Chemiluminescence Measurement of the Generation of Reactive Oxygen Species

Bernd Hipler, BMG LABTECH GmbH, Germany
Uta-Christina Hipler, Department of Dermatology, FSU Jena, Germany

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- Very low ROS concentrations detectable
- Chemiluminescence signals in candida species at c×10^8 blastospores / mL
- Linear correlation between ROS and blastospores concentration

Introduction

Phagocytosis is one of the oxygen depending processes in organisms. During the unspecific immunological defense, the activity of pentosephosphate cyclus is dramatically increased forming NADPH. NADPH is needed to reduce the oxygen which is bonded to membrane-based cytochromes. Therefore, the oxygen demand is strongly increased (respiratory burst). During this process, oxygen is converted into superoxide anions, hydrogen peroxide, monomolecular oxygen and hydroxyl radicals by means of several kinds of phagocytic cells (e.g. neutrophil, eosinophil and basophil leucocytes, macrophages). These extracellular highly reactive oxygen species (ROS) cause many biological effects such as destruction of bacterial cells, parasites and tumor cells, promoting inflammation and modulating the immune reaction (Fig. 1).

Materials and Methods

Reactive oxygen species are formed by phagocytic cells under various conditions:
- Most of the cells are able to form ROS by themselves without any stimulation
- During the phagocytosis of foreign matter
- During cell proliferation and/or cell cytotoxicity processes
- By stimulation with soluble stimuli (e.g. immuno complexes, anaphylatoxins C3a/C5a, chemotactic peptides, ionophores, lectines and phorbol ester)

The generation processes of reactive oxygen species can be monitored using luminescence analysis. Measurements of chemiluminescence (CL) are highly sensitive and specific, owing the possibility to investigate the different kinds of reactive oxygen species simultaneously (HO·, O2·-, H2O2, 1O2). Because of the very weak native luminescence phenomena, luminol or lucigenin dependent chemiluminescence have been used frequently for the detection of superoxide radical anions in biological systems. Luminol reacts in its univalently oxidized form and lucigenin reacts in its univalently reduced form with O2·-. In both cases, light production depends on the formation of an unstable endoperoxide or dioxetane, which decomposes to an electronically excited product. This product releases a photon as it falls to the ground state. In the case of luminol, hydrogen peroxide is more reactive than O2·-, but the superoxide radicalanions were detected faster by lucigenin than hydrogen peroxide (Fig. 2).

The following strains of Candida were used for the measurement of cellular luminescence:
- The strains of fungi were cultured 24 h on Sabouraud-Glucose-Agar in the presence of penicillin and gentamicin.
- Yeast suspensions were prepared in RPMI-1640 medium with concentrations of 10^7 to 10^11 blastospores/mL or in isotonic NaCl-solution with the same concentration.
- The cell counts were assessed either with a counting chamber or a CASY 1 device (Schärfe-System, Germany).

Fig. 1: Reactive oxygen species and oxidative damage

Fig. 2: Luminescence phenomena caused by the reaction with ROS
200 µL of several yeast suspensions were used. 10 µL of the Lucigenin solution (10⁻⁴ mol/L) was added into each well at 30°C or 37°C by an injection pump. The chemiluminescence measurements were carried out by means of a BMG LABTECH microplate reader, such as LUMiStar, NOVOstar, POLARstar, or FLUOstar. For the measurement, the slow kinetic method was used. The results are mean values of accumulated single readings over a period of 24 minutes. The statistics of the chemiluminescence counts and the calculations of correlation coefficients were performed by means of Microsoft Excel and the dedicated BMG LABTECH evaluation software. P < 0.05 was considered statistically significant.

Results and Discussion

The results of lucigenin dependent chemiluminescence measured in suspensions of Candida albicans, Candida glabrata, Candida guilliermondii, Candida parapsilosis, Candida tropicalis are shown in Table 1.

<table>
<thead>
<tr>
<th>Candida albicans</th>
<th>Candida guilliermondii</th>
<th>Candida parapsilosis</th>
<th>Candida tropicalis</th>
<th>Candida glabrata</th>
<th>Trichosporon capitatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells</td>
<td>RLU</td>
<td>RLU</td>
<td>RLU</td>
<td>RLU</td>
<td>RLU</td>
</tr>
<tr>
<td>E+7/mL</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>E+8/mL</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>E+9/mL</td>
<td>2</td>
<td>12</td>
<td>19</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td>E+10/mL</td>
<td>30</td>
<td>48</td>
<td>61</td>
<td>90</td>
<td>126</td>
</tr>
<tr>
<td>E+11/mL</td>
<td>64</td>
<td>122</td>
<td>156</td>
<td>102</td>
<td>189</td>
</tr>
<tr>
<td>r = 0.91</td>
<td>r = 0.96</td>
<td>r = 0.96</td>
<td>r = 0.74</td>
<td>r = 0.84</td>
<td>r = 0.97</td>
</tr>
<tr>
<td>p = 0.02</td>
<td>p = 0.01</td>
<td>p = 0.009</td>
<td>p = 0.10</td>
<td>p = 0.07</td>
<td>p = 0.004</td>
</tr>
</tbody>
</table>

(RPMI-medium, T=30°C, Lucigenin-solution 100 µL)

It was notable that detectable CL signals can be found in candida species at concentrations of >10⁸ blastospores/mL. A linear and direct proportion between ROS levels and blastospore concentrations could be found. ROS production may contribute to the inflammatory reaction in the initial phase of Candida infections and may cause tissue damage to the host (induction of lipid peroxidation and formation of leukotrienes). The activated oxygen species are aggressive and toxic depending on their concentration.

Table 2: Comparison of different yeast species

<table>
<thead>
<tr>
<th>Candida albicans</th>
<th>Candida glabrata</th>
<th>Trichosporon capitatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells</td>
<td>RLU</td>
<td>RLU</td>
</tr>
<tr>
<td>E+5/mL</td>
<td>60</td>
<td>51</td>
</tr>
<tr>
<td>E+6/mL</td>
<td>67</td>
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<td>E+7/mL</td>
<td>81</td>
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<td>E+8/mL</td>
<td>93</td>
<td>75</td>
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<tr>
<td>E+9/mL</td>
<td>306</td>
<td>148</td>
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<tr>
<td>E+10/mL</td>
<td>415</td>
<td>248</td>
</tr>
<tr>
<td>r = 0.84</td>
<td>p = 0.07</td>
<td>r = 0.90</td>
</tr>
<tr>
<td>r = 0.90</td>
<td>p = 0.003</td>
<td>r = 0.98</td>
</tr>
<tr>
<td>p = 0.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(NaCl-solution, T=30°C, Lucigenin-solution 100 µL)

These experiments show that the RPMI medium is not able to stimulate the ROS generation, but more likely to inhibit because of its anti-oxidizing constituents (vitamins, glutathione).

Conclusion

The ability of various candida yeasts and blastomyces to generate ROS can be monitored by means of lucigenin-dependent chemiluminescence. The CL method is sensitive enough to detect very low ROS concentrations produced by several fungi without any stimulation. It was notable that detectable CL signals can be found in candida species at concentrations of 10⁴ blastospores/mL. A linear and direct proportion between ROS levels and blastospore concentrations could be found. ROS production may contribute to the inflammatory reaction in the initial phase of Candida infections and may cause tissue damage to the host (induction of lipid peroxidation and formation of leukotrienes). The activated oxygen species are aggressive and toxic depending on their concentration.

References


Headquarters:
Germany: BMG LABTECH GmbH
Australia: BMG LABTECH Pty. Ltd.
China: BMG LABTECH Co. Ltd.
France: BMG LABTECH SARL
UK: BMG LABTECH Ltd.
USA: BMG LABTECH Inc.

Tel: +49 781 962020
Tel: +61 3 59734744
Tel: +86 10 6424063
Tel: +33 1 48862020
Tel: +44 1296 336650
Tel: +1 919 806 1735
www.bmglabtech.com
info@bmglabtech.com