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Insight into mode-of-action and structural determinants of the compstatin family of peptidic complement inhibitors

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The complement system as “first line of defence” gets activated by invaders or injurious stimuli and leads to pathogen clearance and opsonic cell killing. Yet complement may also be inadvertently triggered on human cells or biomaterial surfaces, thereby contributing to clinical complications in the pathogenesis of various autoimmune, inflammatory and age-related diseases as well as transplant rejection. The involvement of dysregulated complement activation in inflammatory and autoimmune diseases is now widely recognized[1] and has therefore gained increasing interest as potential target for intervention. Given the central role of complement protein C3 in complement response amplification and effector generation, C3 inhibitors are of particular interest. It was only in May 2021 that, with pegcetacoplan (Empaveli, Apellis), a second class of complement-specific drugs has been approved by the FDA. Pegcetacoplan is based on the peptidic C3 inhibitor compstatin, which was originally identified by phage display at the University of Pennsylvania[3]. Meanwhile, the compstatin family has grown impressively and several derivatives have reached clinical development for the treatment of paroxysmal nocturnal hemoglobinuria (PNH), age-related macular degeneration (AMD), periodontal disease, and COVID-19-induced acute respiratory distress syndrome[2]. Among those is Cp40 (AMY-101, Amyndas), an analog with prolonged target residence that omits the PEGylation used in pegcetacoplan. In this study, we combined a newly resolved co-crystal structure of Cp40 in complex with C3b with molecular dynamics simulations and direct binding studies to arrive at a detailed structure-activity-relationship profile. We identified dTyr-1, (1Me)W-5, Gln-6, Trp-8, Sar-9, Ala-10, His-11, mIle-14 as key contact residues that determine affinity and/or target residence. Interestingly, our analysis also revealed major contributions of intramolecular interactions and structural water. We employed surface plasmon resonance studies to investigate the molecular mechanism of Cp40 and provide a more detailed insight into the mode-of-action of compstatin. We could show that C3 is recruited to surface-bound C3 convertases and that Cp40 prevents recruitment and activation of C3 by blocking the dimerization interface between the C3 substrate and the convertase component C3b. The results of our study may not only guide the future development of a promising class of complement inhibitors but also offers new details about the molecular mechanisms of complement activation and regulation.

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