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Development of a Leech Protein that Inhibits the Classical and Lectin Pathways of the Complement System

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Introduction: The complement system is comprised of more than 50 proteins serving as a first line of defence mechanism against pathogens. While pivotal for homeostasis, inappropriate complement activation is a detrimental contributor to various clinical conditions. A better understanding of the initiation of the cascade and its involvement in diseases may therefore pave the way for novel therapeutic approaches [1]. Complement can be activated via various pathways; the classical (CP) and lectin pathway (LP) are both mediated by serine proteases, i.e. C1s/C1r (CP) and MASP1/MASP2 (LP). These pathways have a vital role in recognizing pathogens but also contribute to autoimmune diseases such as haemolytic anaemia and ischemia-reperfusion injuries. *Haementera ghiliani*, the Giant Amazon leech, produces a protein called BD001 inhibiting both C1s and MASP1/2 as part of its immune evasion strategy [1,2]. The protein of interest could therefore serve as a lead structure for future therapeutics.
Aim: Express correctly folded and active BD001 in bacteria and determine inhibitory potential.
Methods: The BD001 gene was amplified using PCR, ligated into the pET15b vector and transformed into the bacterial strain *Rosetta-gami*. The protein was purified via metal ion affinity chromatography via its histidine-tag. Expression yield and purity were tested on SDS-page gel. Inhibitory potential was evaluated in different biochemical assays. In a C4 cleavage assay, C1s and its natural substrate C4 were incubated and cleaved C4a visualized by SDS-PAGE. In a chromogenic substrate assay, the ability of different inhibitors to prevent C1s-mediated cleavage of a chromogenic substrate was assessed by spectrophotometry. Finally, in a haemolytic assay, IgM-coated sheep erythrocytes were incubated with 1% normal human serum and a concentration series of inhibitors. Erythrocytes lysis was determined by measuring the absorbance of free haemoglobin.
Results: We were able to express this highly challenging 122 amino acid long protein, featuring 10 disulphide bridges, in the bacterial strain *Rosetta-gami*. In the bacterial system high yields and fast production as well as high purity could be achieved. After successful purification the protein showed potent activity in all biochemical assays.
Conclusion: Not only we were able to produce the protein in a prokaryotic system, but also determine its activity and therefore its inhibitory potential. Our findings establish BD001 as an interesting lead structure for drugs treating diseases such as autoimmune haemolytic anaemia or ischemia-reperfusion injuries (e.g., transplantation or stroke).

Keywords: complement system, immunology, protein therapeutics

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