

Detection of O₂⁻ production by chemiluminescence (CL) assay

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Method Article

Keywords: superoxide ion, chemiluminescence, Cypridina luciferin analog

Posted Date: October 31st, 2007

DOI: <https://doi.org/10.1038/nprot.2007.485>

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Abstract

Introduction

Hemoglobin is auto-oxidized from ferrous-hemoglobin to ferric hemoglobin (methemoglobin, metHb) with the production of H_2O_2 . H_2O_2 initiates the catalytic cycle between the ferric (HbFeIII) and ferryl (HbFeIV) hemes, thus activating the pseudoperoxidase activity of metHb, which eliminates the H_2O_2 , producing the superoxide¹. The chemiluminescence (CL) of Cypridina luciferin analog indicates the generation of $O_2^{\cdot-}$ or singlet oxygen, but not that of ozone, hydroxyl radicals or H_2O_2 ². To further confirm that the ROS species produced by metHb and indicated by CLA-CL is superoxide anions but not singlet oxygen, superoxide dismutase (SOD) is applied as a diagnostic confirmation of superoxide anion production.

Procedure

The pseudoperoxidase activity is measured as described², with modifications. 1. Add metHb into a substrate mixture containing 5.3 μM of CLA and 3.3 mM of H_2O_2 in 150 μl of PBS (pH 7.4). 2. Continuously monitor the chemiluminescence, indicated by relative luminescence units (rlu), at one reading per s, for 1 min, with Sirius luminometer (Berthold Detection Systems GmbH, Germany). 3. The pseudoperoxidase activity of the metHb is designated as the relative luminescence units per second (rlu/s). 4. To prove that the ROS species indicated by CLA-CL is superoxide anions, 3 Units/ml of SOD is applied to specifically quench the superoxide anions.

Anticipated Results

The read out of CLA-LA varies from 10^5 - 10^7 rlu/s depending on the concentration of the metHb and the H_2O_2 as the substrate as well as that of CLA as the indicator.

References

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Figures

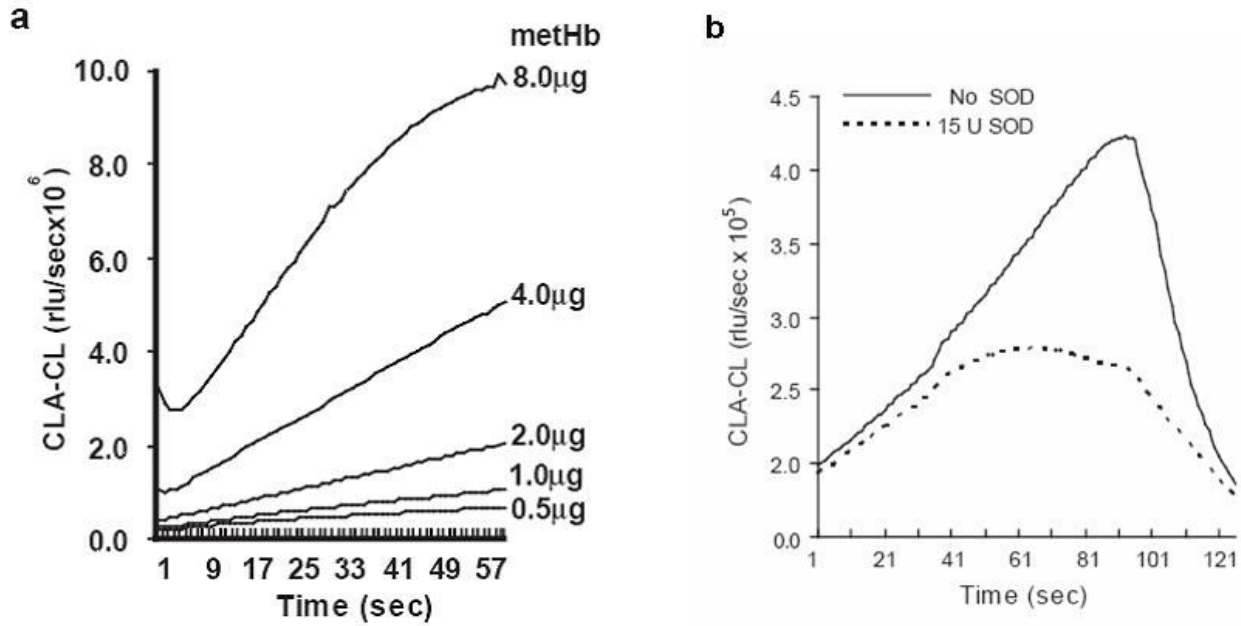


Figure 1

The pseudoperoxidase activity of methemoglobin (metHb) was demonstrated by CLA-CL. (a) metHb catalyzes the production of $O_2^{\cdot -}$, as shown by the chemiluminescence (CL) assay. (b) SOD significantly reduces metHb-mediated CLA-luminescence, thereby confirming the superoxide anion.