Chemical Characterization of a Resazurin-Based Method to Detect Dissolved Oxygen

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Resazurin is a redox-active molecule with three states: a colorless reduced form called dihydroresorufin, a reversibly-oxidized red-colored form called resorufin, and an irreversibly-oxidized purple-colored form called resazurin. Because of this redox activity, resazurin has long been used to test for oxygen. In the classic assay, a sample is combined with an indicator solution containing the colorless dihydroresorufin; the presence of oxygen will result in a purple color while anoxic samples remain colorless. While this assay is widely noted, there has been almost no reported characterization of the underlying chemistries. Contrary to common assumptions, UV-visible spectroscopy indicates that, in the classic assay, the dihydroresorufin form is oxidized to a mixture of resazurin and resorufin. Experiments using different ratios of aerobic and anerobic water show a classic spectrophotometric titration curve with a clear end-point. Stability studies show that, once formed, the absorbance was stable for at least 30 minutes. While the reaction between oxygen and dihydroresorufin is nearly instantaneous, the reverse reaction in which resazurin is reduced by sodium dithionite was surprisingly slow. Kinetic analysis indicates the reaction is 1st order with respect to both dithionite and resazurin (2nd order overall), but that secondary reactions involving the reduction of resorufin to dihydroresorufin occur and follow more complex kinetics. Consistent with the slow reaction, the 1 M half-life of the first step is only 1.3 min^{-1}. Addition of the dihydroresorufin-containing indicator to whole cells of *Heliomicrobium modesticaldum* results in negligible effects on the UV-visible spectrum of the cells and only small effects on light-driven P800 oxidation. Conversely addition of resazurin results in a dramatic increase in the amount of P800 oxidation, although this effect was lost after sufficient dark incubation due to reduction of the resazurin by whole cells. Future work is directed towards continued characterization of the indicator and of its interaction with the cellular electron transfer chain.