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Structural models for the complex of chemotaxis inhibitory protein of *Staphylococcus aureus* with the C5a receptor

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ABSTRACT

The study presents structural models for the complex of the chemotaxis inhibitory protein of *Staphylococcus aureus*, CHIPS, and receptor for anaphylotoxin C5a, C5aR. The models are based on the recently found NMR structure of the complex between CHIPS fragment 31–121 and C5aR fragment 7–28, as well as on previous results of molecular modeling of C5aR. Simple and straightforward modeling procedure selected low-energy conformations of the C5aR fragment 8–41 that simultaneously fit the NMR structure of the C5aR 10–18 fragment and properly orient the NMR structure of $CHIPS_{31-121}$ relative to C5aR. Extensive repacking of the side chains of $CHIPS_{31-121}$ and $C5aR_{8-41}$ predicted specific residue–residue interactions on the interface between CHIPS and C5aR. Many of these interactions were rationalized with experimental data obtained by site-directed mutagenesis of CHIPS and C5aR. The models correctly showed that CHIPS binds only to the first binding site of C5a to C5aR not competing with C5a fragment 59–74, which binds the second binding site of C5aR. The models also predict that two elements of CHIPS, fragments 48–58 and 97–111, may be used as structural templates for potential inhibitors of C5a.

Introduction

CHIPS, a 121-membered chemotaxis inhibitory protein of *Staphylococcus aureus*, is a potent inhibitor of neutrophil and monocyte chemotaxis involving C5a anaphylatoxin or formylated peptides [1]. CHIPS selectively binds both C5a receptor (C5aR) and formylated peptide receptor (FPR) with nanomolar affinities [2], which makes it a promising lead for development of new anti-inflammatory compounds. Mutational studies have previously demonstrated that different epitopes of CHIPS inhibit signaling through C5aR or FPR: the first six residues of CHIPS blocked FPR but not C5aR [3], whereas the truncated CHIPS fragments, 31–121 and 31–113, inhibited C5a binding to C5aR as effectively as the entire CHIPS, but lacked FPR antagonism [4,5].

C5a binds and activates the C5aR utilizing two distinct binding sites, the first located in the N-terminus of C5aR and the second located in a helical crevice between the extracellular loops which accommodate the C-terminus of the C5a ligand (residues 65–74) [6–8]. Mutational studies of C5aR have mapped CHIPS binding to the N-terminal fragment of C5aR (residues 1–38) and demonstrated an essential role for the C5aR fragment 10–18 [9]. In addition, CHIPS does not affect activation of C5aR by a peptide mimic of the C5a 65–74 fragment [9]. These studies demonstrated also that CHIPS blocks C5a activation by inhibiting the binding of the intact ligand to the N-terminus of the C5a receptor and also implied that CHIPS does not interact appreciably with the extracellular loops of the C5aR.

Previously, NMR spectroscopy provided the three-dimensional solution structure of the isolated CHIPS 31–121 [4] and, very recently, in complex with the C5aR 7–28 fragment (Tyr^{11} and Tyr^{14} of C5aR were sulfated) [10]. The NMR structure of the $C5aR_{7-28}$:$CHIPS_{31-121}$ complex confirmed the important role of C5aR fragment 10–18 in binding CHIPS. The C-terminal portion of the $C5aR_{7-28}$ peptide was highly flexible, which prevented elucidation of specific interactions between residues 22–28 of $C5aR_{7-28}$ with CHIPS in the NMR structure [10]. Also, mutational studies using tethered N-terminal peptides that either introduced point mutations into this region or deleted it altogether did not impact binding of CHIPS, thereby supporting the notion that this region does not interact directly with CHIPS [9]. However, in the context of the intact C5aR, residues 22–28 may adopt a very different set of conformations, especially when one considers that this region is directly connected to the transmembrane helix of C5aR (TM1, residues 38–63 [11]).

Our previous studies developed 3D models of the C5aR:C5a complex involving various possibilities for flexible extracellular loops and the N-terminal fragment of C5aR [11]. The models were validated by comparison with the available data of site-directed

Abbreviations: GPCR, G-protein-coupled receptor; C5a, anaphylotoxin C5a; C5aR, C5a receptor; CHIPS, chemotaxis inhibitory protein of *Staphylococcus aureus*

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mutagenesis of C5aR [11] and the results of a novel technique of disulfide trapping by random mutagenesis [12]. In the present study, we employed the results of the previous modeling of the C5aR and the NMR data on the $C5aR_{7-28}$:$CHIPS_{31-121}$ complex to generate new models for interaction between CHIPS and the C5aR. Our modeling procedure selected low-energy conformations of the C5aR fragment 8–41 that simultaneously fit the NMR structure of the C5aR 10–18 fragment and properly orient the NMR structure of $CHIPS_{31-121}$ relative to C5aR. Extensive repacking of the side chains of $CHIPS_{31-121}$ and $C5aR_{8-41}$ predicted specific residue–residue interactions on the interface between CHIPS and C5aR. Many of these interactions are rationalized with experimental data obtained by site-directed mutagenesis of CHIPS and C5aR.

Materials and methods

All energy calculations were performed using the ECEPP/2 force field with rigid valence geometry [13,14] and *trans*-conformations of Pro residues; residues Arg, Lys, Glu and Asp were regarded as charged species. The 3D structures of the $C5aR_{8-41}$:$CHIPS_{31-121}$ complex were obtained by energy minimization of the system consisting of two components, namely the selected conformation(s) of $C5aR_{8-41}$ and the NMR-derived structure of $CHIPS_{31-121}$. The modeling procedure was essentially the same as that applied earlier for determining the 3D structures of the transmembrane regions of GPCRs (see, e.g. [11] for details). The backbone structures of each component were regarded as rigid bodies (with the fixed values of the backbone dihedral angles), while the side chains of both $C5aR_{8-41}$ and $CHIPS_{31-121}$ were extensively repacked prior to energy minimization. For CHIPS mutants, the corresponding side chains were replaced in the NMR structure. The algorithm of side chain repacking [15,16] involved a stepwise grid search (with the grid step of 30°) in the space of the dihedral angles of the side chains, χ_i. To ensure thorough sampling of the χ_i space, two options of the search were applied independently. The first option sampled the grid varying sequentially all χ_1 angles of the side chains of $CHIPS_{31-121}$ and $C5aR_{8-41}$, then all χ_2 angles, then all χ_3 angles, *etc*. until all side chain angles of both components were rotated. The second option sampled all χ_i angles for each side chain in order of their number in protein sequence, i.e., from 31 to 121 for $CHIPS_{31-121}$ and from 8 to 41 for $C5aR_{8-41}$. In both cases, sampling was performed until convergence to energetically optimal packing of side chains was achieved. If necessary, specific side chains (as those on CHIPS:C5aR interface) were repacked additionally. Finally, energy minimization in the space of possible rotations and translations of the two components as well as in the space of the dihedral angles of the side chains followed. Results of all options were pooled together to cover energetically plausible conformational possibilities for the side chains. To account to some extent for the absence of solvent and membrane environment, energy calculations were performed using two values of macroscopic dielectric constant, $\varepsilon = 2$ (the generic value for the ECEPP force field) and $\varepsilon = 80$, which is closer to the water dielectric constant. In most cases, distributions of the side chain rotamers obtained by calculations with both ε values were identical.

Results and discussion

Computational models of the $CHIPS_{31-121}$:$C5aR_{8-41}$ complex

Previous modeling of the isolated N-terminal fragment $C5aR_{8-41}$ identified 185 low-energy backbone structures [11]; it was reasonable to assume that possible conformations of $C5aR_{8-41}$ in complex with CHIPS should be selected from this pool of structures. Fifty-nine of 185 structures contained spatial positions of fragment C5aR 10–18 that fit to that observed in the $C5aR_{7-28}$:$CHIPS_{31-121}$ complex by NMR spectroscopy (according to the rms cut-off of 2 Å calculated for the Cα atoms of residues Asp^{10}, Tyr^{11}, Gly^{12}, Tyr^{14}, Asp^{15} and Asp^{18}; NMR model #1 from the PDB entry 2K3U was used for fitting). In turn, only 17 of 59 structures showed no steric clashes with the NMR structure of $CHIPS_{31-121}$ (i.e., no intermolecular Cα–Cα distance was less than 3 Å). When these 17 structures were fit to the helical stem of TM1 (residues 38–41) of our model of C5aR, only four structures did not contain steric clashes with either the TM region of C5aR or with all possible conformations of the extracellular loops that have been found previously [11]. Visual inspection showed that, in two of the four structures, $CHIPS_{31-121}$ was significantly embedded into the membrane space, which is highly unlikely; those two structures were discarded from subsequent considerations.

The remaining two models of the $C5aR_{8-41}$:$CHIPS_{31-121}$ complex are depicted in Fig. 1A and B. The interfaces between C5aR and CHIPS were established by subjecting both models to extensive side chain repacking and energy calculations (see Materials and methods). The system of possible residue–residue contacts on the interface in both models is described in Table 1 (a contact was defined as situation when at least one distance between atoms belonging to the corresponding side chains was less than 5.5 Å). The two models feature significantly different potential strong salt bridges and/or hydrogen bonding at the interfaces. The strongest interactions in the first model were between Y48/K51 of CHIPS and Asp^{10} of C5aR (the corresponding residues are shown in Table 1 in bold; different notations are applied to distinguish between amino acid residues of C5aR and CHIPS). In the second model, the strongest interactions were K51–Thr^{8}, K54–Ser^{30}, K100–Thr^{29} and Y108–Thr^{29}. Importantly, many of interactions on the interface listed in Table 1 should be regarded as possibilities, since some side chains possess more conformational freedom and therefore may also interact with residues not on the interface (see also below).

Rationalizing site-directed mutagenesis data by computational models of the $CHIPS_{31-121}$:$C5aR_{8-41}$ complex

Interfaces between C5aR and CHIPS suggested by both models agree well with the two independent sets of data on NMR titration of $C5aR_{1-37}$ in presence of ^{15}N–$CHIPS_{0-121}$ [17] and of the sulfated $C5aR_{7-28}$ in presence of ^{15}N–$CHIPS_{31-121}$ [10]. Both studies demonstrated that two segments of CHIPS (*ca.* 45–61 and 98–111) directly interact with the C5aR fragments; these segments figure prominently in the binding interfaces of both computational models. Mutations of K95 (K95A–$CHIPS_{1-121}$ [4], K95S–$CHIPS_{31-113}$ [5]) or Y97 (Y97A–$CHIPS_{31-121}$ [10], Y97K–$CHIPS_{31-113}$ [5]) significantly lowered the ability of mutants to block activation of C5aR by C5a. K95 and Y97 likely are directly involved in interactions with the C5aR residues on C5aR:CHIPS interface, since CD spectroscopy showed that the above mutations do not disturb structural integrity of CHIPS (see Fig. S3 in Ref. [10]). At the same time, interactions K95–Asp^{18} and Y97–Asp^{18} are available in the first computational model but not in the second one that features interaction K105–Asp^{18} (see Table 1 and Fig. 1). Other experimental data on CHIPS mutants also support the first model. Specifically, mutations at Y48A–$CHIPS_{31-121}$ [10] and K51A–$CHIPS_{1-121}$ [4] led to significant loss of CHIPS ability to block C5aR activation by C5a by likely affecting the strong Y48–Asp^{10} and K51–Asp^{10} interactions present in the first model. Alternatively, the K51A mutation may interrupt the strong K51–Thr^{8} interaction characteristic for the second model. Also, S107A–$CHIPS_{31-121}$ [10] and S107N–$CHIPS_{31-113}$ [5] only partially inhibit C5aR interaction with C5a, which may be due to loss of the possible interaction S107–Lys^{28} present in both models (Lys^{28} may alternatively interact either with S107 or with Asp^{15}, which is shown in Fig. 1A). In both models, S106 closely contacts