Facts and artefacts in measurements of H_2O_2 production using Amplex UltraRed assay as a function of oxygen pressure

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Oxidative stress occurs when there is an imbalance between generation and elimination of reactive oxygen species (ROS) leading to oxidative damage of the tissue. Excessive amount of ROS can be initiated 1.) after anoxia or tissue hypoxia ($[O_2]$<10 µM), 2.) by accumulation of reducing equivalents, which called reductive stress, and 3.) by ROS via a self-amplifying process called ROS-induced ROS release. Amplex UltraRed (AmR) assay is one of the most frequently used methods to detect the most stable form of ROS, hydrogen peroxide (H_2O_2) with its strength and limitations.

In the present study, we aimed to shed light on the anoxia-reoxygenation-triggered H_2O_2 production and reinvestigate the oxygen dependence of H_2O_2 generation on yeast in different respiration medium using the AmR assay.

H_2O_2 production during anoxia-reoxygenation transition was measured on commercially available dry baker´s yeast (*Saccharomyces cerevisiae*) by High-Resolution FluoRespirometry (Oroboros Instruments, Innsbruck, Austria) which allows to detect oxygen concentration, oxygen flux and H_2O_2 flux simultaneously. Different respiration media: MiR05-Kit (Oroboros Instruments, Innsbruck, Austria), commercially available Dulbecco´s Phosphate Buffered Saline (DPBS; ThermoFisher Scientific, Waltham, MA, USA) and KCl-based medium were tested.

In DPBS and KCl-based medium an exponential increase of the H_2O_2 production was observed after each reoxygenation on yeast, however, the H_2O_2 formation was negligible in MiR05-Kit under the same condition. We have found that the a medium-specific fluorescence background slope of AmR assay was increasing over the experimental time and was higher at low oxygen creating artefacts in the presence and absence of sample in DPBS and KCl-based medium. Interestingly, in MiR05-Kit the fluorescence background slope was stable at different oxygen regime and linearly increasing over time. These results would suggest that the components of the respiration medium may interact with the AmR assay leading to artefact formation.

Our results show the relevance of setting appropriate experimental conditions for the elimination of recurrent artefacts in H_2O_2 measurements. In KCl-based medium and DPBS methodological artefacts are created with the AmR assay, which are not related to physiology.

In contrary, our measurements in MiR05-Kit disagree with the theory of the ROS- and reductive stress-induced ROS release.