SPR Binding Affinity Determination of Novel Peptide Inhibitors to the Innate Immune activator C1q

The complement system is an essential component of the human innate immune system, playing a critical role as a defense mechanism against invading pathogens, priming adaptive immune responses and helping to remove immune complexes and apoptotic cells. Three different pathways comprise the complement system: the classical, lectin and alternative pathways [1]. While the complement system plays a central role in many protective immune functions, complement activation is a significant mediator of tissue damage in a wide range of autoimmune and inflammatory disease processes [2]. Consequently, in the past few years much effort has focused on developing small molecule inhibitors of the complement system [3].

Our research group has recently identified virally derived peptides of 15-30 amino acids that can potently inhibit the classical pathway of complement [4-6]. These peptides have been demonstrated to bind C1q, and are hypothesized to disrupt its interaction with the cognate serine protease complex (C1r-C1s-C1s-C1r) that is required to activate the classical pathway (Fig. 1).

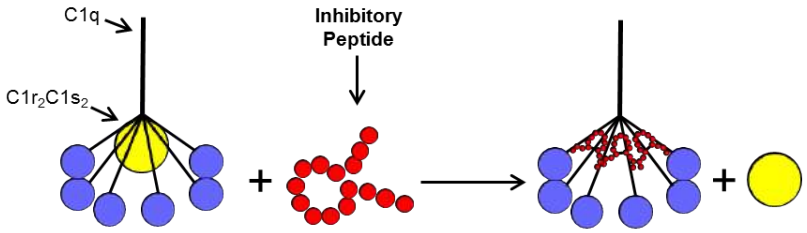

Figure 1. A model of the inhibitory peptide interfering with the normal association of C1q with its cognate serine proteases ($C1r_2C1s_2$). The inhibitory peptide functionally displaces the $C1r_2C1s_2$ by binding to C1q.

C1r-C1s-C1s-C1r has been previously shown by SPR to bind immobilized C1q with a K_D of 2.72 nM [7]. To evaluate the interaction of the peptide with C1q by SPR, we utilized a BI-3000 instrument to measure the affinity of three inhibitory peptides E23A, Δ8-22, and PA (ligand) with human C1q (analyte). The peptide ligands were immobilized on a dextran coated sensor chip with PBS running buffer. The analyte binding was measured in PBS-Tween 20 (0.01%) at a flow rate of 20 µL/min. Surface regeneration after C1q injection was accomplished by injecting 10 mM NaOH. The mean equilibrium dissociation constants (K_D) were 6.08 nM for E23A, 5.43 nM for Δ8-22, and 33.3 nM for PA (Table 1), respectively. Figure 2 depicts the SPR sensorgrams, which were fitted by the 1:1 model with the mass transport. These dissociation constants show that the peptides bind C1q with high affinity. The binding of E23A, Δ8-22, and PA to C1q with similar affinity for C1s-C1r-C1r-C1s (*i.e.,* nM range) is consistent with our hypothesis that these peptides may function to inhibit classical pathway activation by competitively displacing, at least partially, C1s-C1r-C1r-C1s from C1q. SPR data such as this not only informs our understanding of the mechanism by which these peptides inhibit C1q activation, but will also lay the foundation for preclinical therapeutic development of these peptides [8].

Table 1. Kinetic properties of inhibitory peptide binding to C1q

Immobilized Protein	Soluble analyte	k_{on} (×10^4) M^{-1}s^{-1}	k_{off} (×10^{-4}) s^{-1}	K_D[a] (nM)
E23A	C1q	11.1 ± 2.34	6.52 ± 0.351	6.08 ± 0.966
Δ8-22	C1q	19.8 ± 0.395	10.6 ± 3.94	5.43 ± 2.10
PA	C1q	2.58 ± 0.596	8.08 ± 0.206	33.3 ± 8.49

[a] K_D values were calculated from k_{on}/k_{off} for each experiment and were averaged from at least two separate experiments.

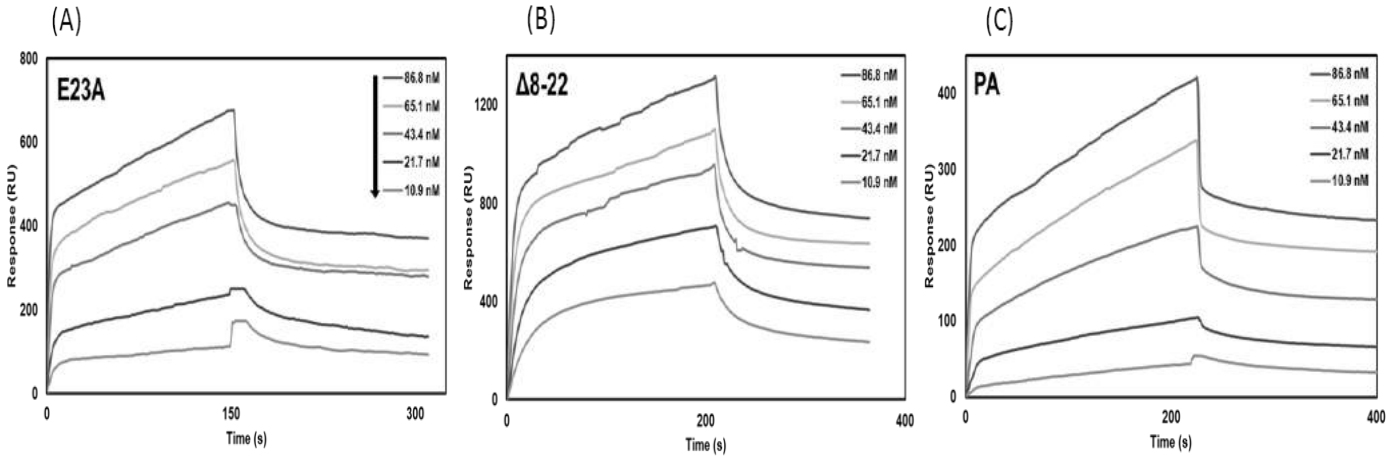

Figure 2. Interactions between inhibitory peptides and C1q measured by SPR. Peptide were immobilized onto the surface of a dextran sensor chip and C1q was injected at 86.8, 65.1, 43.4, 21.7 and 10.9 nM. SPR sensorgrams for C1q interaction with immobilized E23A (A), Δ8-22 (B) and PA (C). Data analysis consisted of globally fitting the SPR sensorgrams for different C1q concentrations with a 1:1 model taking into account the mass transport.

References

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