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Arranged Sevenfold: Structural Insights into the C-Terminal Oligomerization Domain of Human C4b-Binding Protein

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Abstract

The complement system as a major part of innate immunity is the first line of defense against invading microorganisms. Orchestrated by more than 60 proteins, its major task is to discriminate between host cells and pathogens and to initiate immune response. Additional recognition of necrotic or apoptotic cells demands a fine-tune regulation of this powerful system. C4b-binding protein (C4BP) is the major inhibitor of the classical complement and lectin pathway. The crystal structure of the human C4BP oligomerization domain in its 7α isoform and molecular simulations provide first structural insights of C4BP oligomerization. The heptameric core structure is stabilized by intermolecular disulfide bonds. In addition, thermal shift assays indicate that layers of electrostatic interactions mainly contribute to the extraordinary thermodynamic stability of the complex. These findings make C4BP a promising scaffold for multivalent ligand display with applications in immunology and biological chemistry.

Introduction

The complement system as part of the innate immune response is one of the oldest sections of multicellular organisms' defense repertoire. It is the first line of protection against invading microorganisms that paves the way for the adaptive immunity to react via initiation of antibody production or T-cell response. In addition, complement activity on apoptotic and necrotic cells was reported.[1] The highly conserved complement system is found in all kinds of invertebrates and vertebrates[2] and is orchestrated by approximately 60 membrane-bound and soluble plasma proteins.[3] Once activated, a membrane attack complex is formed by components of the complement via a proteolytic cascade that eventually mediates lysis of pathogens by osmotic shock. The relevance of the complement system is underlined by the fact that congenital defects in activation and regulation increase the susceptibility to inflammatory diseases.[4]

Human C4b-binding protein (hC4BP) is a plasma glycoprotein complex of 570 kDa, which is mainly produced in the liver. C4BP is the major inhibitor of complement activation (Fig. 1). For hC4BP, four different isoforms are known. The major isoform consists of 7α and one β-chain, while less abundant isoforms exist as 6α1β, 7α, or 6α, respectively. Each

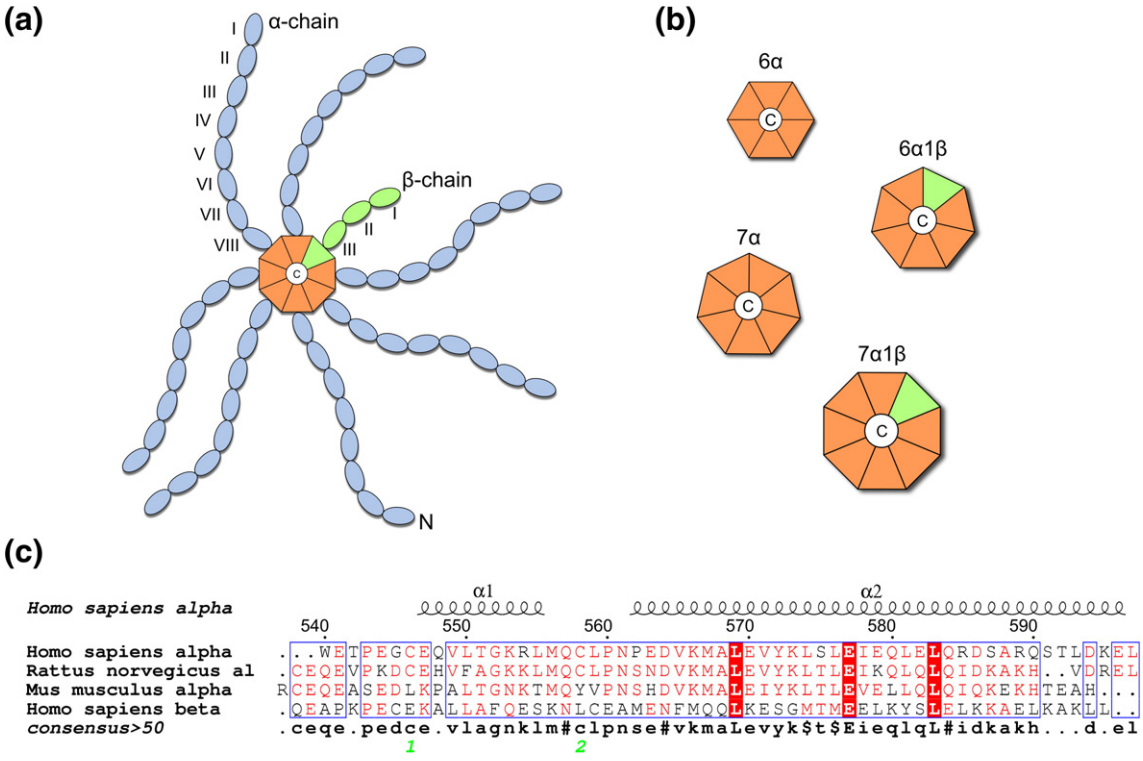

Fig. 1. (a) Schematic drawing of the major 7α1β isoform of hC4BP. Seven α-chains and one β-chain containing eight and three CCPs, respectively, are arranged around the C-terminal oligomerization domain. (b) Overview of all naturally occurring oligomerization domains of C4BP. The following are the subunit compositions (from top to bottom): 6α, 6α1β, 7α, and 7α1β, respectively. (c) Alignment of C4BP α-chains and β-chain from *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus*. Multiple sequence alignment was performed with MultAlin[45] and rendered using ESPript 2.2.[46] Secondary-structure information was obtained from hC4BP$^{540–597}$ coordinates (this study). Amino acids marked in red are identical with the hC4BP sequence; amino acids highlighted in red are conserved for all aligned sequences. Sequence labels on top of the alignment refer to the hC4BP α-chain. The blue frames show the consensus of at least two amino acids. The consensus sequence (bottom line) was calculated with a threshold of 0.5. Consensus sequence: uppercase is identity, lowercase is consensus level >0.5, $ is any one of LM, # is any one of NDQEBZ. Green numbers indicate the position of disulfide bridges in respect to the template.

α-chain comprises eight complement control domain proteins (CCPs) and a C-terminal oligomerization domain with a molecular mass of 75 kDa. In contrast, the smaller β-chain (40 kDa) of hC4BP has only three CCPs and a C-terminal oligomerization domain. The assembly results in a characteristic spider- or octopus-like structure of the C4BP protein complex.[5] Both the α-chains and the β-chain of the core domain consist of approximately 60 amino acids and are linked together in most organisms by intermolecular disulfide bonds. Interestingly, the β-chain shares no sequence homology with the α-chain and it remains largely unclear how the assembly of this heterooligomeric complex occurs (Fig. 1c).[6]

C4BP modulates the lectin pathway that proceeds through lectin-mediated binding of complement factors to sugars residing on the target surface as well as the classical complement pathway by binding to C4b via a cluster of positively charged residues that are located in the first three CCP domains of the α-chains.[7] C4b protein interacts with other components of the complement system as C2a and C3b. Binding of C4BP results in increased accessibility for proteolytic cleavage of C4b by factor I and inhibition of assembly of C3/C5 convertase (C4b, C2a and C3bC4b, C2a).[8] Each C4BP α-chain alone is fully capable of binding C4b molecules, but due to steric hindrances, up to four C4b can be bound simultaneously.[9] The β-chain of hC4BP does not bind to C4b but has high affinity to protein S, which is part of the coagulation system. It also is a cofactor for activated protein C that is important for degradation of coagulation factors Va and VIIIa. C4BP-bound protein S is not able to act as a cofactor in coagulation. Interestingly, about 70% of protein S in human serum circulates in complex with C4BP, directly linking the complement to the coagulation system.[5] While the CCPs of C4BP interact with a variety of proteins, for the C-terminal core domain and parts of CCP8, only