The function of the heavy chain of complement factor I
Implications for atypical haemolytic uraemic syndrome (aHUS)

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Background

Atypical haemolytic uraemic syndrome is a disease of complement over activation. The complement system is one of the first line defence mechanisms of the innate immune system.

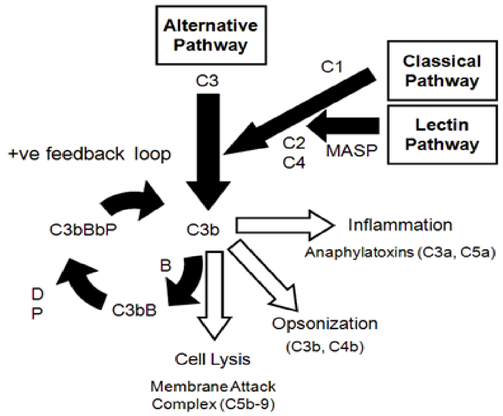


Figure 1: To rapidly attack invading organisms, the alternative pathway of the complement cascade is a positive amplification loop.

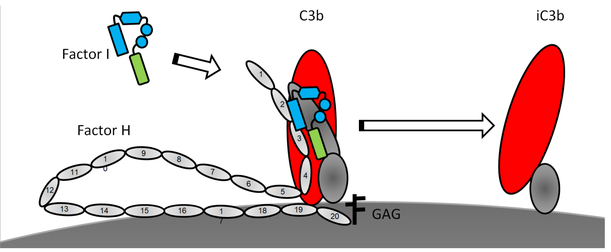
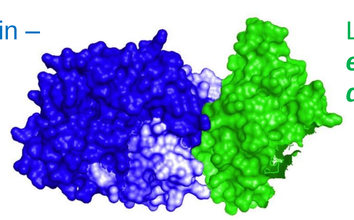


Figure 2: Control of this potent pathway is achieved by several regulators, especially factor I and factor H. Active C3b is cleaved by Factor I to its inactive form iC3b.

Mutations in the complement regulatory factors I, H and membrane cofactor protein predispose to atypical haemolytic uraemic syndrome (aHUS).

Heavy chain – ***function unknown***



Light chain ***enzymatic domain***

Figure 3: Complement regulatory factor I has a heavy and a light chain.

The *light chain (LC)* of factor I is the enzymatic part (serine protease – SP domain) responsible of for the cleavage of C3b whilst the function of the *heavy chain (HC)* is unknown.
Some patients with aHUS have mutations in the heavy chain. It is therefore important to determine its function.

Aim:

To determine the function of the heavy chain of complement factor I.

Methods:

- The cDNA of the light chain of factor I was cloned into the vector pPicZalpha.
- The plasmid was then transformed into *E. coli* and colonies selected using Zeocin.
- Veracity of the vector was confirmed by DNA sequencing.
- The vector was then transformed into *Pichia pastoris.*
- Small scale cultures were grown and analysed for expression of the light chain of factor I.

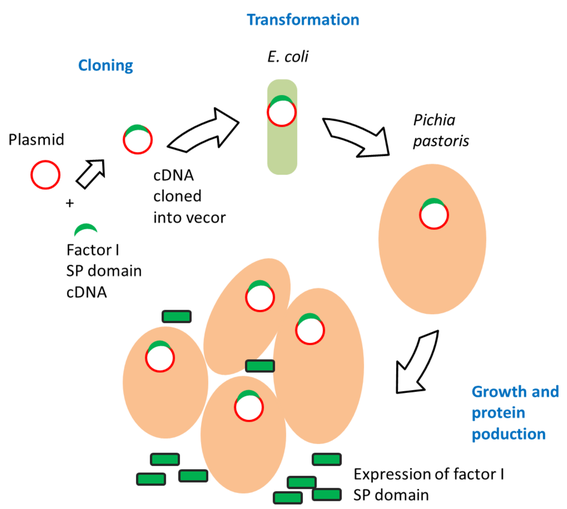


Figure 4: Diagram of methods involved in the generation of a P. pastoris clone expressing recombinant factor I LC (SP domain).

- Both anion and cation exchange chromatography were optimised for purification.
- Western Blotting and SDS-Page analysis were used during optimisation to verify column binding
- A large scale (4l) culture was grown
- Crude ion exchange was initially undertaken.
- Polishing purification using cation exchange with a NaCl gradient elution was then performed (figure 5).
- SDS page and mass spectrometry were used to confirm purity.
- A cofactor assay with C3b and cofactors was then performed and analysed with Western Blotting (figure 6).

Results

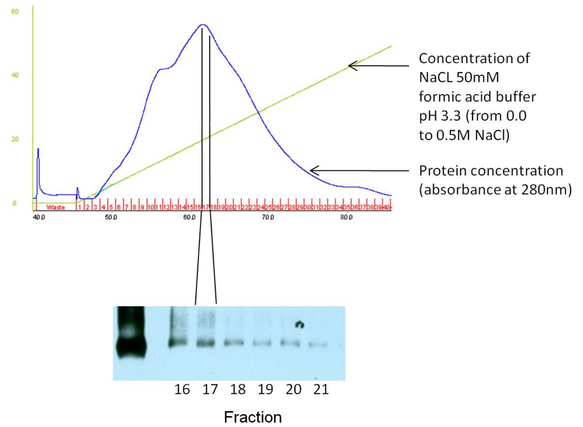


Figure 5: Polishing purification: Gradient elution of the LC of Factor I after cation exchange. LC fractions were detected using Western Blotting.

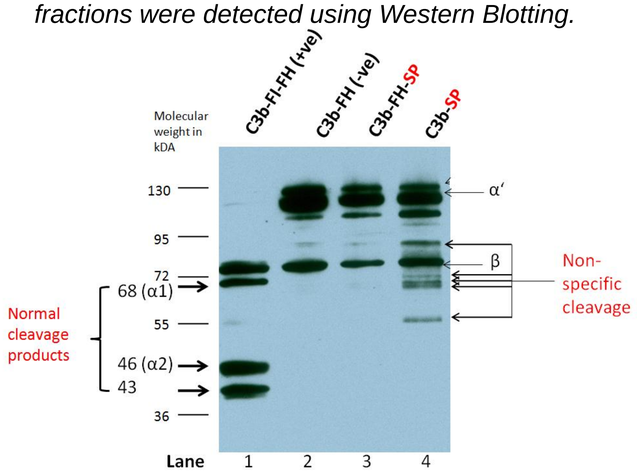


Figure 6: Complement assay demonstrating non-specific cleavage of C3b by the light chain (serine protease/SP domain) of Factor I.

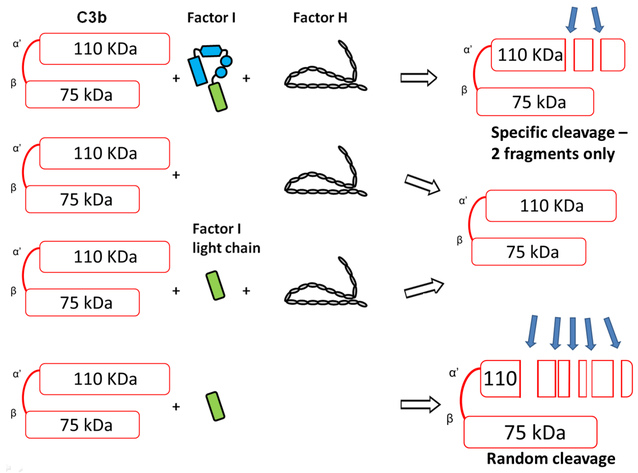


Figure 7: Diagrammatic representation of the results in figure 6.

This suggests that:

- The light chain can cleave C3b in the absence of factor H. This cleavage was different to the cleavage by full length factor I and factor H.
- Full length factor I cannot cleave C3b on its own.
- The light chain in in the presence of Factor H cannot cleave C3b.

Conclusions

- The heavy chain of factor I has a role in the tripartite reaction of factor I, factor H and C3b.
- In the presence of cofactor, the heavy chain is required for the cleavage of C3b
- The heavy chain confers specificity to the cleavage of C3b
- This functional data will facilitate the interpretation of the genetic data of patients with aHUS.