Modeling Molecular Mechanisms of Binding of the Anaphylatoxin C5a to the C5a Receptor

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Additional details of computational procedures

Force field. Arg, Lys, Glu and Asp side chains were considered as ionized. Aliphatic and aromatic hydrogens were generally included in united atomic centers of CH_n type. H^{α}-atoms and amide hydrogens were described explicitly.

Build-up procedures for C5a 59-74 and C5aR 8-41.

For C5a 59-74, the single conformation of the peptide backbone for the fragments C5a 59-62 and C5a 66-69 was selected based on the NMR structure of C5a, the PDB entry 1KJS (*1*) and the NMR structures of the C5a agonist YSFKPMPLaR (*2, 3*), respectively. This conformation was used as the starting point for energy minimization together with all combinations of local minima of peptide backbone of individual residues in fragments C5a 63-65 and C5a 70-74, i.e., with the ϕ,ψ values (-140º, 140º), (-75º, 140º), (-75º, 80º), (-60º, -60º), and (60º, 60º) for residues Ala63, Asn64, Ile65, Met70, Gln71, Leu72 and Arg74; for Gly73, the ϕ,ψ values (140º, -140º), (75º, -140º) and (75º, -80º) were considered additionally. Only conformations with the distance $C^{\alpha}_{59} – C^{\alpha}_{74}$ larger than 22 Å (those allowing the unobstructed attachment of the rest of C5a) were selected as

starting points for energy calculations. Energy calculations with ε=80 were performed for each resulting starting point of the peptide backbone of fragment C5a 59-74; along with energy minimization, the side chains were re-packed to achieve their most favorable spatial arrangements employing an algorithm described earlier (*4*).

For C5aR 8-41, the initial steps of the build-up procedure involved energy calculations with ε=2 for all oligopeptide fragments of C5aR 8-24, from hexapeptides to 17-membered peptides, where all hexapeptides were regarded as the initial fragments for further elongation (a similar procedure was employed earlier (*5*)). The local minima of peptide backbone listed above were considered for all L-amino acid residues and for glycines; for prolines, the corresponding ϕ,ψ values were (-75º, 140º), (-75º, 80º) and (-75º, -60º). At each step of elongation, the low-energy conformations with re-packed side chains were selected according to energy cut-offs of N kcal/mol, where N was number of residues in the corresponding fragment (the energy cut-off was equal to 15 kcal/mol also for 16- and 17-membered peptides). Upon elongation from C5aR 8-24 to 8-28 to 8-31 to 8-34 to 8-41, all energy cut-offs were of 15 kcal/mol, and the ϕ,ψ values of the residues I38-L39-A40-L31 were fixed in the values found for these residues during building of the TM region of C5aR.

Restoring the EC loops. Geometrical sampling of the individual loops involved starting conformations of individual residues and overall sampling procedure as described earlier (*6*) with limitations on the residue-residue contacts within the loop (C^{α}-C^{α} distances ≥ 4 Å) and on the contacts between the loop and the template (C^{α}-C^{α} distances ≥ 6 Å); the values of coefficients EL and DEL were 3.0 and 0.0, respectively (see (*6*)). Elongation

steps were as follows: a single step from residue 98 to residue 107 for EC1; from 267 to 276 to 278 to 281 for EC3, and two separate steps for EC2, namely from 172 to 181 to 184 to 186 to 188 (spatial position of the target C^{α}_{188} was selected close to C^{α}_{109} in the TM region, since residues C109 and C188 are connected by the disulfide bridge) and then from 188 to 191 to 193 to 195 to 197. After geometrical sampling selected the set of potentially loop-closing conformations for a specific loop, the selected structures were subjected to energy minimization with ε=80. All loops were capped from both sides with the four-residue TM helical stems where the ϕ,ψ values were fixed as they were in the corresponding TM helices; the proper residue-residue distances between stems were ensured by adding the parabolic potentials between C^{α} atoms with U_o = 10 kcal/mol.

References

1. Zhang, X., Boyar, W., Toth, M. J., Wennogle, L., and Gonnella, N. C. (1997) Structural definition of the C5a C terminus by two-dimensional nuclear magnetic resonance spectroscopy, *Proteins 28*, 261-7.
2. Finch, A. M., Vogen, S. M., Sherman, S. A., Kirnarsky, L., Taylor, S. M., and Sanderson, S. D. (1997) Biologically active conformer of the effector region of human C5a and modulatory effects of N-terminal receptor binding determinants on activity, *Journal of Medicinal Chemistry 40*, 877-84.
3. Vogen, S. M., Prakash, O., Kirnarsky, L., Sanderson, S. D., and Sherman, S. A. (1999) Determination of structural elements related to the biological activities of a potent decapeptide agonist of human C5a anaphylatoxin, *Journal of Peptide Research 54*, 74-84.
4. Nikiforovich, G. V., Hruby, V. J., Prakash, O., and Gehrig, C. A. (1991) Topographical Requirements for Delta-Selective Opioid Peptides, *Biopolymers 31*, 941-955.
5. Nikiforovich, G. V., Andersen, N. H., Fesinmeyer, R. M., and Frieden, C. (2003) Possible Locally Driven Folding Pathways of TC5b, A 20-Residue Protein, *Proteins: Structure, Function, and Genetics 52*, 292-302.
6. Nikiforovich, G. V., and Marshall, G. R. (2005) Modeling Flexible Loops in the Dark-Adapted and Activated States of Rhodopsin, a Prototypical G-Protein Coupled Receptor, *Bioph. J. 89*, 3780-3789.

Table S1. Outline of building 3D model of C5a/C5aR complex

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1. Results of energy calculations for specific fragments:

C5a 59-74: 30 clusters of low-energy conformations
C5aR 8-41: 185 low-energy conformations
EC loops: 29 clusters of low-energy conformations for the EC1+EC2+EC3 package
TM region of C5aR: modeled by homology to rhodopsin

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2. Final C5aR model:
TM region + 29 conformations of EC1+EC2+EC3 + 44 conformations of the N-terminal segment 8-41 not clashing with each other.

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3. Building of C5a/C5aR complex

Results of finding orientations of C5a fragments within the partial model of C5aR (the TM region + the most open conformation of the EC loops):

C5a 65-69:
5184 orientations were sampled;
76 were of low-energy (simplified energy calculations within the TM region of C5aR);
20 were not clashing with C5aR

C5a 59-74:
600 orientations were sampled (20 orientations for C5a 65-69 times 30 clusters of low-energy conformations of C5a 59-74);
20 were not clashing with C5aR;
11 were of low energy (simplified energy calculations within the TM region of C5aR);
5 were facing the N-terminal segment of C5aR;
4 were of reasonable energy (full energy calculations within the partial model of C5aR, i.e., within the TM region + the most open conformation of the EC loops)

C5a 1-74:
selection of orientation **B.**

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4. Final model of C5a/C5aR complex with orientation **B** for C5a 1-74:
TM region + 11 conformations of EC1+EC2+EC3 + 21 conformations of the N-terminal segment 8-41 not clashing with each other and with C5a.