Characterization of the Binding Site on the Formyl Peptide Receptor Using Three Receptor Mutants and Analogs of Met-Leu-Phe and Met-Met-Trp-Leu-Leu*

John S. Mills, Heini M. Miettinen, David Cummings, and Algirdas J. Jesaitis
From the Department of Microbiology, Montana State University, Bozeman, Montana 59717

The formyl peptide receptor (FPR) is a chemotactic G protein-coupled receptor found on the surface of phagocytes. We have previously shown that the formyl peptide binding site maps to the membrane-spanning region (Miettinen, H. M., Mills, J. S., Grippentrog, J. M., Dratz, E. A., Granger, B. L., and Jesaitis, A. J. (1997) J. Immunol. 159, 4045–4054). Recent reports have indicated that non-formylated peptides, such as MMWLL can also activate this receptor (Chen, J., Bernstein, H. S., Chen, M., Wang, L., Isahi, M., Turck, C. W., and Coughlin, S. R. (1995) J. Biol. Chem. 270, 23398–23401.) Here we show that the selectivity for the binding of different NH$_2$-terminal analogs of MMWLL or MFL can be markedly altered by mutating Asp-106 to asparagine or Arg-201 to alanine. Both D106N and R201A produced a similar change in selectivity with our previous finding that the leucine side chain of MFL is probably bound to FPR near PFR $^3$VRK$^6$ (Mills, J. S., Miettinen, H. M., Barnidge, D., Vlases, M. J., Wimer-Mackin, S., Dratz, E. A., and Jesaitis, A. J. (1998) J. Biol. Chem. 273, 10428–10435.), indicate that the most likely position of MFL in the binding pocket of FPR is approximately parallel to the fifth transmembrane helix with the formamide group of MFL hydrogen-bonded to both Asp-106 and Arg-201, the leucine side chain pointing toward the second transmembrane region, and the COOH-terminal carboxyl group of MFL ion-paired with Arg-205.

The formyl peptide receptor (FPR)$^1$ is a chemoattractant G protein-coupled receptor found on the surface of phagocytes. It is thought to play an important role in allowing phagocytic cells to recognize the presence of bacteria (1), which are a source of formyl peptides (2, 3). In addition, it recognizes and is activated by peptides derived from the GP-41 envelope protein of the human immunodeficiency virus type I (HIV-1) (4, 5). Recent studies with FPR-deficient mice indicate that they exhibit an increased susceptibility to infection with Listeria monocytogenes and that neutrophils from these knockout mice fail to exhibit chemotaxis in response to MFL (6).

The formyl peptide receptor was originally identified based on its ability to bind the formylated peptide, MFL (1). Six different chemotactic peptides have been isolated from Escherichia coli, including /MLF (3), but the only similarity between them was an NH$_2$-terminal formyl methionine, suggesting that this moiety is highly important in binding to FPR. Studies using NH$_2$-terminal analogs of MFL have indicated that the formyl group has significant effects on the ligand binding affinity for neutrophil FPR. Free amino, desamino, and acetylated derivatives of MFL were all 3000-fold lower in affinity than /MLF (7). Substitution of the formyl group of /MLF with a tert-butyloxycarbonyl group made the ligand an antagonist of low affinity (8). However, the formyl group may be less essential than originally thought. N-Butyloxycarbonyl MFL exhibits agonist activity (9) with an affinity similar to /MLF, and phenyl and tolyl isourea derivatives of MFL exhibit activity similar to or greater than /MLF (10). On the other hand, most aliphatic isourea derivatives of MFL exhibit low affinity antagonist activity similar to what is observed with tert-butyloxycarbonyl-MFL (10). This difference indicates that FPR exhibits a high degree of specificity for NH$_2$-terminal modifications of MFL, and that the specificity for the formyl group is not absolute. In addition, other reports have indicated that non-formylated pentapeptides can activate FPR. Both MNeleLFF and MMWLL are effective activators of FPR (11, 12), and acetyl-MNeleLFF is more potent than /MLF (12), indicating that these pentapeptides exhibit somewhat different NH$_2$-terminal specificities than does MFL.

We have previously shown that the formyl peptide binding site maps to several membrane-spanning regions (13–14). Ten residues which affect /MLF binding have been mapped to transmembrane domains II–VII (13), including residues Leu-78 (II-17, helix II, residue 17 of 26 transmembrane domains II–VII (13), including residues Leu-78 (II-17, helix II, residue 17 of 26 transmembrane-spanning residues in the nomenclature used throughout this text), Asp-106 (III-8), Leu-109 (III-11), Thr-157 (IV-18), Arg-201 (V-2), Ile-204 (V-5), Arg-205 (V-6), Trp-254 (VI-16), Tyr-257 (VI-19), and Phe-291 (VII-11). In addition, photo-cross-linking data suggest that the leucine side chain of /MLF is probably located close to FPR $^3$VRK$^6$, which is at the COOH terminus of helix II (14).

Human FPR is one of three receptors in the human FPR family, which includes, FPR, the lipoxin A$_4$ receptor (15–18) (also called FPR1 and recently shown to bind and be activated by serum amyloid A (Ref. 19) and several other peptide ligands (Refs. 5, 20, and 21)), and FPR2. Asp-106 is found in most

* This work was supported by grants from the Rocky Mountain Chapter of the Arthritis Foundation and the National Arthritis Foundation (to J. S. M.), a grant from the National Arthritis Foundation (to H. M. M.), and Public Health Service Grants 2RO1AI0108-4 and 2RO1AI22735-14 (to A. J. J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Microbiology, Montana State University, 109 Lewis Hall, Bozeman, MT 59717. Tel.: 406-994-6506; Fax: 406-994-4926; E-mail: umhj@montana.edu.

‡ The abbreviations used are: FPR, formyl peptide receptor; CHO, Chinese hamster ovary; wt, wild type; HIV, human immunodeficiency virus; GTP-$\gamma$S, guanosine 5’-3-O(thio)triphosphate.

This paper is available on line at http://www.jbc.org
species variants of FPR and FPRL1 receptors sequenced so far (15–16, 22–25). It is also found in FPRL2 of primates (24). Arg-201 and Arg-205 (or their equivalents) and all residues in between \(^{201-205}\) are conserved in most species variants of both FPR and FPRL1. However, the FPRL2 receptors contain FLILH in these positions (24). The mouse does not have a homolog of FPRL2 but instead has six genes related to FPR and FPRL1 (25). Three of these genes (named Fpr1, Fpr-rs1, and Fpr-rs2) conserve residues Asp-106, Arg-201, and Arg-205, while Fpr-rs3, Fpr-rs4, and Fpr-rs5 have His or Asn in place of Arg-201; and a Ser or Asn in place of Arg-205. Thus, Asp-106, Arg-201, and Arg-205 might be important in determining ligand specificity in the FPR family.

Here we show that the selectivity for the binding of different NH\(_2\)-terminal analogs of MMWLL and MLF can be markedly altered by mutating Asp-106 (III-8) or by mutating Arg-201 (V-2). Both D106N and R201A exhibit a very similar change in ligand specificity, including an enhanced affinity to the HIV derived peptide, DP178. In contrast, the mutation R205A (V-2) resulted in altered selectivity at the COOH terminus of fMLF.

**Materials and Methods**

**Peptide Synthesis**—MMWLL and DP-178 (4) (HIV-1-strain B.FR.HXB2, GP-41 residues 643–678) were synthesized by Macromolecular Resources, Fort Collins, CO. fMLF, fMLF-O-methyl, and tert-butoxycarbonyl-MLF were obtained from Sigma. Analogs of MMWLL or MLF were synthesized as follows. Peptide in 95% dimethylformamide, 5% triethylamine (pH 2–4) was added to a 3-fold molar excess of tolyl isocyanate, tolyl isothiocyanate, or formic acid with 3-fold molar excess (9-fold with respect to peptide) of diisopropylcarbodiimide, and incubated at 20 °C for 1 h. fMLF esters were synthesized by incubating the free acid with 9-fold molar excess of diisopropylcarbodiimide in the presence of either 100% butanol or 60% dimethylaminolethanol, 40% dimethylformamide. N-Formyl-MMWLL butyl ester was synthesized by incubating the free acid with 9-fold molar excess of diisopropylcarbodiimide in the presence of 100% butanol. The peptides were separated by high performance liquid chromatography, and the products were identified using matrix-assisted time of flight mass spectrometry.

**Ligand Binding Analysis**—The binding constants of the ligands were determined on intact cells expressing different forms of FPR by analysis of their ability to inhibit binding of f(Nle-Leu-Phe-Nle-Tyr-Lys)-fluorescein as described previously (13). The \(K_b\) was calculated using the previously determined binding constants for the different forms of FPR (wild type FPR, \(K_b = 4 \text{ nM, } 9.9 \times 10^5 \text{ molecules/cell; D106N, } K_b = 110 \text{ nM, } 4.0 \times 10^5 \text{ molecules/cell; R201A, } K_b = 130 \text{ nM, } 4.6 \times 10^5 \text{ molecules/cell; and R205A, } K_b = 50 \text{ nM, } 5.0 \times 10^5 \text{ molecules/cell; Ref. 13).}

**Guanine Nucleotide Binding Analysis**—Stimulation of GTP-S binding by fMMWLL of CHO membranes expressing wt FPR, D106N, R201A, or R205A was determined as described previously (14), using a buffer containing 5 mM HEPES, pH 7.4, 1 mM Mg\(^{2+}\), 3 \(\mu\)M GDP (Buffer A). Effects of Na\(^+\), Li\(^+\), and K\(^+\) on GTP-S binding to CHO cell membranes were determined using a buffer containing 75 mM Tris-Cl, pH 7.4, 12.5 mM Mg\(^{2+}\), 1 mM EDTA, 3 \(\mu\)M GDP (Buffer B) to maintain the conditions previously described by Wenzel-Seifert et al. (26) and Gierisch et al. (27). Incubations were carried out at 30 °C for 10 min, and GTP-S binding never exceeded more than 30% of the total added to assure that binding was in the linear range.

**Chemotaxis Assays**—Chemotaxis assays were done as described previously by Miettinen et al. (28).

**Results and Discussion**

**D106N FPR Mutant Localizes NH\(_2\)-terminal Specificity**—We previously observed that the mutation D106N exhibited modest reductions in affinity to f(Nle-Leu-Phe-Nle-Tyr-Lys)-fluorescein (35-fold) but was completely inactive when tested with fMLF for its ability to stimulate GTP-\(S\) binding (13). Previous analysis of the thyrotropin-releasing hormone receptor, which binds the tripeptide pyrog glutamic acid-histidine-proline-amide, indicated that a tyrosine in the analogous position to Asp-106 of FPR was the likely interaction site with the cyclic amide (between the side chain carboxyl and the amino terminus) at the NH\(_2\) terminus of this tripeptide (29). To determine whether Asp-106 of FPR might likewise be an interaction site with the NH\(_2\) termini of its ligands, we synthesized four NH\(_2\)-terminal analogs of MMWLL, which is active in its free amino form, and two NH\(_2\)-terminal analogs of MLF (the structures of these NH\(_2\)-terminal modifications are shown in Fig. 1) and compared wt FPR’s and D106N’s ability to bind these analogs. Table I shows the effects of NH\(_2\)-terminal modifications of MLF and MMWLL on ligand binding affinity. D106N exhibited markedly reduced affinity for fMLF, N-formyl-MMWLL (fMMWLL), and N-acetyl-MMWLL as compared with wt FPR, but D106N bound tolylsirena-MLF, tolylsirena-MMWLL, tolylsirena-MMWLL, and MMWLL better than did wt FPR. This behavior indicated that Asp-106 is important in NH\(_2\)-terminal selectivity, agreeing with the above mentioned thyrotropin-releasing hormone receptor results (29).

**Roles for Arg-201 and Arg-205 in FPR Function**—Modeling of FPR suggests that both Arg-201 and Arg-205 are located sufficiently close to Asp-106 that either one of them might form an ion pair with Asp-106 (30). A direct interaction between helix III and helix V in FPR would be similar to what has been shown for rhodopsin, where Glu-122 (III-12) in helix III was shown to directly interact with His-211 (V-10) of helix V using Fourier transform infrared spectroscopy (31). Arg-205 (V-6) is at a membrane depth more similar to Asp-106 (III-8) than is Arg-201 (V-2) and was considered the more likely candidate. However, analog analysis on two G protein-coupled receptors, the C5a receptor and the angiotensin I receptor, which have an Arg and Lys in the position analogous to Arg-205 of FPR, respectively, indicated that this position was the likely interaction site between the COOH-terminal carboxylate of these peptide ligands and their respective receptors (32–33). These observations, extended to FPR, suggest that Arg-205 might be the site of interaction between the COOH-terminal carboxylate of MLF with FPR.

**NH\(_2\)-terminal Analogs of MMWLL and MLF Suggest that Arg-201 Ion-Pairs with Asp-106**—We attempted to determine whether Arg-201 or Arg-205 was the more likely ion-pairing partner with Asp-106 by comparing their spectrum of binding of NH\(_2\)-terminally modified analogs of MLF and MMWLL. Table I compares the effects of NH\(_2\)-terminal modifications of MLF and MMWLL on binding to wt FPR, D106N, R201A, and R205A. Formylation of MMWLL produced a 1500-fold enhance-

**Fig. 1. Structures of NH\(_2\)-terminal modifications of MMWLL and MLF.**
Identification of Formyl Binding Site in FPR

TABLE I

|                | fMLF | Tolylisourea MMWLL | Tolylisothiourea MMWLL | fMLF |
|----------------|------|-------------------|-----------------------|------|
| wt             | 0.16 | 10 ± 3            | 490 ± 200             | 38 ± 10|
| D106N          | 29 ± 5| 15.0 ± 0.2        | 2.1 ± 0.1             | 2500 ± 500|
| R201A          | 140 ± 45| 1140 ± 200        | 160 ± 22              | 16,000 ± 1000 |
| R205A          | 36 ± 3 | 108 ± 12          | 9500 ± 1600           | 19,000 ± 1000 |

Fig. 2. Binding interactions. A, Asp-106 and Arg-201 interaction with the NH2 terminus of N-formylated peptides. B, Arg-205 interaction with the COOH terminus of fMLF.

Carboxyl Modifications of fMLF Suggest Arg-205 Interacts with the Carboxyl Terminus of fMLF—Since analog analysis on the C5a receptor and the angiotensin I receptor indicate that position 6 of the fifth transmembrane-spanning region is the likely interaction site between the COOH-terminal carboxylate of these peptide ligands and their respective receptors (30, 31), we attempted to ascertain whether Arg-205 (V-6) of FPR might interact with the COOH terminus of fMLF. We therefore synthesized several COOH-terminal analogs of fMLF and tested wt FPR, D106N, R201A, and R205A for their ability to bind these analogs. Table II shows the effects of COOH-terminal modifications of fMLF on ligand binding affinity. D106N, R201A, and R205A all exhibited markedly reduced affinity for fMLF, with a much greater reduction than was previously observed with fNle-Leu-Phe-Nle-Tyr-Lys-fluorescein (13). This effect was especially true for R205A, which exhibited only a 12-fold reduction of affinity for fNle-Leu-Phe-Nle-Tyr-Lys-fluorescein but a 500-fold reduction for fMLF. Substitution of the free carboxy group of fMLF with a methyl ester enhanced its ability to bind to R205A (25-fold) and to R201A (7-fold). Moreover, substitution of a butyl ester for the free carboxyl group of fMLF improved the binding affinity for R205A to similar to that seen with wt FPR while having only a modest effect on either D106N or R201A. These effects suggest replacement of the hydrophilic, charged, and bulky arginine with the smaller hydrophobic alanine improves the binding of the bulkier more hydrophobic carboxyl substituents on fMLF. Thus, it appears that Arg-205 is the likely site of interaction with the carboxyl
group of fMLF bound to FPR. If the carboxyl group of fMLF interacts with Arg-205 (see Fig. 2B for proposed interaction), one would expect that substituting the negatively charged carboxyl group with a positive charge would markedly affect activity. We therefore synthesized the dimethylaminoethylester of fMLF and tested it for activity. The substitution of a positive charge for the carboxyl group abolished activity for both wt FPR and R205A. This result was expected for wt FPR, as juxtaposition of two positive charges near one another would be expected to destabilize. The fact that R205A is unable to bind the positively charged ester group, and binds the negatively charged carboxylate group very poorly, probably indicates that the COOH terminus of the peptide binds in a relatively hydrophobic environment, which could not stabilize either a positive or a negative charge. Indeed, the 500-fold reduction in affinity of R205A for fMLF compared with wt FPR is similar to what would be expected if fMLF bound to R205A with its COOH group in its un-ionized form (assuming a $pK_a$ of ~4.8, 0.0025% would be unionized at pH 7.4). A hydrophobic environment with a low dielectric constant would be expected to enhance the binding energy between the fMLF COOH-terminal carboxylate and Arg-205 and contribute to high affinity binding of a small peptide.

The butyl ester of fMMWLL did not exhibit enhanced binding to R205A, indicating that enhanced binding was observed when the butyl group was added to the third but not the fifth residue.

D106N and R201A Bind a Peptide Derived from HIV (DP178) More Tightly than Does wt FPR—Recently, a peptide derived from the second heptad repeat region from HIV was shown to activate FPR (5). Since this peptide, like MMWLL, is active without having a formyl group on its NH$_2$ terminus (5, 12), our mutant analysis might suggest a mode of binding for this very different peptide. Therefore, we tested the ability of the NH$_2$COOH terminally acetylated and NH$_2$-terminally amidated form of this peptide to bind wt FPR, D106N, and R201A. DP178 bound to wt FPR, D106N, and R201A with $K_d$ values of 1200, 30, and 600 nM, respectively, indicating that D106N bound DP178 30-fold more tightly than wt FPR. In addition, R201A bound DP178 twice as tightly as wt FPR. This binding behavior is similar to that observed with MMWLL with relative affinity increases of 40- and 3-fold, respectively, for these two mutants. This is much different than that seen with acetyl-MMWLL where wt FPR bound acetyl-MMWLL 40- and 500-fold more tightly than did D106N or R201A, respectively. This would indicate that the acetyl group on DP178 is situated away from the Asp-106–Arg-201 ion pair in wt FPR. DP178, despite its much larger size compared with MMWLL, nonetheless appears to bind to FPR in a manner similar to MMWLL.

Consequences of FPR Mutation to FPR Function—Since our previous studies had indicated that 1 gM fMLF was unable to stimulate GTP$_\gamma$S binding in the mutants D106N, R201A, or R205A, we examined them for their ability to be stimulated by 1 gM MMWLL, which has 80–500-fold higher affinity for these mutants (see Table I for respective binding constants). The results are shown in Fig. 3. R201A and R205A, unlike with fMLF (13), were stimulated to similar extents as wt FPR by fMMWLL. D106N, however, remained unresponsive. Interestingly, we also found that 1 gM tertbutyloxycarbonyl-MLF, which is a known antagonist for FPR (8), stimulated R201A to a similar extent as was observed with 1 gM MMWLL (data not shown), again suggesting altered NH$_2$-terminal specificity. 1 gM tertbutyloxycarbonyl-MLF did not stimulate GTP$_\gamma$S binding in CHO cells expressing wt FPR (data not shown).

Other studies have indicated that FPR exhibits a high level of constitutive activity that can be inhibited by Na$^+$ and to a lesser degree by $K^+$ (26–27). It is believed that Na$^+$ reduces FPR’s affinity for fMLF by reducing the number of high affinity binding sites. This effect is cation-specific with NaCl > LiCl > KCl > choline chloride (27), suggesting that the ion pairing implied by our experiments might play a role in this inhibition.

Therefore, we tested GTP$_\gamma$S binding in CHO cells expressing wt FPR, D106N, R201A, and R205A, and compared their activity to that observed in CHO cells that did not express FPR. The results are shown in Fig. 4. wt FPR and the three mutants all exhibited high levels of constitutive activity, 5–8-fold greater GTP$_\gamma$S binding than that seen with non-expressing CHO cell membranes. The constitutive activity of wt FPR, R201A, and R205A were readily inhibited by physiologic levels of Na$^+$ ($K_{50}$ of 50, 120, and 110 mM, respectively), whereas the $K_{50}$ for D106N was > 1000 mM. In addition, wt FPR exhibited cation selectivity of Na$^+$ > Li$^+$ > K$^+$ (80%, 63%, and 48% inhibition at 100 mM, respectively), whereas all three cations inhibited D106N similarly (~50% inhibition at 100 mM for all three cations.) This result indicates that Asp-106, in addition to being important in NH$_2$-terminal selectivity, may also be important for Na$^+$ binding. Na$^+$ competition with Arg-201 for interaction with Asp-106 might explain the Na$^+$ regulation of ligand affinity observed by Giercke et al. (27). However, we were unable to detect any Na$^+$-dependent changes in fNle-Leu-Phe-Nle-Tyr-Lys-fluorescein binding to CHO cells expressing wt FPR. No difference in binding was observed in the absence of Na$^+$, as compared with that seen in the presence of 100 mM Na$^+$, nor was there any difference in binding between 100 mM Na$^+$, 100 mM Li$^+$, K$^+$, or choline as reported previously by

**Table II**

|          | fMLF-OH | fMLF-O-CH$_3$ | fMLF-O-butyl | fMLF-O-CH$_2$CH$_2$NH$^+$ (CH$_3$)$_2$ | fMMWLL | fMMWLL-O-butyl |
|----------|---------|---------------|--------------|--------------------------------------|--------|----------------|
| wt       | 37 ± 10 | 170 ± 20      | 136 ± 50     | No effect at 10000                    | 0.16 ± 0.04 | 2.2 ± 0.8 |
| D106N    | 2500 ± 500 | 3800 ± 400   | 1100 ± 400   | Not determined                       | 29 ± 5     | 390 ± 70       |
| R201A    | 16,000 ± 1000 | 2400 ± 450   | 2100 ± 450   | Not determined                       | 140 ± 46   | 2150 ± 250     |
| R205A    | 19,000 ± 1000 | 730 ± 250    | 180 ± 70     | No effect at 10,000                  | 36 ± 3     | 80 ± 20        |

**Stimulation of GTP$_\gamma$S binding by 1 gM MMWLL**

![Graph showing GTP$_\gamma$S binding]
D106N exhibited a 4-fold increase (high degree of “random migration” in the absence of ligand).

We previously developed a model for constitutive activity.

Gierchek et al. (27). This result may reflect a difference between cation regulation of binding to intact cells versus that seen using membrane preparations (27).

We also analyzed D106N for its ability to mediate chemotaxis toward either fMLF or MMWLL. Fig. 5 shows that D106N exhibited no chemotaxis toward fMLF, whereas MMWLL stimulated chemotaxis ~5-fold at 100 nM. D106N also exhibited a high degree of “random migration” in the absence of ligand.

In rhodopsin, an ion pair exists between Glu-113 (III-3) and the Schiff base of retinal in rhodopsin, marked by the presence of charged residues or 50° rotation for Glu-122 or 400° rotation versus 5 residues for Glu-113 and 50° for Glu-113. This ion pair is in close proximity to the Schiff base of retinal and the fact that mutation of Phe-291 (VII-11), which is analogous to the lysine in rhodopsin, markedly affects binding (13).

In addition, our previous cross-linking data suggest that the Schiff base of retinal in rhodopsin interacts with His-209 (III-12) of rhodopsin via a water molecule bound to the retinal Schiff base (35–38). This ion pair is also known to be important in retinal binding (13); and 3) a photoaffinity label of fMLF binding to FPR (28) based on: 1) the structural similarity of G protein-coupled receptors (38), that Asp-106 and Arg-205 are sufficiently close to ion-pair with one another; the side chain of Asp is ~1 Å longer than Thr and Arg ~1.5 Å longer than Phe, so that the Asp-Arg distance would be expected to be ~3.5 Å. In the crystal structure of rhodopsin, Phe-203 and Met-207, which are analogous to Arg-201 and Arg-205, respectively, are within 4 Å of one another. Our mutagenesis data suggest that Arg-201 and Arg-205 interact with the formyl group and the COOH terminus of fMLF, respectively. The expected distance between the formyl group and the carboxyl group is, of course, dependent on the peptide conformation. In a helical conformation, the carboxylate of fMLF would be hydrogen-bonded to the formamide nitrogen and the nitrogen to oxygen distance would be ~3 Å. If fMLF bound in an extended conformation, the NH2 and COOH termini could be up to 10 Å apart. Other conformations would be intermediate in separation distance. Thus it seems likely that Arg-201 and Arg-205 are positioned sufficiently close to one another to be able to interact with the formyl group and COOH terminus of fMLF, respectively.

In addition, our previous cross-linking data suggest that the leucine side chain of fMLF is probably located close to FPR R201A VRK95, which is at the COOH terminus of helix II (14).

Therefore, the most likely positioning of fMLF in the binding pocket of FPR is as follows.

1) The backbone of fMLF is approximately parallel to helix V with the formamide oxygen hydrogen-bonded to one of the guanidine nitrogens of Arg-201. One of the Asp-106 oxygen hydrogen-bonds with the formamide nitrogen, and the other carboxylate oxygen interacts with one of the guanidine nitrogens of Arg-201 (see Fig. 2A).

2) The COOH-terminal carboxylate of fMLF is ion-paired with Arg-205 (see Fig. 2B).

3) The leucine side chain is positioned toward helix II.

This new placement of ligand parallel rather than perpendicular to the average helical axes of FPR does not rule out the possibility of remote global effects of the mutations or, for that matter, the existence of more than one position of the ligand. Clearly, the newly discovered promiscuity of FPR for MMWLL and DP-178 suggests the existence of multiple binding locations, one of which might still be compatible with our original retinal in rhodopsin-like placement of fMLF. Clearly, MMWLL, Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein, and other chemotactic peptides that are longer than three amino acids
Identification of Formyl Binding Site in FPR

would not have a free carboxyl group in position three, and might take on a kinked conformation which might enable it to take advantage of the hydrophobic nature of the space between helices II and VII (30).

This positioning of MLF in the binding pocket of FPR is similar to the proposed positioning of the tripeptide pyroglutamic acid-histidine-proline-amide (TRH) when bound to the TRH receptor (39) where the NH₂ terminus was proposed to interact with a tyrosine in the analogous position to Asp-106 (Tyr-106) and the COOH terminus was buried within the receptor. The proposed site for binding the COOH terminus of TRH, Arg-306 (VII-7), is different from what we propose for MLF. Interestingly, the TRH receptor exhibits a marked preference for the COOH-terminal amide (the free acid is a metabolic breakdown product, which exhibits very low activity (Ref. 40)), and this receptor has an aspartic acid (Asp-195) in the carboxyl terminus of MLF. The effects of Asp-195 on TRH binding, however, have not been reported. Thus, there may be important motifs in ligand binding of peptides, and knowledge of these motifs may provide important insights to the structure of the ligand binding pockets of G protein coupled receptors.

The FPRL1 receptor is known to bind /MLF (16), albeit weakly, whereas no binding at all is observed with FPRL2 (17). The mouse formyl peptide receptor (Fpr1) also binds weakly, whereas no binding at all is observed with FPRL2 (17). Our studies with Asp-106, Arg-201, and Arg-205 indicate that FPRL1, Fpr-rs1, and Fpr-rs2 might be expected to bind formylated peptides as well. Recently, Klein et al. (41), identified several peptides, including MMWLL, which could activate FPRL1. Formylation of either MMWLL, which exhibited >200-fold preference for FPR over FPRL1, or SLLWLT-CRPWEM, which exhibited a >500-fold preference for FPRL1 over FPR, enhanced the binding to both receptors, although the effect of formylation was much greater for FPR than FPRL1. This would indicate that, although Asp-106 and Arg-201 are necessary for N-formyl specificity, other residues found only in FPR might be involved in maximizing formyl group binding. Studies are presently under way to identify such groups.

REFERENCES

1. Schiffman, E., Corcoran, B., and Wahl, S. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1059–1062
2. Maraseo, W. A., Phan, S. H., Krutzsch, H., Showell, H. J., Feltner, D. E., Nairn, R., Becker, E. L., and Ward, P. A. (1984) J. Biol. Chem. 259, 5430–5436
3. Broom, M. F., Mellor, D. M., and Chadwick, V. S. (1989) J. Immunol. 142, 1333–1334
4. Su, S. B., Gong, W., Gao, J., Shen, W., Murphy, P. M., Oppenheim, J. J., and Wang, J. M. (1999) J. Exp. Med. 189, 395–402
5. Le, Y., Gong, W., Li, B., Dunlop, N. M., Shen, W., Su, S. B., Ye, R. D., and Wang, J. M. (1999) J. Immunol. 163, 6777–6784
6. Dahlgren, C., Christophe, T., Boulay, F., Madianos, P. N., Rabiet, M. J., and Karlsson, A. (2000) Blood 95, 1810–1818
7. Ye, R. D., Quehenberger, O., Thomas, K. M., Navarro, J., Cavanagh, S. L., and Prossnitz, E. R. (1999) J. Immunol. 163, 1383–1394
8. Gao, J., and Murphy, P. M. (1997) J. Biol. Chem. 272, 24181–24189
9. Wenzel-Seifert, K., Hurt, C. M., and Seifert, R. (1998) J. Biol. Chem. 273, 24181–24189
10. Gierschik, P., Sidiropolous, D., Steissinger, M., and Jakobs, K. H. (1989) Eur. J. Pharmacol. 172, 481–492
11. Miettinen, H. M., Quehenberger, O., and Jesaitis, A. J. (1998) J. Cell Sci. 111, 1921–1928
12. Pearlman, J. H., Thaw, C. N., Laakkonen, L., Bowers, C. Y., Osman, R., and Gershengorn, M. C. (1984) J. Biol. Chem. 269, 1610–1613
13. Mills, J. S., Miettinen, H. M., Vlases, M. J., and Jesaitis, A. J. (1999) in Molecular and Cellular Basis of Inflammation (Serhan, C. N., and Ward, P. A., eds) Humana Press, Inc., Totowa, NJ, pp. 215–245
14. Beck, M., Sakmar, T. P., and Siebert, F. (1998) /Biochemistry 37, 7630–7639
15. Demartino, J. A., Konteatis, Z. D., Siciliano, S. J., Van Riper, G. V., Underwood, D. J., Fischer, P. A., and Springer, M. S. (1995) J. Biol. Chem. 270, 15866–15869
16. Noda, K., Saad, Y., Kinoshita, A., Boyle, T. P., Graham, R. M., Ahsan, H., and Karnik, S. S. (1995) J. Biol. Chem. 270, 2284–2289
17. Belleau, B., Lajoie, G., Sauve, G., Rao, V. S., and di Paola, A. (1989) Int. J. Immunopharmacol. 11, 467–471
18. Weitz, C., and Nathans, J. (1993) Biochemistry 32, 14176–14182
19. Eilers, M., Reeves, P. J., Ying, W., Krishna, G. H., and Smith, S. O. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 487–492
20. Han, B., and Tashjian, A. H. (1995) Biochemistry 34, 13412–13422
21. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., and Miyano, M. (2000) Science 289, 739–745
22. Laakkonen, L. J., Guarnieri, F., Perlman, J. H., Gershengorn, M. C., and Osman, R. (1996) Biochemistry 35, 7651–7663
23. Hedin, H., Hedin, T., Wessberg, P., Lundberg, D., and Jonasson, J. (1983) Acta Physiol. Scand. 119, 427–437
24. Klein, C., Paul, J. I., Sauve, K., Schmidt, M. M., Arcangeli, L., Ransom, J., Trueheart, J., Manfredi, J. P., Bronch, J. A., and Murphy, P. A. (1998) Nat. Biotechnol. 16, 1334–1337