Investigating the Lipopolysaccharide Binding Site on Colicin N

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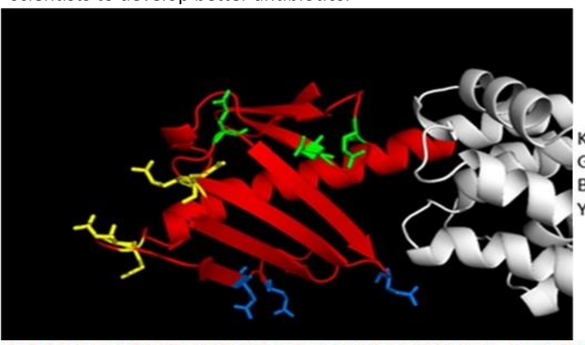
Introduction

Colicin N is a pore forming colicin which belongs to a group of antibacterial toxins produced by *E. coli* that can kill other *E. coli* cells. They do so by using their pore forming domain to form a pore in the inner membrane which results in a K^+ efflux and leads to cell death (1). Unlike other members of the colicin family, colicin N binds to lipopolysaccharide (LPS) on the cell surface instead of binding to the outer membrane protein receptors (2).

Aim

The aim of this research project is to determine the LPS binding site on colicin N by making mutations in the receptor domain without altering the structure of these mutants. Some amino acid residues are being mutated to glutamine because previous studies have shown that colicin are sensitive to negatively charged particles on LPS. This research is important because it would help scientists to develop better antibiotics.



Key:
Green – K105/R108/K121Q
Blue – K129/K155/R157Q
Yellow – R139/K140/K164/K167Q

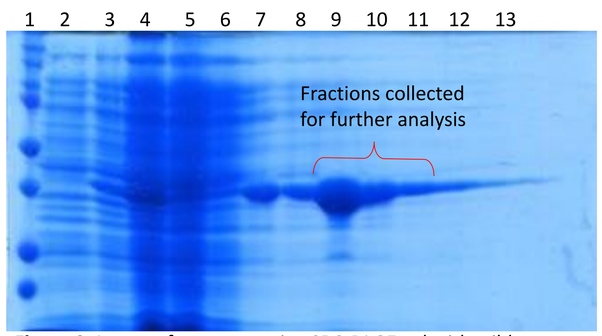
Amino Acid Code

DGASAKVGEITITPDNSQPGQYISSNPEYSLLAQLIDAESIQGTEVYTFHTQQGQ
YVKVTVPDSNIDQMQVDYVNWQGPQYNNKLVKRFVSQFLLFRKEEKEKNEK

Figure 1. 3-D protein structure image produced using PYMOL showing the various mutations made to the colicin N receptor domain (Protein database file number: 1A87). (3 and 4)

Method

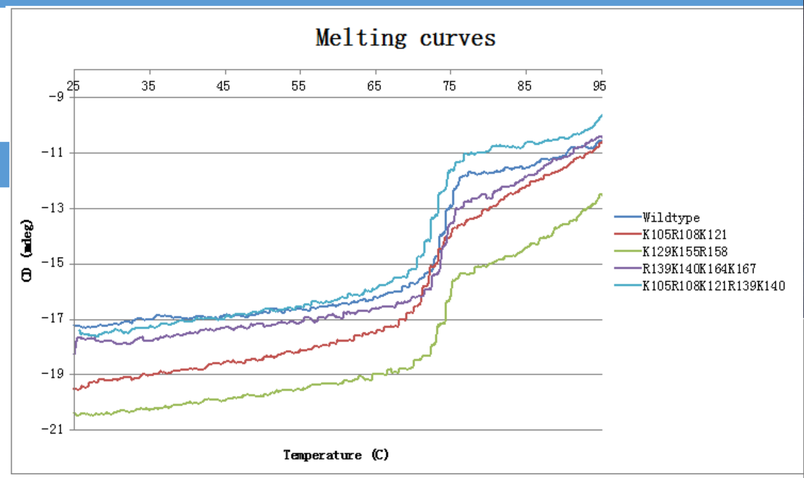
1. Site directed mutagenesis using standard procedures was carried out to produce the following mutations: K105Q/R108Q/K121Q, K129Q/K155Q/R157Q, R139Q/K140Q/K164Q/K167Q, and K105Q/R108Q/K121Q/R139Q/K140Q. (Figure 1.)
2. Mutated plasmids were transformed into competent BL21 AI *E. coli* cells (Life technologies).
3. Successfully transformed cells were grown in double strength LB media, protein expression was induced using arabinose and optical densities were measured using UV spectrophotometer.
4. Proteins were released from cells using One-Shot cell disruptor and purified using an elution column containing Nickel-NTA resin. 20ul of each fraction was loaded onto 12% acrylamide gels. (Figure 2.)
5. Circular Dichroism and thermal melt analyses at 222nm were carried out to determine the secondary structure and melting temperature of mutated proteins. (Figure 3-5.)
6. Spot tests were carried out on LB agar plates containing BE3000 *E. coli* cells and a thin layer of sloppy agar to test the killing ability of these mutated colicins. (Figure 6.)
7. Surface plasmon resonance (Biacore) was used to test the effectiveness of mutated colicins in binding to LPS. (Figure 7.)



Key:
1.Marker
2.Uninduced
3.Induced
4.Loading
5.Flow through
6.Wash 1
7.Wash 2
8.Wash 3
9.Elution 1
10.Elution 2
11.Elution 3
12.Elution 4
13.Elution 5

Figure 2. Image of representative SDS-PAGE gel with wildtype colicin N plasmid, demonstrating effective protein purification.

Results



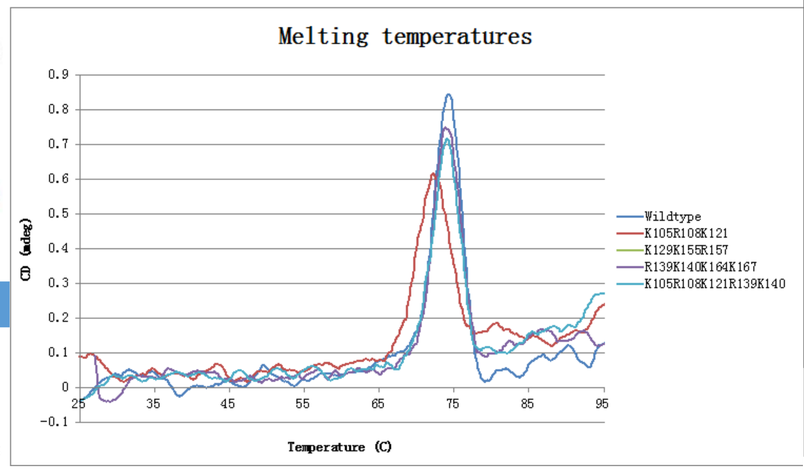


Figure 3 and 4. The above two graphs showing the melting temperatures of the wildtype colicin N and the 4 mutants. There are slight variations in melting temperatures, this could be due to the position of some mutations which makes the bond between certain amino acids weaker and therefore is broken more easily.

Colicin N	Melting temperature (c)
Wildtype	74.2
K105R108K121	72.2
K129K155R157	73.4
R139K140K164K167	73.8
K105R108K121R139K140	73.8

Figure 5. Melting temperature of the wildtype and mutant colicins. Compared to the wildtype colicin N the mutants have slightly lower melting temperatures but this is expected because the mutations changed positively charged amino acids to negatively charged glutamine which alters the bonds and therefore lowers the melting temperatures.

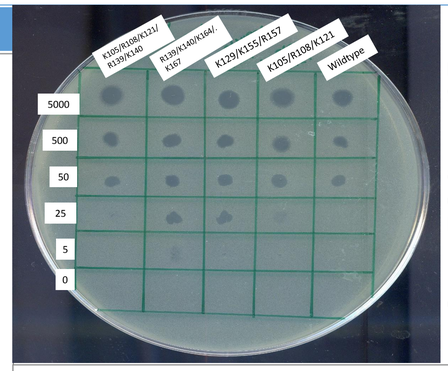


Figure 6. Image demonstrating the spot test assay. Surprisingly, mutant colicin N R139Q/K140Q/K164Q/K167Q and K129Q/K155Q/R157Q have an increased killing activity compared to the wildtype colicin. This could be due to the artificial environment in the lab.

* Larger clear zone indicates higher killing efficiency.

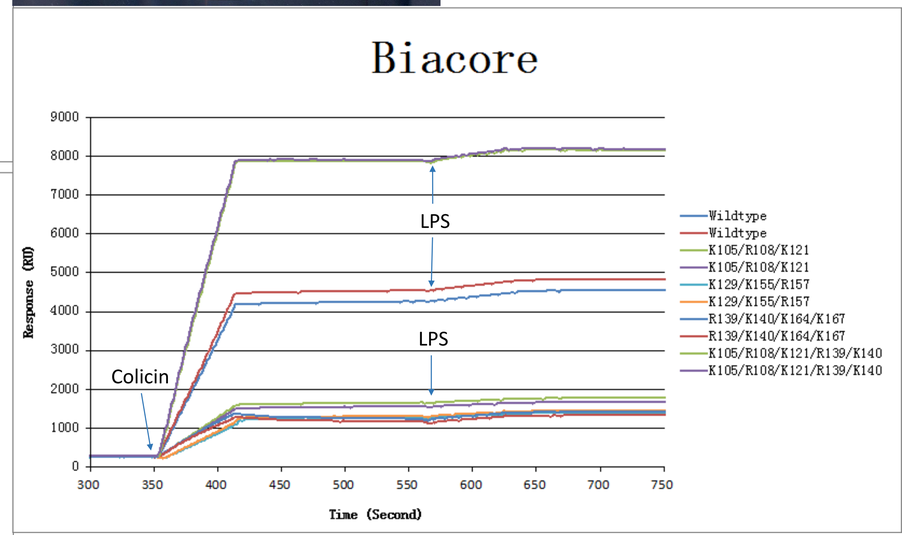


Figure 7. Colicin Binding to LPS. The Biacore result shows that there are variable levels of colicin N binding onto the NTA chip, potentially due to the nature of each mutants. It also shows that LPS was able to bind to all colicin N mutants.

Conclusion

- All mutants have similar melting temperature as the wildtype which confirms that their structure remain relatively unaltered.
- All colicin mutants bind LPS indicating that these mutations did not markedly alter the LPS binding sites on colicin N.
- Further tests are required which should include combining more mutations together in one plasmid to observe any effect produced.

Acknowledgement

I would like to thank Professor Jeremy Lakey and Dr Helen Waller for giving me the opportunity to carry out this research project and their guidance throughout this project. I would also like to thank Newcastle University for awarding me the scholarship.

References

(1) Jakes, K. (2014). Daring to be different: colicin N finds another way. *Molecular Microbiology*, 92(3), pp.435-439.

(2) Johnson, C., Ridley, H., Marchetti, R., Silipo, A., Griffin, D., Crawford, L., Bonev, B., Molinaro, A. and Lakey, J. (2014). The antibacterial toxin colicin N binds to the inner core of lipopolysaccharide and close to its translocator protein. *Molecular Microbiology*, 92(3), pp.440-452.

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(4) The PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC.