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The use of metal ions as a potential inhibitors of ferrochelatase

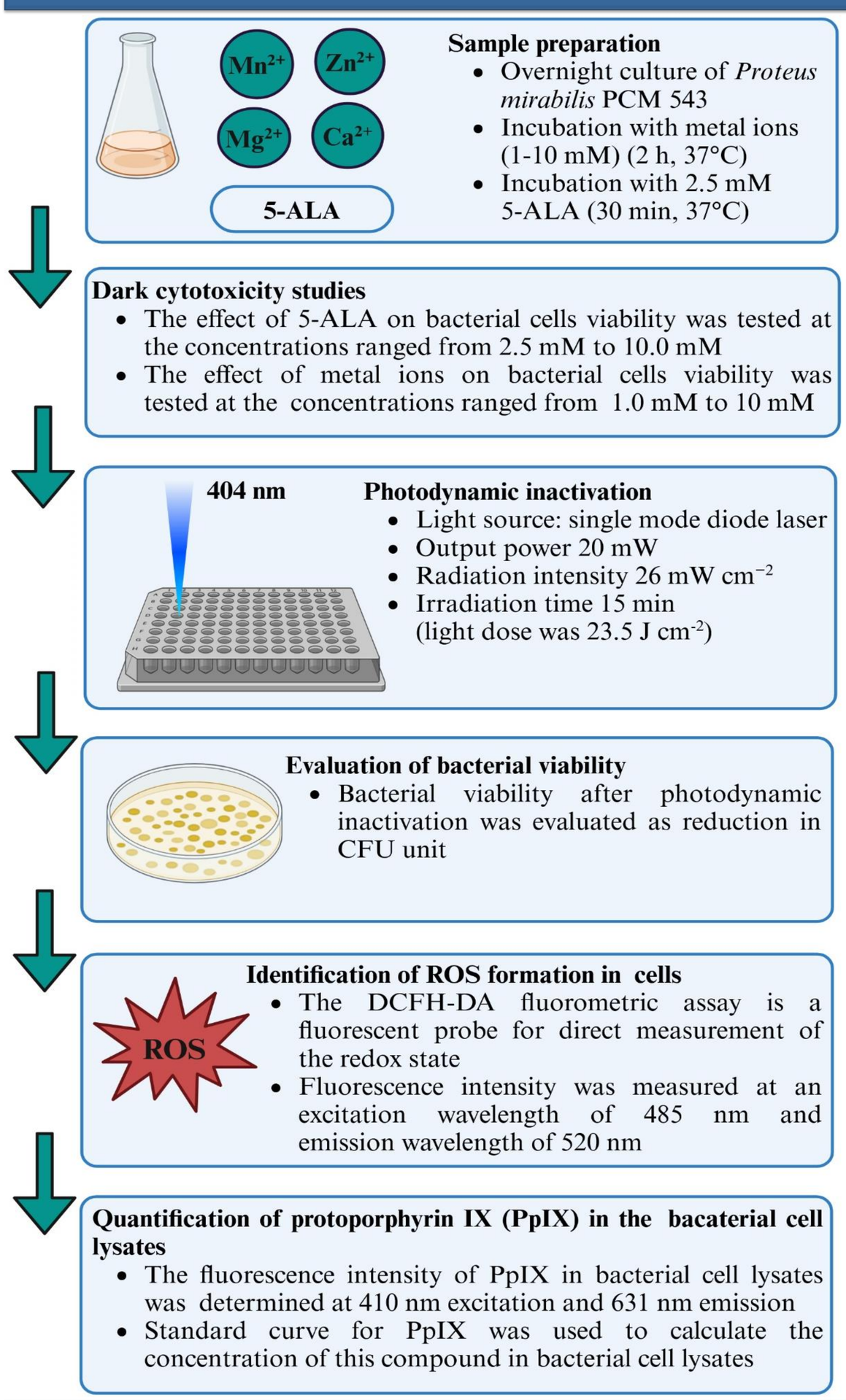
Anna Zdubek, Irena Maliszewska
Wrocław University of Science and Technology, Faculty of Chemistry, Department of Organic and Medicinal Chemistry

INTRODUCTION & AIM

The need for alternative techniques of microbial control is determined by the rapid spread of antibiotic-resistant bacteria. One approach that is gaining popularity is photodynamic inactivation of bacteria (PDI). This method involves the use of a chemical compound (photosensitizer) which is activated by light in the presence of molecular oxygen. The activated photosensitizer will induce the production of reactive oxygen species (ROS), which will lead to cell destruction. Significant attention is directed toward the application of 5-aminolevulinic acid (5-ALA) in PDI. This compound occurs naturally in cells, where it is converted mainly to intracellular photosensitizer protoporphyrin IX (PpIX). Over time, PpIX is transformed into heme (which does not possess photosensitizing properties) by ferrochelatase enzyme [1]. An increased concentration of PpIX in cells can be achieved by inhibiting the activity of ferrochelatase by divalent metal ions (eg, Mn^{2+}, Zn^{2+}) [2].

The aim of the study was to evaluate the combined effect of 5-ALA (2.5 mM), light and metal ions on the photosensitization of bacteria. The microorganism tested was antibiotic resistant *Proteus mirabilis* (PCM 543). Irradiation was performed with a 404 nm diode laser for 15 minutes (the light dose was 23.5 J cm^{-2}). For the research, ions of manganese (1 mM), zinc (1 mM), calcium (10 mM), and magnesium (10 mM) were tested as potential competitive inibitors of ferrochelatase. All compounds were used at a nontoxic concentrations (approximately 20% of bacterial viability reduction).

METHOD



RESULTS & DISCUSSION

The most effective approach was found to involve the use of 5-ALA with zinc ions (Zn^{2+}), resulting in the destruction of almost 99% of bacterial cells (reduction in the viability of *P. mirabilis* by 2.02 log_{10} after 15 min of exposure to light), while the use of 5-ALA alone led to the eradication of approximately 90% (reduction in viability by 1.0 log_{10}) of cells after the same exposure time (Fig.1). Unfortunately, preincubation of bacterial cells with the other ions tested did not significantly improve the efficiency of PDI. The results may suggest that Zn^{2+} competes with Fe^{2+} for the ferrochelatase enzyme active site, while the other ions do not, and therefore only zinc ions may have the ability to inhibit the activity of the ferrochelatase.

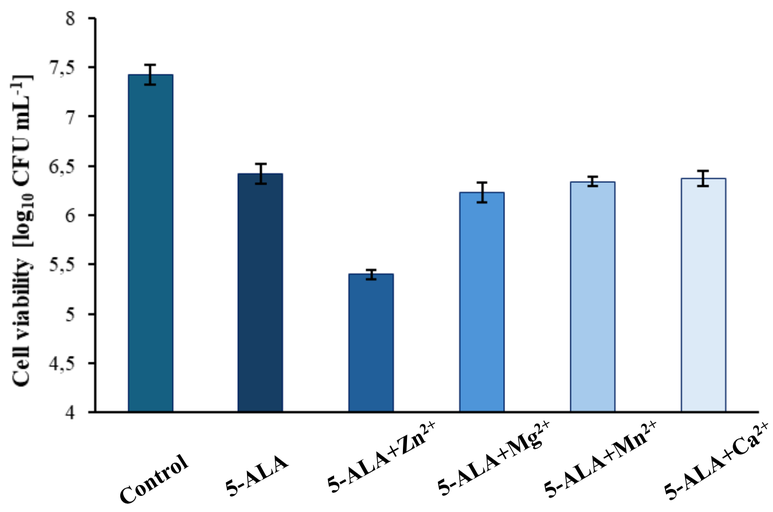


Figure 1. The effect of 5-ALA and metal ions on the viability of *P. mirabilis* after 15 min exposure to blue light (404 nm).

In addition, the quantification of protoporphyrin IX in *P. mirabilis* cell lysates after incubation with 5-ALA and divalent metal ions was performed. In Fig. 2, it can be observed that only the exogenous administration of 5-ALA into bacterial cells, preceded by preincubation with zinc ions, significantly increased the amount of PpIX in cells. The results presented may indicate the inhibition of ferrochelatase activity by the mentioned ions. It is probable that they compete with iron ions for the enzyme's active site, effectively displacing them and thus preventing heme formation, which results in an increased concentration of PpIX.

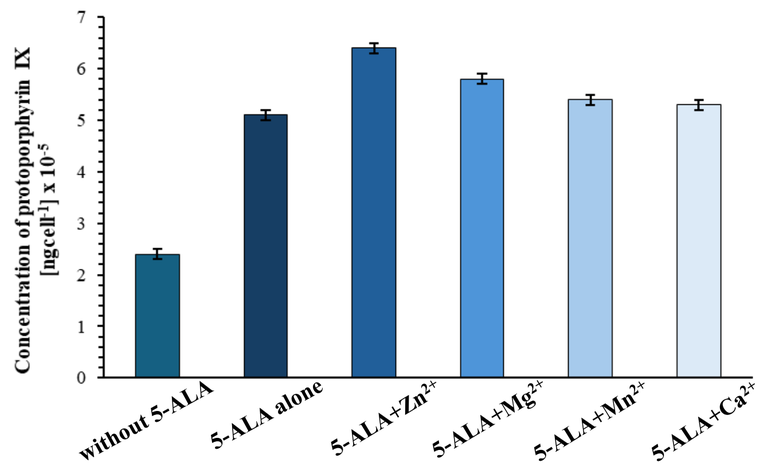


Figure 2. The effect of 5-ALA and metal ions on protoporphyrin IX concentration in *P. mirabilis*.

The DCFH-DA assay measures the redox state using a fluorescent probe. Inside cells, DCFH-DA is deacetylated to DCFH, which reacts with ROS to form fluorescent DCF (excitation: 485 nm, emission: 520 nm). The results showed increased concentration of oxidizing compounds in bacterial cells after light exposure, leading to enhanced DCF formation. A significant increase in DCF fluorescence intensity was observed after preincubation of bacteria mainly with zinc ions. That may also confirm the potential of Zn^{2+} to be a competitive inhibitor of ferrochelatase activity.

Tabela 1. Effectiveness of increasing the oxidation rate of the bacterial cells (oxidative stress) by 5-ALA alone and exogenous administration of 5-ALA after pretreatment with metal ions. Dichlorodihydrofluorescein diacetate (DCFH-DA) was used as a fluorescent probe.

Control	5-ALA	5-ALA+Zn^{2+}	5-ALA+Mg^{2+}	5-ALA+Mn^{2+}	5-ALA+Ca^{2+}
Fluorescence intensity values of DCF (Ex = 485 nm, Em = 520 nm)					
369.5 ±5.5	600.0 ±20	847.5 ±30.5	654.0 ±29	587.5 ±14.5	613.0 ±19

CONCLUSION

The results obtained suggest that metal ions, especially zinc, may compete with iron ions for the ferrochelatase binding site, leading to inhibition of the enzyme activity, and at the same time, accumulation of PpIX in cells. This explained the enhancement of the photodynamic inactivation efficiency of *P. mirabilis* using 5-ALA, after preincubation with selected metal ions.

FUTURE WORK / REFERENCES

[1] doi:10.3390/ijms25073590 [2] doi:10.1074/jbc.M803372200