Expression and Purification of Complement Factor H (CFH) to Understand Bridge Between Coagulation and Inflammation

Grace Stryker, Dylan Webber, and Julia R. Koeppe
Department of Chemistry, SUNY Oswego, Oswego, NY 13126

Introduction

Complement and Coagulation are intertwined, innate blood defense systems that crosstalk in disease. The coagulation system features a tightly regulated cascade of proteolysis that generates a fibrin clot in response to stimulus [1]. Complement protects against pathogens by inducing a series of inflammatory responses. Complement works via three pathways: classical, mannose binding lectin, and alternative. All pathways converge on the enzymatic cleavage of component 3 (C3) forming fragments C3b and C3a [2].

Complement Component 3 (C3) is an essential protein in the activation of complement. When C3 is cleaved, one resulting fragment, C3b, acts as an opsonin, binding to both pathogens and adjacent host cells [2]. Since C3b can interact with other surface proteins to continue the complement response, its cascade activity must be carefully controlled (Figure 1).

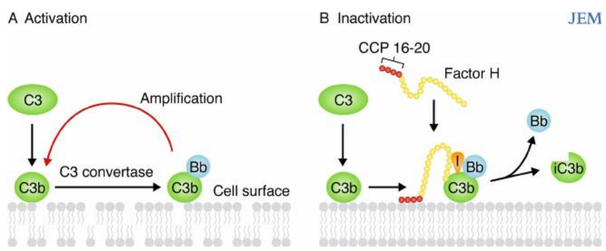

Figure 1. Proposed events in the complement alternate pathway. A) C3 is activated to C3b, which can bind to Bb to start an amplification loop. B) CFH decays Bb from the C3b, inactivating the system [3].

Complement Factor H (CFH) is a protein involved in negatively regulating the alternative pathway (AP) of the complement system. AP is viewed as constitutively active, which is analogous to the coagulation system [1]. CFH accelerates the dissociation of C3 convertase and acts as a co-factor for factor I, helping to prevent damage to self tissue by inactivating C3b [2].

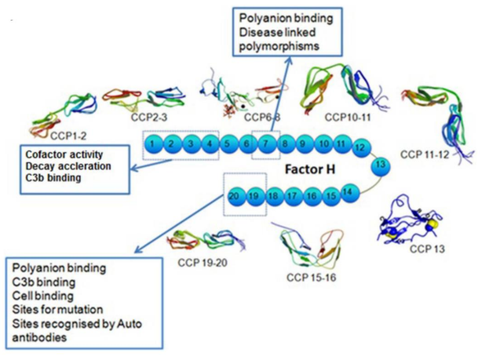

Figure 2. Illustration of CFH molecule having 20 CCP modules with different binding properties [4].

Expression of CFH

Expressing CFH

To characterize binding interactions between C3, CFH, and thrombomodulin, CFH was expressed from *Pichia pastoris* yeast. Growth of yeast cultures, SMD1168 and GS115, was done by inoculating glycerol-rich media (BMGY), then transferring cultures into methanol-rich (BMMY) induction media.

Purification of CFH

Isolating CFH

a. Centrifugal separation and filtration of supernatant
b. Ion exchange column with step gradient (Figure 3)

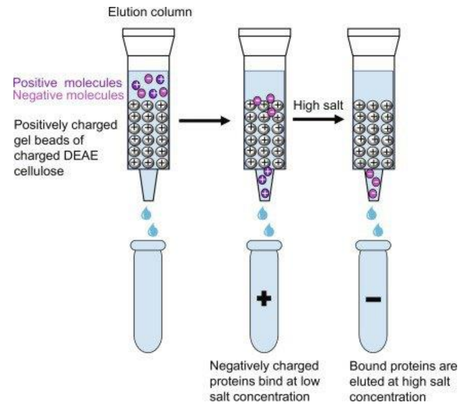

Figure 3. Schematic of DEAE anion exchange column [5].

c. Concentrate samples and run SDS PAGE gel electrophoresis
d. BCA protein concentration assay & combining fractions
e. Size exclusion column (SEC) separation
f. BCA protein concentration assay of SEC fractions (Figure 4)

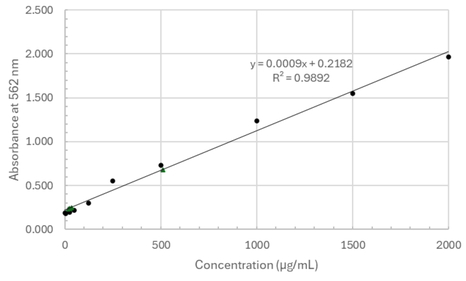

Figure 4. Standard curve (●) used to determine the concentration of CFH (▲) in the BCA protein concentration assay.

g. Biotinylation: a biotin tag was added to CFH to eventually be immobilized for SPR analysis (Figure 5)

Future Work

Optimizing CFH Expression and Purification

Pichia pastoris stocks will be screened for MC insertion and protein expression. Purified CFH fractions will be concentrated, and a Western blot will be used to confirm the protein's identity.

Surface Plasmon Resonance (SPR)

SPR experiments will be performed by immobilizing biotin-labeled CFH on a streptavidin sensor chip surface. Once immobilized, binding kinetics and strength of interactions will be investigated at different C3 concentrations and eventually in combination with thrombomodulin domain 1.

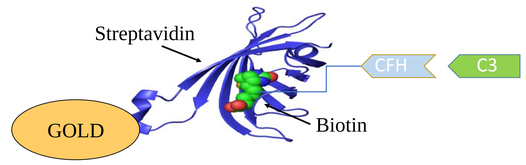

Figure 5. SPR experimental setup depicting CFH bound to the gold sensor chip while C3 flows over the surface.

Thrombomodulin (TM) enhances CFH-mediated activity, thus modulating the complement system through C3b inactivation [1]. The molecular basis for this occurrence is not yet completely understood but is clinically relevant for the introduction of anti-complement drugs for devastating thrombotic disorders.

References

1. Edward L.G. Pryzdial; Leatherdale, A.; Conway, E. M. Coagulation and Complement: Key Innate Defense Participants in a Seamless Web. *Frontiers in Immunology* **2022**, *13*. doi.org/10.3389/fimmu.2022.918775.
2. Heurich, M.; Preston, R. J. S.; O'Donnell, V. B.; Morgan, B. P.; Collins, P. W. Thrombomodulin Enhances Complement Regulation through Strong Affinity Interactions with Factor H and C3b-Factor H Complex. *Thrombosis Research* **2016**, *145*, 84–92. doi.org/10.1016/j.thromres.2016.07.017.
3. Atkinson, J. P.; Goodship, T. H. J. Complement Factor H and the Hemolytic Uremic Syndrome. *The Journal of Experimental Medicine* **2007**, *204* (6), 1245–1248. doi.org/10.1084/jem.20070664.
4. Kouser, L.; Abdul-Aziz, M.; Nayak, A.; Stover, C. M.; Sim, R. B.; Kishore, U. Properdin and Factor H: opposing players on the alternative complement pathway "See-Saw." *Frontiers in Immunology* **2013**, *4*. doi.org/10.3389/fimmu.2013.00093.
5. *Everything You Need to Know About Ion exchange chromatography*. microbiozindia.com.

Acknowledgements

We would like to thank past students who have contributed to this project in various ways. Thank you to donors who have generously given to SUNY Oswego's Research Fund for Chemistry and Math/Science Students. Special thanks to the SUNY Oswego Chemistry Department for the opportunity and Dr. Thomas C. Mahl for supporting summer research.