Rat and human neutrophil *N*-formyl-peptide chemotactic receptors

Species difference in the glycosylation of similar 35–38 kDa polypeptide cores

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Rat and human neutrophil *N*-formyl-peptide chemotactic receptors were subjected to glycosidase and proteinase treatments to determine the extent and species differences of glycosylation and the carbohydrate requirement in the high-affinity ligand binding. *N*-Formyl-Nle-Leu-Phe-Nle-¹²⁵I-Tyr-Lys was attached to rat and human neutrophils either before or after glycosidase and proteinase treatments, and the labelled receptors were solubilized after glutaraldehyde cross-linking and analysed by SDS/PAGE and autoradiography. Both the rat and human *N*-formyl-peptide chemotactic receptors contain only *N*-linked oligosaccharides, as demonstrated by their sensitivity to peptide *N*-glycosidase F (PNGase F) and resistance to *O*-glycanase treatment. The *N*-linked oligosaccharides seem to be of the complex type rather than the high-mannose or hybrid type and lack terminal sialic acid, as demonstrated by their resistance to endoglycosidases D and H and neuraminidase treatments. This sensitivity pattern was similar in both species, and the shift in the molecular size of the receptors to 35–38 kDa after PNGase F treatment occurred through one intermediate product, suggesting that both receptors contain a similar 35–38 kDa polypeptide core with two *N*-linked complex-type oligosaccharides, the heterogeneity of which is responsible for the species difference in receptor size. Papain treatment alone or followed by PNGase F produced in both species a 33–36 kDa membrane-bound fragment that was still able to bind the ligand, suggesting that the oligosaccharides are located on the approx. 2 kDa papain-cleavable polypeptide fragment of the receptors. The cleavage sites for both papain and PNGase F were hidden in occupied receptors, suggesting a conformational or topographical change in these upon ligand binding. Scatchard analyses and cross-linking experiments demonstrated that carbohydrates are not required for high-affinity ligand binding and that the 33–36 kDa membrane-bound papain fragment of both receptors contains the ligand-binding site.

INTRODUCTION

The *N*-formyl-peptide chemotactic receptor that has been demonstrated for several inflammatory cell types [1–4] mediates chemotaxis [1], increased adherence to endothelial cells [5], lysosomal enzyme release [6] and superoxide production [7] in neutrophils, thus playing a major role in the defence mechanisms of the host against acute inflammation [8]. The receptor has been well characterized in terms of its biochemical properties using radiolabelled synthetic analogues [9–11] of the *N*-formyl-peptide products of bacterial metabolism [12–14]. Although its primary structure in humans has recently been resolved [15], the structural and functional relationships of the receptor are not yet fully understood. We have demonstrated (J. J. Remes, K. P. Keinänen, U. E. Petäjä-Repo & H. J. Rajaniemi, unpublished work) that the rat *N*-formyl-peptide chemotactic receptor (82–88 kDa) is about 23 kDa larger than the human one (59–65 kDa). Characterization of the biochemical basis for this difference may provide an insight into those domains which are conserved and therefore likely to be essential for the functioning of these receptors. Observations implying that the human receptor is glycosylated [16] prompted us to search for possible differences in the glycosylation of these receptors and the effects of glycan moieties on ligand-binding properties by employing a series of deglycosylating enzymes and a well-established glutaraldehyde cross-linking technique [17] to achieve covalent radiolabelling of the receptor. In addition, papain was used to compare the polypeptide cores of the receptors and to locate the glycosylation and ligand-binding sites within the polypeptide core. Our results

provide a molecular explanation for the size difference between the rat and human *N*-formyl-peptide chemotactic receptors.

MATERIALS AND METHODS

Materials

N-Formyl-Nle-Leu-Phe-Nle-Tyr-Lys (hexapeptide), BSA, dextran T-500, phenylmethanesulphonyl fluoride (PMSF), papain and high-molecular-mass standards for SDS/PAGE were products of Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bio-Gel P-2 and equipment and reagents for electrophoresis were obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Peptide *N*-glycosidase F (PNGase F; EC 3.2.2.18), endo- α -*N*-acetylgalactosaminidase (*O*-glycanase; EC 3.2.1.97), endo- β -*N*-acetylglucosaminidase D (endoglycosidase D; EC 3.2.1.96), endo- β -*N*-acetylglucosaminidase H (endoglycosidase H; EC 3.2.1.96) and dithiothreitol (DTT) were from Boehringer-Mannheim Biochemica (Mannheim, Germany). Trypsin and Brewer thioglycolate medium (dehydrated) were purchased from Difco Laboratories (Detroit, MI, U.S.A.), and NaN₃, dimethyl sulphoxide (DMSO) and EDTA were from Merck (Darmstadt, Germany). Na¹²⁵I (carrier-free; 5 mCi) and neuraminidase (from *Vibrio cholerae*) were products of Amersham International (Amersham, Bucks., U.K.) and Calbiochem (San Diego, CA, U.S.A.) respectively. Glass microfibre GF/C filters were from Whatman International (Maidstone, Kent, U.K.), and glutaraldehyde was from Leiras (Turku, Finland). All the other reagents were of analytical grade and were purchased from commercial sources.

Abbreviations used: PMSF, phenylmethanesulphonyl fluoride; PNGase F, peptide *N*-glycosidase F; DTT, dithiothreitol; DMSO, dimethyl sulphoxide; PBS, phosphate-buffered saline.

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The hexapeptide was diluted to 1 mM with DMSO and was stored at -20°C until use. Glutaraldehyde was diluted to a 10% stock solution with distilled water and kept at 22°C until used.

Neutrophil isolation

Sprague–Dawley rats (3 months old) were injected intraperitoneally with 3 ml of sterile 8% (w/v) thioglycolate solution in distilled water. After 24 h the animals were killed in CO_2 gas, and the peritoneal cavities were opened and the exudates washed out with 10 ml of 0.9% NaCl containing 1 unit of heparin/ml. The exudates were carefully layered on to 3 ml of Lymphoprep (Nycomed, Oslo, Norway) and centrifuged at 1000 *g* for 20 min at 4°C . Thereafter, the supernatant containing the monocytes was removed and the neutrophil pellet was suspended in 10 ml of 10 mM-phosphate buffer, pH 7.4, containing 140 mM-NaCl (PBS), and repelleted at 200 *g* for 10 min at 4°C . The wash was repeated once and the final pellet was suspended in the appropriate buffer for the assays. The cells from at least two rats were pooled for each experiment.

Human neutrophils were isolated from the blood of healthy volunteers by a modification of the method of Boyum [18]. Venous blood (25 ml) was taken in a heparinized syringe and mixed with 25 ml of PBS containing 2% dextran. The erythrocytes were allowed to sediment at 37°C in a 5% CO_2 atmosphere for 30 min, and then 7 ml of the supernatant was aspirated and layered on to 3 ml of Lymphoprep and centrifuged at 1000 *g* for 20 min at 4°C . The contaminating erythrocytes were lysed by adding 1 ml of 0.21% NaCl solution and vortex-mixing for 50 s, and then adding another 1 ml of 1.5% NaCl solution. The cell suspension was pelleted at 200 *g* for 10 min, and the procedure was repeated once. The resulting neutrophil pellet was suspended in the appropriate buffer, pelleted as above, and the wash was repeated.

Radioiodination of the hexapeptide

Radioiodination of the hexapeptide was performed by the method of Niedel *et al.* [9]. Briefly, 1 mM-hexapeptide in DMSO was diluted to 0.1 mM with methanol. Then 10 μl of 0.1 mM-hexapeptide, 2 mCi of carrier-free ^{125}I and 10 μl of 1.0 mM-chloramine-T (diluted in distilled water just before use) were added to 100 μl of 250 mM-sodium phosphate, pH 7.6. The reaction was allowed to proceed for 10 min at 22°C with constant vigorous stirring. The reaction was stopped by adding 100 μl of 20% glycerol in distilled water. Free ^{125}I was separated out by fractionating the mixture on a Bio-Gel P-2 column (1.4 cm $\times$ 15.5 cm) equilibrated and eluted with 25 mM-NaOH. The fractions were counted for ^{125}I radioactivity, and those containing the highest amounts of ^{125}I -hexapeptide were pooled. The pool was neutralized with 1 M-HCl, and a part of it was diluted to 1 mM-hexapeptide with 15 mM-sodium phosphate buffer, pH 6.75, containing 123 mM-NaCl, 1 mM- CaCl_2 and 0.1% BSA and stored at -20°C . The specific radioactivity of the iodinated peptide, determined by the self-displacement assay [19], was 800 Ci/mmol. The binding capacity of labelled hexapeptide to an excess of isolated neutrophils varied in the range 47–58%, and this was taken into account when calculating the binding data.

Enzymic treatments of the receptor

Neutrophils (1×10^6) for the glycosidase treatments were suspended in a final volume of 200 μl of 0.1 M-sodium phosphate, pH 7.4 (adjusted to pH 5.5 for endoglycosidase D and H treatments), containing 10 mM- NaN_3 , 10 mM-EDTA and 1 mM-PMSF. Next, a further 30 μl of PNGase F (6 units), 30 μl of neuraminidase (30 munits), 30 μl of neuraminidase plus 20 μl of *O*-glycanase together (10 munits), 20 μl of endoglycosidase D

(20 munits), 40 μl of endoglycosidase H (40 munits) or buffer alone (control) was added, and the mixtures were incubated at 30°C for the periods of time indicated. After incubation, the neutrophils were diluted in 1 ml of ice-cold 15 mM-sodium phosphate buffer, pH 6.75, containing 123 mM-NaCl, 1 mM- CaCl_2 , 10 mM-EDTA, 10 mM- NaN_3 and 0.1% BSA (termed the binding assay buffer), and pelleted at 3000 *g* for 10 min at 4°C . The wash was repeated once, and the neutrophil pellet was subjected to the ^{125}I -hexapeptide binding assay as described below.

For the papain and trypsin digestions, neutrophils (1×10^6) were suspended in a final volume of 200 μl of 0.1 M-sodium phosphate, pH 6.75, containing 10 mM- NaN_3 and 1 mM-DTT. Papain or trypsin (7 units) was added and the mixtures were incubated in a water bath with constant stirring at 30°C for 15 or 30 min. The cells were washed with 1 ml of ice-cold binding assay buffer as described above and subjected to ^{125}I -hexapeptide binding assays.

Deglycosylation of the cell-bound receptor after papain treatment was performed as described above, except that the incubation time with papain was 15 min at 30°C and the neutrophils were washed with 1 ml of the ice-cold buffer appropriate for PNGase F as described above. This was followed by a 4 h PNGase F digestion at 30°C as described.

After the enzyme treatments, the neutrophils were suspended in 400 μl of binding assay buffer containing 250 fmol of ^{125}I -hexapeptide, and incubated for 30 min at 22°C . The suspension was centrifuged at 3000 *g* for 10 min at 4°C . The cell pellet was suspended in 1 ml of ice-cold PBS and repelleted as above. The wash was repeated once, and the final pellet was subjected to cross-linking.

Alternatively, PNGase F (4 h) or papain (15 min) treatments were performed after the binding of ^{125}I -hexapeptide to the neutrophils in some experiments. ^{125}I -Hexapeptide (250 fmol) and neutrophils (1×10^6) were incubated for 30 min at 22°C in a final volume of 200 μl of the appropriate buffer, followed by centrifugation at 3000 *g* for 10 min at 4°C . The pellet was suspended in 1 ml of the ice-cold buffer appropriate for the enzyme and repelleted. After the enzyme treatments the cells were subjected to cross-linking as described below.

Cross-linking of ^{125}I -hexapeptide to the receptor

Labelled and washed neutrophils were suspended in 1 ml of PBS containing 2 mM-glutaraldehyde, and the mixture was incubated for 60 min at 22°C . The reaction was stopped by adding 130 μl of 1 M-Tris/HCl, pH 7.4, at 4°C and centrifuging at 3000 *g* for 10 min at 4°C . The cell pellet was suspended in 0.5 ml of 50% SDS sample buffer (pH 6.8) containing 2% SDS, 125 mM-Tris/HCl, 10% glycerol and 0.001% Bromophenol Blue, vortex-mixed thoroughly and heated for 2 min at 95°C in the Eppendorf Thermostat 5320 heater. The suspension was centrifuged at 8000 *g* for 30 min at 4°C , and the supernatant was dialysed against 500 ml of 10% SDS sample buffer overnight at 4°C and subjected to SDS/PAGE and autoradiography.

SDS/polyacrylamide-gel electrophoresis

The electrophoresis was performed according to Laemmli [20], using 10% acrylamide monomer in the separating gel and 4% acrylamide in the stacking gel. The samples were concentrated to one-tenth of their volume (50 μl) by vacuum centrifugation in a Savant Speedvac apparatus. Before electrophoresis, the samples were diluted with SDS sample buffer to obtain the same amount of radioactivity in each well and boiled for 2 min at 95°C in an Eppendorf heater. Following electrophoresis (200 V, 45 min), the gels were stained with Coomassie Brilliant Blue [21], de-

Fig. 1. SDS/PAGE analysis of ^{125}I -hexapeptide-labelled rat (a) and human (b) *N*-formyl-peptide chemotactic receptors after neuraminidase and PNGase F treatment of the neutrophils

Neutrophils (1×10^6) were incubated without (lanes 1 and 2) or with 30 units of neuraminidase (lanes 3–5) or 6 units of PNGase F (lanes 6–8) in a final volume of $200 \mu\text{l}$ at 4°C (lane 1) or 30°C (lane 2–8) for the periods of time indicated. ^{125}I -Hexapeptide was attached to the receptors, and the receptor–ligand complexes were cross-linked, solubilized and analysed by SDS/PAGE as described in the Materials and methods section.

stained, dried and exposed for autoradiography on Kodak XAR-5 film for 7 days at -80°C . The mass standards were: myosin heavy chain, 205 kDa; β -galactosidase, 116 kDa; phosphorylase b, 94 kDa; BSA, 68 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 29 kDa. The apparent molecular masses of the labelled bands were determined from a standard curve based on the mobility of molecular mass markers co-electrophoresed with the samples.

Measurement of ^{125}I -hexapeptide binding to papain-treated neutrophils

For Scatchard [22] analyses, neutrophils (1×10^6 cells/ $200 \mu\text{l}$) were incubated alone or with 10 mM- NaN_3 or 7 units of papain plus 10 mM- NaN_3 for 15 min at 30°C , followed by washing the cells twice with 1 ml of binding assay buffer as described above. The treated cells (4×10^5) were incubated in $200 \mu\text{l}$ of the binding assay buffer in the presence of 10 mM- NaN_3 with an increasing amount of ^{125}I -hexapeptide for 30 min at 22°C . The binding was stopped by placing the test tubes on ice followed by rapid filtration of the cell suspension through a presoaked Whatman GF/C filter under a strong vacuum. The filter spots were washed twice with $400 \mu\text{l}$ of ice-cold binding assay buffer. The filter was allowed to dry in air and the spots were cut out and counted for radioactivity with a LKB Wallac 1280 Ultragamma counter. Non-specific binding was defined as the amount of ^{125}I -hexapeptide bound in the presence of a 1000-molar excess of unlabelled hexapeptide, and was always less than 10% of the total binding. Specific binding throughout this paper refers to total binding minus non-specific binding. All assays were performed using duplicate samples, and the intra-assay variation was always less than 5%.

RESULTS

Deglycosylation of the cell-bound receptor

The ^{125}I -hexapeptide labelled similar-sized polypeptides on the rat (82–88 kDa) and human (59–65 kDa) neutrophils as previously reported and shown to bind this ligand in a saturable manner [9]. Neuraminidase treatment had no detectable effect on the molecular size of the receptors, suggesting that their carbohydrates do not carry terminal sialic acids (Fig. 1). The presence of two *N*-linked carbohydrate side-chains in both receptors was demonstrated by PNGase F and *O*-glycanase treatments. The size of both receptors was decreased to 35–38 kDa by PNGase F treatment (Fig. 1), whereas *O*-glycanase treatment together with neuraminidase did not produce any change in the electrophoretic

Fig. 2. SDS/PAGE analysis of ^{125}I -hexapeptide-labelled rat and human *N*-formyl-peptide chemotactic receptors after neuraminidase and *O*-glycanase treatment of the neutrophils

mobility of the receptors (Fig. 2). Subtle changes cannot be ruled out, however. Longer incubation times did not produce any smaller-sized species, suggesting that deglycosylation had proceeded to completion in the 35–38 kDa product in both species. The control incubations at either 4°C or 30°C did not cause any change in the size of the receptors on SDS/PAGE, thus excluding any proteolytic receptor cleavage under the present conditions.

Both receptors also appeared to be resistant to endoglycosidase D and endoglycosidase H treatments (results not shown) after 4 h incubations at 30°C , suggesting the absence of significant amounts of mannose-rich *N*-linked carbohydrates.

Both receptors also appeared to be resistant to endoglycosidase D and endoglycosidase H treatments (results not shown) after 4 h incubations at 30°C , suggesting the absence of significant amounts of mannose-rich *N*-linked carbohydrates.

Effect of proteinases on the structure of the receptor

Proteinase treatments were carried out to compare the polypeptide cores of the receptors and to locate the glycosylation and ligand-binding sites. The enzyme treatments and ligand binding were carried out in the presence of NaN_3 to prevent internalization and degradation of the receptor–ligand complexes. Papain treatment of the neutrophils produced a 33–36 kDa membrane-bound fragment from both rat and human receptors. As this fragment was affinity-labelled, it must have contained the ligand-binding site. Trypsin treatments, on the other hand, had no effect on the electrophoretic mobility of the receptors (Fig. 3). Doubling of the incubation time from 15 to 30 min did not produce smaller fragments capable of ligand binding, and similar treatment with

Fig. 3. SDS/PAGE analysis of the ^{125}I -hexapeptide-labelled rat (a) and human (b) *N*-formyl-peptide chemotactic receptors after proteinase treatment of the neutrophils

Neutrophils (1×10^6) were incubated with 7 units of papain (lanes 3 and 4) or trypsin (lanes 5 and 6) at 30 °C for the periods of time indicated. The receptors were analysed as described in the legend to Fig. 1. Controls were incubated without enzymes at 4 °C (lane 1) or 30 °C (lane 2).

Fig. 4. SDS/PAGE analysis of ^{125}I -hexapeptide-labelled rat (a) and human (b) *N*-formyl-peptide chemotactic receptors after sequential treatment of the neutrophils with papain and PNGase F

Neutrophils (1×10^6) were treated with 6 units of PNGase F (lane 3), 7 units of papain (lane 4) or 7 units of papain followed by 6 units of PNGase F (lane 5). The incubations were performed at 30 °C for the periods of time indicated, and the receptors were analysed as described in the legend to Fig. 1. Controls were incubated without enzymes at 4 °C (lane 1) or 30 °C (lane 2).

Fig. 5. Ability of papain and PNGase F to digest *N*-formyl-peptide chemotactic receptor- ^{125}I -hexapeptide complexes of rat (a) and human (b) neutrophils

Neutrophils (1×10^6) were labelled with ^{125}I -hexapeptide, washed carefully and subjected to PNGase F (6 units; lane 2) or papain (7 units; lane 3) treatment at 30 °C for the periods of time indicated. The receptor-ligand complexes were cross-linked, solubilized and analysed as described in the Materials and methods section. Controls were incubated without enzymes at 4 °C (lane 1) or 30 °C (lane 2).

trypsin was without effect (Fig. 3). To disclose whether the papain-cleavable 2 kDa polypeptide fragment contains all the glycosylation sites, the papain-pretreated neutrophils were further subjected to PNGase F treatment. Fig. 4 shows that the treatment caused a slight decrease in the size of the 33–36 kDa fragment. The result is, however, not repeatable, and may in this

particular experiment be due to a slight twisting of the SDS/PAGE run.

Interestingly, subsection of neutrophils bearing occupied receptors to papain or PNGase F treatments had no effect on the size of the receptor-ligand complexes (Fig. 5).

Table 1. Binding of ^{125}I -hexapeptide to *N*-formyl-peptide chemotactic receptors after papain treatment of rat and human neutrophils

Neutrophils (1×10^6) were incubated in the absence (control) or the presence of 10 mM- NaN_3 alone or together with 7 units of papain. After washing, the cells were incubated with an increasing amount of ^{125}I -hexapeptide for 30 min at 22 °C, and the free and receptor-bound ligand pools were separated by filtration. Equilibrium dissociation constants (K_d) and the numbers of receptors/cell were derived from Scatchard plots [22]. The data are presented as means $\pm$ S.E.M. of three independent assays.

	Rat		Human	
	K_d (nM)	Receptor number/cell	K_d (nM)	Receptor number/cell
Control	0.41 ± 0.44	$67\,000 \pm 4000$	0.32 ± 0.18	$97\,000 \pm 5400$
NaN_3	0.42 ± 0.34	$60\,000 \pm 3500$	0.23 ± 0.09	$83\,000 \pm 2700$
NaN_3 + papain	0.33 ± 0.71	$10\,000 \pm 2100$	0.17 ± 0.07	$52\,000 \pm 2200$

Effect of deglycosylation on the ligand-binding properties of the receptor

The experiments described above demonstrated that the asparagine-linked oligosaccharides represent an essential part of the receptor molecules (57% in rat and 41% in human). The carbohydrate moieties can be cleaved with papain together with a 2 kDa peptide core without disappearance of the ligand-binding activity of the 33–36 kDa fragment remaining on the membrane. This notion was utilized to evaluate the role of the small peptide core and carbohydrates in high-affinity ligand binding. These experiments were all performed in the presence of NaN_3 to prevent both internalization and externalization. Table 1 shows that NaN_3 itself did not affect the binding affinities of the receptors, although their number was decreased by about 10% in the rat and 14% in the human material. These may in fact represent the portions of the receptors that are situated within the cells [23,24]. Similarly, papain did not affect the ligand-binding affinities of the receptors, but the number of receptors was decreased by about 85% in the rat and 46% in the human. Thus the 33–36 kDa papain fragment of both receptors binds the ligand with the same affinity as the intact receptor, indicating that glycan moieties are not involved in high-affinity ligand binding.

DISCUSSION

Plasma membrane receptors for hormones and growth factors have been shown to be glycoproteins [25–27]. Among these, the *N*-formyl-peptide chemotactic receptor on human neutrophils represents a 59–65 kDa glycoprotein with *N*-linked complex-type carbohydrates attached to a polypeptide core of about 35–38 kDa. We also demonstrate here that the size difference between the rat 82–88 kDa and human 59–65 kDa receptors is most likely due to post-translational modifications on similar-sized polypeptide backbones. The size of the oligosaccharides, the type of glycosidic linkage and the location of the glycosylation sites within the polypeptide core were demonstrated by cleavage of the receptors by glycosidases of different specificities and by proteinases. The presence of NaN_3 in all incubations to prevent both internalization and degradation of receptor– ^{125}I -hexapeptide complexes [28] and externalization of intact receptors present in specific granules [23,24] allowed us to lower the protein load on the SDS/acrylamide gels, resulting in a markedly improved resolution of the proteins and more conclusive autoradiograms.

The sensitivity of the rat and human receptors to PNGase F treatment and their resistance to *O*-glycanase demonstrated that both of them contain only *N*-linked oligosaccharides. These are of the complex type rather than the high-mannose or hybrid type and lack terminal sialic acid, as demonstrated by their resistance to endoglycosidase D and H and neuraminidase treatments. As this sensitivity pattern was similar in both species and the shift in the molecular size of the receptors to 35–38 kDa after PNGase F treatment occurred clearly through one intermediate product (rat, 65–73 kDa; human, 48–55 kDa), both receptors must contain a similar 35–38 kDa polypeptide core with two *N*-linked complex-type carbohydrate side-chains, with the heterogeneity of the latter contributing to the species difference in receptor size. Heterogeneity of carbohydrates has also been shown to be responsible for molecular mass differences in the *N*-formyl-peptide chemotactic receptors on monocytes, HL-60 cells and neutrophils [16]. Similarly, other receptors display species or tissue differences in their carbohydrate side-chains [29–31]. The possible species differences in the location of the two oligo-

saccharide side-chains within the polypeptide core of the *N*-formyl-peptide chemotactic receptors was elucidated by proteinase and PNGase F treatments. Although there are clear glycosylation differences between the two species, the oligosaccharides seem to be located solely in the distal 2 kDa papain-cleavable peptide core of the receptors, as the molecular size of the 33–36 kDa fragment remaining on the membrane fragment was unchanged after the additional PNGase F treatment. This result is consistent with recently published cloning data on the human receptor, which reveal two potential *N*-linked glycosylation sites at positions 4 and 10 on the polypeptide [15].

The function of the carbohydrates of glycoprotein receptors in terms of ligand binding and activation of the signal transduction pathways is not well understood. Addition of *N*-linked oligosaccharides to the insulin receptor appears to be required for the expression of any insulin-binding activity [32–34]. In turn, acetylcholine and β -adrenergic receptors and the receptors for luteinizing hormone, immunoglobulin E and cholecystokinin, for instance, do not require glycosylation for expression of high-affinity ligand-binding activity [25,35–38]. The species differences between the rat and human *N*-formyl-peptide chemotactic receptor carbohydrates observed here suggest that they are not critical for high-affinity ligand binding and receptor activation. This view is supported by our finding that the 33–36 kDa papain fragment of both receptors remaining on the membrane, which is devoid of oligosaccharides, bound the ligand with the same affinity as the glycosylated receptor. In addition to carbohydrates, the *N*-terminal 2 kDa papain-cleavable fragment of the 35–38 kDa polypeptide core similarly seems not to be required for high-affinity ligand binding. It is also likely that this fragment represents the extracellular domain of the receptor and that the ligand-binding site is located proximally to the papain cleavage site, possibly in the intramembrane portion of the receptor. Interestingly, Dolmatch & Nidel [39] have shown that a 35 kDa papain fragment of the human receptor (corresponding to the 33–36 kDa fragment identified here) is capable of transmitting a signal for chemotactic-peptide-stimulated β -glucuronidase release, suggesting that the carbohydrates in the *N*-formyl-peptide chemotactic receptor are likewise not required for signal transduction. Before any final conclusions can be drawn regarding the role of carbohydrates in signal transduction, however, their effect upon different signalling pathways used by the receptor should be determined directly.

Conformational changes have been demonstrated to occur upon ligand binding in a number of plasma membrane receptors, and it has been suggested that these may be related to receptor activation [40–42]. Interestingly, the ligand-occupied *N*-formyl-peptide chemotactic receptor in both rat and human neutrophils was resistant to both papain and PNGase F treatment. Since internalization of the receptor–ligand complexes was prevented by NaN_3 , the most conceivable explanation for this result is an altered conformation that leads to masking of the cleavage sites for papain and PNGase F. Our finding is in contrast with that of Malech *et al.* [16], who showed digestion of occupied receptors on plasma membranes.

In summary, we have characterized the glycosyl residues of rat and human *N*-formyl-peptide chemotactic receptors and demonstrated that they contain a similar 35–38 kDa polypeptide core with two *N*-linked complex-type oligosaccharides that contribute to the species difference in receptor size and are not required for high-affinity ligand binding.

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REFERENCES

1. Sciffmann, E., Corcoran, B. A. & Wahl, S. M. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1059–1062
2. Weinberg, J. B., Muscato, J. J. & Niedel, J. E. (1981) *J. Clin. Invest.* **68**, 621–629
3. Snyderman, R. & Fudman, E. J. (1980) *J. Immunol.* **124**, 2754–2757
4. Niedel, J., Kahane, I., Lachman, I. & Cuatrecasas, P. A. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1000–1004
5. Charo, I. F., Yuhlen, C., Perez, H. D. & Goldstein, I. M. (1986) *J. Immunol.* **136**, 3412–3419
6. Showell, H. J., Freer, R. J., Zigmond, S. H., Sciffmann, E., Aswanikumar, S., Corcoran, B. & Becker, E. (1976) *J. Exp. Med.* **143**, 1154–1169
7. Becker, E. L., Sigman, M. & Oliver, J. M. (1979) *Am. J. Pathol.* **95**, 81–97
8. Lehrer, R. I., Ganz, T., Selsted, M. E., Babior, B. M. & Curnutte, J. T. (1988) *Ann. Intern. Med.* **15**, 127–142
9. Niedel, J., Wilkinson, S. & Cuatrecasas, P. (1979) *J. Biol. Chem.* **254**, 10700–10706
10. Aswanikumar, S., Corcoran, B., Sciffmann, E., Day, A. R., Freer, R. J., Showell, H. J., Becker, E. L. & Pert, C. B. (1977) *Biochem. Biophys. Res. Commun.* **74**, 810–817
11. Benyunes, M. C. & Snyderman, R. (1984) *Blood* **63**, 588–592
12. Sciffmann, E., Showell, H. V., Corcoran, B. A., Ward, P. A., Smith, E. & Becker, E. (1975) *J. Immunol.* **114**, 1831–1817
13. Bennett, J. P., Hirth, K. P., Fuchs, E., Sarvas, M. & Warren, G. B. (1980) *FEBS Lett.* **116**, 57–61
14. Marasco, W. A., Phan, S. H., Kruttsch, H., Showell, H. J., Feltner, D. E., Nairn, R., Becker, E. L. & Ward, P. A. (1984) *J. Biol. Chem.* **259**, 5430–5439
15. Boulay, F., Tardif, M., Brouchon, L. & Vignais, P. (1990) *Biochem. Biophys. Res. Commun.* **168**, 1103–1109
16. Malech, H. L., Gardner, J. P., Heiman, D. F. & Rosenzweig, S. A. (1985) *J. Biol. Chem.* **260**, 2509–2514
17. Petäjä, U., Kellokumpu, S., Keinänen, K., Metsikkö, K. & Rajaniemi, H. (1987) *J. Recept. Res.* **7**, 809–827
18. Boyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21**, 77–88
19. Ketelslegers, J. M., Knott, G. D. & Catt, K. J. (1975) *Biochemistry* **14**, 3075–3083
20. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
21. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2616
22. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
23. Fletcher, M. P. & Gallin, J. I. (1980) *J. Immunol.* **124**, 1585–1588
24. Fletcher, M. P. & Gallin, J. I. (1983) *Blood* **62**, 792–799
25. Keinänen, K. P. (1988) *Biochem. J.* **256**, 719–724
26. Hedo, J. A., Harrison, L. C. & Roth, J. (1981) *Biochemistry* **20**, 3385–3393
27. Lin, C. R., Chen, W. S., Kruiger, W., Stolarsky, L. S., Weber, W., Evans, R. M., Verma, I. M., Gill, G. N. & Rosenfeld, M. G. (1984) *Science* **224**, 843–848
28. Niedel, J. E., Kahane, I. & Cuatrecasas, P. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 1085–1089
29. Schojoldager, B., Molero, X. & Miller, L. J. (1990) *Regul. Pept.* **28**, 265–272
30. Grigoriadis, D. E. & De Souza, E. B. (1989) *Endocrinology (Baltimore)* **125**, 1877–1887
31. Heidenreich, K. A. & Brandenburg, D. (1986) *Endocrinology (Baltimore)* **118**, 1835–1842
32. Reed, B. C., Ronnett, G. V. & Lane, M. D. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2908–2912
33. Ronnett, G. V. & Lane, D. M. (1981) *J. Biol. Chem.* **256**, 4704–4707
34. Ercolani, L., Brown, T. J. & Ginsberg, B. H. (1984) *Metab. Clin. Exp.* **33**, 309–316
35. Prives, J. & Bar-Sagi, D. (1983) *J. Biol. Chem.* **258**, 1775–1780
36. Stiles, G. L., Benovic, J. L., Caron, M. G. & Lefkowitz, R. J. (1984) *J. Biol. Chem.* **259**, 8655–8663
37. Hempstead, B. L., Kulczycki, A., Jr. & Parker, C. W. (1981) *Biochem. Biophys. Res. Commun.* **120**, 815–822
38. Rosenzweig, S. A., Madison, L. D. & Jamieson, J. D. (1984) *J. Cell Biol.* **99**, 1110–1116
39. Dolmatch, B. & Niedel, J. (1983) *J. Biol. Chem.* **258**, 7570–7577
40. King, A. C. & Cuatrecasas, P. (1981) *N. Engl. J. Med.* **305**, 77–88
41. Goldstein, J. L., Anderson, R. G. W. & Brown, M. (1979) *Nature (London)* **279**, 679–685
42. Niedel, J., Wilkinson, S. & Cuatrecasas, P. (1979) *Science* **204**, 1412–1414

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