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# Identification of a complement receptor 1 peptide for inhibition of immune hemolysis ☆

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**Abstract**

Complement sensitization of red blood cells (RBCs) can cause life-threatening hemolytic anemias. We have previously shown that complement receptor 1 (CR1) derivatives specifically the N-terminal region with decay accelerating activity (DAA) for inactivation of a key enzyme in the complement cascade can reduce complement-mediated RBC destruction *in vitro* and in an *in vivo* mouse model of hemolytic transfusion reaction. In the present study, we have modeled the N-terminal CR1 molecule based on the X-ray crystal structure of decay accelerating factor and the NMR structure of a homologous CR1 domain. Based on the homology model, we identified a 34-mer peptide encompassing the putative DAA which *in vitro* reduced hemolysis, C3a release and surface C3 deposition. More importantly, this peptide at 0.6 mM was effective in prolonging survival of transfused incompatible RBCs *in vivo*. Our results indicate that CR1-based structure–function studies may provide insights for developing structure-derived transfusion therapeutics in the future.


*Keywords:* Complement receptor 1; CD35; Complement inhibition; Transfusion therapy; Hemolysis; Homology modeling; Mouse model; Transfusion reactions; CR1 peptides; C3a; C3b

The complement system is an important part of the innate immune system for fighting infections and foreign molecules before an adaptive response is developed. To execute its functions, the system has to be activated. A key step in the complement activation cascade is the formation of the C3 convertase complex on the surface of target cells which cleaves C3 into C3b, the main effector of complement, and C3a, a potent anaphylatoxin that is released into the medium. C3b and its degradation products on target cells act as opsonins marking them for removal from the circulation by phagocytes. In addition, if sufficient quantities of C3b are formed on the cell surface, they can result in serial activation of complement proteins (C5–C9) that form the C5b-9 complex, also known as the membrane attack complex. Once adequate number of C5b-9 complexes form, the target cell is lysed. As expected, inappropriate complement activation can have deleterious effects. In the transfusion medicine setting, complement sensitization of red blood cells (RBCs) can result in life-threatening hemolytic transfusion reactions as well as hemolytic anemias [1]. There is thus a critical need for a therapeutically applicable complement inhibitor. To date several inhibitors have been described [2], but none of these has yet been adopted as a therapeutic agent. A strategy to develop complement inhibitors has been to manipulate naturally occurring complement regulatory proteins that act at various steps in the activation cascade and control

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inappropriate complement activation for therapeutic use. Complement receptor 1 (CR1) is the most versatile member of the complement regulatory proteins through its ability to bind complement split products (C3b and C4b) and possessing decay accelerating and cofactor activities that can inactivate the two critical enzymes (C3 and C5 convertases) of both the classical and alternative complement activation pathways [3–6]. A recombinant soluble form of CR1 (SCR-1) by inactivating the convertases in the complement cascade has successfully inhibited the complement activation and prevented complement-mediated tissue injury in several animal models [7,8]. More importantly, SCR-1 has been in human clinical trials for the treatment of acute respiratory distress syndrome and to reduce tissue damage in myocardial infarction and lung transplantation [9–11] with possible favorable outcomes [10].

Our studies were the first to demonstrate a potential for SCR-1 for inhibiting complement-mediated red cell destruction following transfusion immunization events [12,13]. We reasoned that by identifying the functional regions of CR1 (decay accelerating and cofactor activities), structure-based small synthetic molecules with more specific inhibitory activities, but lacking immunogenecity can be developed, thereby permitting the administration of lower drug doses and better efficacy. A combined *in vitro* and *in vivo* structure–function analysis of CR1 domains responsible for complement inhibition has been our approach towards the future design of such molecules. The extracellular 1930 residue long domain of CR1 can be divided into 30 short consensus repeats (SCRs), each of 59–72 amino acids (aa) with sequence homology between SCRs ranging from 60% to 90% [14]. Through structure–function analysis we identified a 254 aa domain at the N-terminus of CR1, consisting of 4 SCRs (SCRs-1–4), that has anti-hemolytic activity both *in vitro* and *in vivo* in a mouse model of complement-mediated hemolytic transfusion reaction [15]. This region mediates inhibition of the classical pathway and possesses decay acceleration of the C3 convertases [16]. Previous mutagenesis data of SCRs-1–4 have identified several critical residues for decay accelerating activities in the N-terminal domain [16–20]. For example, three positively charged amino acids Arg59, Arg60, and Lys61 at SCR-1/SCR-2 junction, Arg64, Asn65, Thr103, and Thr110 in SCR-2 as well as Gly35 in SCR-1 were found to be important for both decay accelerating activity while Phe82 was required primarily for decay accelerating activity [20]. To date, no structural data for the N-terminal domain of CR1 is available. However, the NMR structure of a homologous region (SCRs-15–17) in CR1 has been reported [21]. In addition, there is crystallographic data on the structure of the 4 SCRs of decay accelerating factor (DAF)/CD55 [22]. In the present study, we have modeled the amino terminal SCRs based on the crystal structure of DAF [22] and the NMR structure of SCRs-15–17, designed peptides based on this homology model and tested their anti-hemolytic activities. Using this rational approach, we have identified a 34-mer peptide in SCR-2 which has anti-complement activity both *in vitro* and *in vivo*.

## Methods and materials

*Modeling CR1 based on DAF crystal structure and SCRs-15–17 NMR structure*. Homology models of the first 3 N-terminal SCRs of CR1 were built based on the X-ray crystal structure of DAF/CD55 (pdb code: 1ojw) and on the average NMR structure of the SCRs-15–16 of CR1 (CD35) (pdb code: 1gkn) using the automated software Modeller [23] within Quanta 2000 (Accelrys, San Diego, CA) running on a Silicon Graphics Octane with a dual R12000 processor (sgi, Mountain View, CA). 'Refine 3' option in Modeller, which uses conjugated gradient together with molecular dynamics by simulated annealing technique, was used to optimize the models. Five models for each run were developed and the model with the lowest Objective Function was selected. The resulting models were evaluated for its stereochemical properties by Procheck software [24] at 2.0 Å and by Quanta Protein Health programs within Quanta 2000.

*Synthetic peptides*. The peptides in Fig. 2 were synthesized by Genemed Synthesis Inc. (South San Francisco, CA) and by the Biomolecular Synthesis Laboratory of the New York Blood Center. A standard solid-phase Fmoc method was used for peptide synthesis. Peptides were purified to

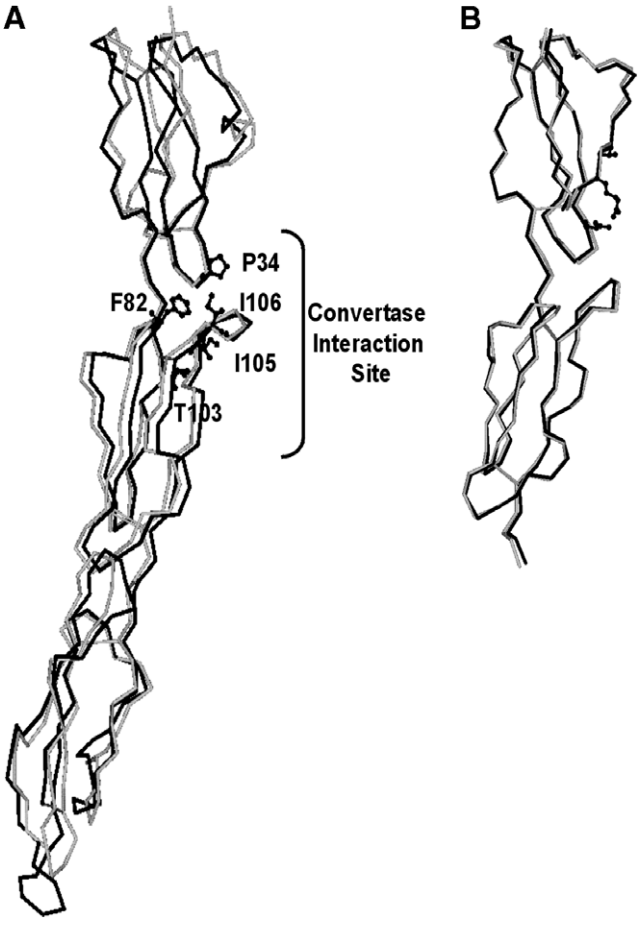


Fig. 1. Homology model of N-terminal domain of CR1. (A) C-α tracing of SCR-1–SCR-3 domains of CR1 (black) superimposed on the X-ray crystal structure (grey) of human complement regulator CD55. The residues, forming the hydrophobic patch and responsible for interaction with convertase, are shown as ball-and-stick model. (B) C-α tracing of SCR-1 and SCR-2 domains of CR1 (black) superimposed on the NMR structure (grey) of SCRs-15–16 of CR1.