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Project: Interplay between enterobactin and myeloperoxidase during infections with E coli

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Sponsor: Canterbury Scientific Limited

Introduction:
Iron is an essential element for bacterial growth. To achieve infection, bacterial pathogens release iron binding agents known as siderophores to competitively uptake iron from their host organism. Enterobactin, released by Escherichia coli and Salmonella enterica bacteria, is one of the strongest iron-binding siderophores known. A recent publication in the scientific journal Nature Communications has proposed that enterobactin may inhibit chlorine bleach production by the enzyme myeloperoxidase (MPO) in humans. MPO is present within neutrophils, a type of white blood cell important in host defence against microbial infections. Neutrophils employ their MPO to produce chlorine bleach to kill invading bacterial pathogens. Enterobactin-mediated inhibition of MPO has been proposed as an auxiliary mechanism by which E. coli achieve infection. Although it has been suggested that enterobactin may serve as an inhibitor of MPO, it should also be considered that enterobactin may serve as an oxidisable substrate for human MPO. It is thus possible that neutrophils releasing MPO may degrade enterobactin thereby disrupting bacterial pathogen growth. This is proposed to constitute an additional host-defence mechanism for neutrophils aside from direct killing of bacteria.

Aim:
The overall aim of this research was to examine the interaction between enterobactin and MPO by establishing whether enterobactin can inhibit chlorine bleach production by MPO or act as its substrate.

Method:
To examine whether enterobactin is an inhibitor of chlorine bleach production by MPO, two enzyme activity assays, were employed.

These involved spectrophotometric detection of the formation of taurine chloramine and NADH bromohydrin by purified MPO. The potential for enterobactin to act as a substrate of MPO was investigated using spectrophotometry to assess oxidation product formation. The analysis of oxidation by MPO was compared with that achieved by horseradish peroxidase, an enzyme similar to MPO, and also performed using hydroquinone, a known substrate of MPO, as a control.

Results:
Taurine chloramine and NADH-bromohydrin activity assay studies both showed that enterobactin does not inhibit chlorine bleach production by MPO. Rather, chlorine bleach production was observed to be enhanced in the presence of enterobactin suggesting that enterobactin is capable of serving as a substrate that promotes the cycling of MPO. Spectrophotometry studies indicated that enterobactin was oxidisable in the presence of horseradish peroxidase. At low levels of MPO however, oxidation of enterobactin was not observed.

Conclusion:
Enterobactin does not act as an inhibitor of human MPO chlorine bleach production, despite the previous claim. The Nature Communications publication used an assay that measured peroxidation activity, not the production of chlorine bleach. Both their and my results are consistent with enterobactin serving as a substrate for MPO.

There was no evidence of a significant oxidation product being formed by MPO but this will require further investigation. An interaction between MPO and enterobactin during a bacterial infection will not result in the inhibition of MPO. However, the consequence of enterobactin serving as a substrate of MPO, has yet to be determined, in order to assess how it may contribute to the pathogens ability to evade killing by neutrophils.