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Evolution of the complement system C3 gene in Antarctic teleosts

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ABSTRACT

Notothenioidei are typical Antarctic teleosts evolved to adapt to the very low temperatures of the Antarctic seas. Aim of the present paper is to investigate sequence and structure of C3, the third component of the complement system of the notothenioid *Trematomus bernacchii* and *Chionodraco hamatus*. We determined the complete nucleotide sequence of two C3 isoforms of *T. bernacchii* and a single C3 isoform of *C. hamatus*. These sequences were aligned against other homologous teleost sequences to check for the presence of diversifying selection. Evidence for positive selection was observed in the evolutionary lineage of Antarctic teleost C3 sequences, especially in that of *C. hamatus*, the most recently diverged species. Adaptive selection affected numerous amino acid positions including three residues located in the anaphylatoxin domain. In an attempt to evaluate the link between sequence variants and specific structural features, we constructed molecular models of Antarctic teleost C3s, of their proteolytic fragments C3b and C3a, and of the corresponding molecules of the phylogenetically related temperate species *Epinephelus coioides*, using human crystallographic structures as templates. Subsequently, we compared dynamic features of these models by molecular dynamics simulations and found that the Antarctic C3s models show higher flexibility, which likely allows for more pronounced movements of both the TED domain in C3b and the carboxyl-terminal region of C3a. As such dynamic features are associated to positively selected sites, it appears that Antarctic teleost C3 molecules positively evolved toward an increased flexibility, to cope with low kinetic energy levels of the Antarctic marine environment.

1. Introduction

The complement system (CS) participates in host protection acting as a first line defensive molecule, promoting local inflammatory reactions, and coordinating the adaptive immune response (Carroll and Isenman, 2012; Ricklin et al., 2010). It consists of over 30 secreted or membrane-bound proteins and can be activated through three different pathways (classical, alternative, and lectin). CS activation pathways converge in the formation of the C3 convertase, which cleaves the third component C3. The proteolytic cleavage of C3 generates a small bioactive fragment, the anaphylatoxin C3a, which mediates many biological activities, and a large fragment, C3b, which undergoes an extensive conformational change resulting in the exposure of the thioester group that, in turn, interacts with bacterial matrices (Gros et al., 2008; Law and Dodds, 1997). C3b proteolytic break down, initiated by Factor I, produces multiple fragments; among them iC3b and its portion C3d, are the ligands of complement receptor 2 (CR2) on the B cells surface. Ligand–receptor binding mediates the adaptive immune response (Kalli et al., 1991).

The C3 molecular structure has been determined at atomistic level (Janssen et al., 2005) allowing the comprehension of the complex molecular functions exerted by this molecule. A single primary transcript encodes the C3 pro-protein that undergoes relevant post-translational modifications consisting in the glycosylation at two asparagine residues (Hirani et al., 1986), the loss of the signal peptide, the cleavage in two polypeptide chains (β and α) with the loss of four arginine residues, the formation of 13 disulfide bridges (Dolmer and Sottrup-Jensen, 1993; Huber et al., 1980), and the formation of the internal thioester bond (Tack et al., 1980).

Abbreviations: α′NT, α′ N-terminal tag; ANATO, anaphylatoxin domain; C3, third complement system component; C345C, C3, C4, C5 domain; CS, complement system; CUB, complement C1r/C1s, Uegf, Bmp1; LINCS, linear constraint solver; LNK, linker region; MD, molecular dynamics; MG, α_2 macroglobulin domain; RACE, rapid amplification of cDNA ends; RMSD, root mean square deviation; RMSF, root mean square fluctuation; SAR, short anchor region; TED, thioester containing domain.

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