

The Interactions of the N-formyl Peptide Chemoattractant Receptor with Guanyl Nucleotide-binding Proteins

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Human neutrophils are the body's first line of defense against bacterial infection. They are activated by a variety of agents including inflammatory cytokines, serum proteins, and bacterial protein synthesis initiation products. The latter N-formyl methionyl peptides stimulate chemotaxis, superoxide production, degranulation, and adherence, processes central to neutrophil microbicidal function. The receptors for these peptides, called formyl peptide chemoattractant receptors (FPR) exist in the plasma membrane and are stored in intracellular specific granule or endosomal storage sites. Upon occupancy, the FPR interact with guanyl nucleotide-binding G-proteins triggering the biochemical cascades that initiate these complex cellular processes. They also interact with regulatory proteins to control their function and organization in the plasma membrane.

The interaction of the formyl peptide receptor and G-protein (G_i) has been reconstituted in detergent extracts, permitting a biochemical analysis of the FPR- G_i complexes. At nanomolar concentrations of photoaffinity labeled, occupied FPR, either bovine or neutrophil G_i associates with FPR at an EC_{50} of approximately 100nM. Physical complexes of the proteins sediment in detergent-containing sucrose gradients at 7S and are disrupted by guanyl nucleotides, pertussis toxin-mediated ADP ribosylation of G_i , and by synthetic peptides mimicking certain FPR sequences. To identify receptor interfacial contact sites, peptides from the predicted cytoplasmic tail, cytoplasmic loops, and transmembrane alpha-helical regions of FPR were synthesized and used to inhibit 7S complex formation. Synthetic peptides having sequences identical to portions of cytoplasmic tail, each of the predicted cytoplasmic loops, and from certain transmembrane regions inhibited FPR- G_i complex formation at IC_{50} of 20 μ M to 1 mM. Other peptides mimicking the most hydrophilic portions of the predicted cytoplasmic loops or extracellular domains did not inhibit complex formation. Our results suggest that there is extensive contact between FPR and G_i that may approximate a ball in glove model, where the finger shafts (transmembrane alpha helices) of the glove but not the fingertips (most hydrophilic portions of the the predicted cytoplasmic loops) contact G_i (see figure, page 47).

The active regions may be exploited to develop specific inhibitors of FPR-activated pathways and gain important structural information about FPR. This information may also be useful in the design of anti-bacterial and anti-inflammatory drugs. We are currently attempting to determine the three dimensional structure of these regions using transferred nuclear Overhauser Effect spectroscopy (see abstracts by Dratz et al, on pp. 17-19 in the department of Chemistry and Biochemistry) of receptor peptides bound to their signal transduction partners, the G proteins. Such structures will also provide

important information about the overall structure of these important class of integral membrane proteins and more precisely define how they interact with regulatory proteins.

Recent Publications

Schreiber, R.E., Prossnitz, E.R., Ye, R.D., Cochrane, C.G., **Jesaitis, A.J.**, and Bokoch, G.M. Reconstitution of recombinant chemotactic peptide receptor with G-protein. *J.Leuk.Biol.* **3**: 470-474, 1993.

Bommakanti, R.K., **Klotz, K.-N.**, **Dratz, E.A.**, **Jesaitis, A.J.** Carboxyl-terminal tail peptide of neutrophil chemotactic receptor disrupts its physical complex with G-protein. *J.Leuk.Biol.* **54**: 572-577, 1993.

Bommakanti, R.K., **Dratz, E.A.**, Siemsen, D.W., **Jesaitis, A.J.** Characterization of complex formation between Gi2 and octylglucoside-solubilized neutrophil N-formyl peptide chemoattractant receptor by velocity sedimentation submitted *Biochim. Biophys. Acta.*, In press, 1994.



A model of the molecular interaction of agonist occupied FPR and Gi2. Our results suggest that the juxtamembrane and intramembrane spans of FPR interact with Gi2 but the most hydrophilic portions of the predicted cytoplasmic loops of FPR do not. One way this interaction can be interpreted is to imagine that when the FPR becomes occupied, it opens its cytoplasmic face much like a glove opening to catch a ball. The fingertips of the glove (middle of cytoplasmic loops, open areas) do not mediate the interaction but the finger barrels (juxta-membrane and intramembrane alpha helical regions) do. The arrangement of

N-formyl peptide chemoattractant receptor (FPR) transmembrane helices and their interaction with Gi is based on a modification of the model originally proposed for the rhodopsin Dratz et al, *Nature* 363:176-179, 1993). The G protein subunits are represented by the circles labeled a, b, g with zig-zags projecting from the subunits into the membrane representing posttranslational lipid modifications to Gi2. The darkly shaded areas on or near the FPR cytoplasmic face correspond to peptide regions that dissociate FPR-Gi2 complexes while the open areas denote the inactive peptide regions that do not interfere with physical coupling of FPR and Gi2. The putative cytoplasmic loop regions of FPR that are behind the plane of the diagram are shown in outline by light shading.