a strong oxidant, but relatively unreactive in comparison to other ROS [2]. The peroxide bond is nonetheless prone to cleaving due to heating, photolysis or

[*] *Abbreviations:* H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; FIA, flow injection analysis; MDP, microdialysis probe; MDP-FIA, flow injection system coupled to a microdialysis probe; GOX, glucose oxidase; CL, chemiluminescent reagent; AE, acridinium ester; PMT, photomultiplier tube; ID, inner diameter; OD, outer diameter; LOD, limit of detection; LOQ, limit of quantification; DI, deionized water; LB medium, Luria-Bertani broth; PBS, saline phosphate buffer, F2 medium, enriched seawater medium; ASW, artificial seawater; FSW, filtered seawater

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