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Solubilization of a functionally active platelet-activating factor receptor from rabbit platelets

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Binding of platelet-activating factor (PAF) to a specific high-affinity membrane receptor has been demonstrated in numerous cell types, but very little is known about the molecular nature of this receptor. The receptor from rabbit platelets was solubilized using CHAPS, digitonin, octyl glucoside, Nonidet P-40 or sodium cholate, either with pre-bound [^{3}H]PAF or in the absence of ligand. We have been able to demonstrate for the first time that the receptor solubilized with CHAPS, in the absence of ligand, could retain its binding activity. It migrated as a high molecular mass complex (> 350 kDa) on a Bio-Gel A-0.5 m gel filtration column. Binding to solubilized receptor rapidly reached an equilibrium at room temperature, but was much slower at 0 °C. Scatchard plots were used to calculate the number (approx. 100 per cell) and the affinity (K_d 2.5 ± 1.4 nM) of the solubilized receptors. These values were comparable with those obtained from whole-cell binding experiments. Competition by PAF antagonists also verified that the assay was measuring PAF receptor binding activity. The presence of a protein in the receptor complex was demonstrated by heat and trypsin inactivation of binding activity. Trypsin had no effect on binding of PAF to whole cells, but was able to decrease binding activity in solubilized receptor preparations. Attempts to demonstrate the involvement of a glycoprotein by use of various lectin columns proved unsuccessful. The latter results are consistent with findings suggesting that the binding site of the PAF receptor may not be exposed at the cell surface.

INTRODUCTION

Platelet-activating factor (PAF) is a multi-functional molecule that has been shown to have effects on numerous cell types (Vargaftig & Benveniste, 1983; Hanahan, 1986; Braquet *et al.*, 1987). The existence of a specific membrane receptor for PAF was initially supported by studies demonstrating that only the naturally occurring *R* stereoisomer of the molecule had biological effects, whereas the *S* form was inactive (Wykle *et al.*, 1981; Blank *et al.*, 1982). Subsequent binding studies in numerous target cell lines and tissues have demonstrated the high-affinity saturable binding of PAF to whole cells and to membranes. The affinity of this binding varies considerably, but some generalizations can be made. Platelets from various species (Kloprogge & Akkerman, 1984; Hwang *et al.*, 1986; Tahraoui *et al.*, 1988; Duronio *et al.*, 1990) have been shown to bind PAF with affinities ranging from 0.1 to 2 nM. Receptor numbers in platelets are generally estimated to be in the range 200–1300 per cell. The other cell types in which PAF binding has been characterized are primarily neutrophils or granulocytes (Hwang *et al.*, 1983; O'Flaherty *et al.*, 1986; Hwang, 1988; Marquis *et al.*, 1988; Vallari *et al.*, 1990). In these cell types, the binding of PAF has been found to be of slightly lower affinity than in platelets, with K_d values ranging from 0.3 to 7 nM, and the receptor number has been reported to be between 5000 and 10000 per cell.

There have been relatively few studies describing the biochemical characterization of the PAF receptor, so very little is known about its molecular size and its orientation in the plasma membrane. Two reports have shown that membranes in which PAF was pre-bound to its receptor could be solubilized using detergents. Nishihara *et al.* (1985) solubilized human platelet membranes pre-incubated with [^{3}H]PAF using Triton X-100 and showed that the label was associated with a complex having a molecular mass of 160 kDa. Chau & Jii (1988) attempted a similar procedure with rabbit platelet membranes using digitonin, and showed that the label migrated with a molecular mass of 220 kDa. In another study, Chau *et al.* (1989) attempted to use a photoaffinity-labelling technique to label the PAF receptor. They showed that a polypeptide with a molecular mass of 52 kDa was labelled using this technique.

While the above-mentioned reports have shown that a receptor complex bound to PAF could be solubilized, there was no indication that the receptor could first be solubilized and still retain its ability to bind PAF. The latter situation would provide a sensitive assay for the presence of active receptor which could be used to detect receptors through various steps of purification. We undertook an evaluation of a number of detergents to test their ability to solubilize the PAF receptor, and a protocol has been developed that allows a reproducible assay of PAF-binding activity. This study demonstrates for the first time that the PAF receptor consists of a protein-containing complex, which is not a common glycoprotein containing *N*-linked oligosaccharides, that can be solubilized with detergents and retain a conformation that allows it to bind PAF with a similar affinity to that seen in whole cells.

MATERIALS AND METHODS

Materials

PAF, detergents, poly(ethylene glycol) (PEG), BSA, protease inhibitors, concanavalin A (Con A)–agarose and ricin–agarose were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) Wheat-germ agglutinin (WGA)–agarose came from E-Y Labs (San Mateo, CA, U.S.A.). Gel filtration media and protein standards were from Bio-Rad Laboratories (Mississauga, Ont., Canada). Cellulose acetate filters (HAWP, 0.45 μm) were from

Abbreviations used: PAF, platelet-activating factor (1-*O*-alkyl-2-acetylglycero-3-phosphocholine); Con A, concanavalin A; ricin, agglutinin RCA_{120}; PEG, poly(ethylene glycol) 8000; PMSF, phenylmethanesulphonyl fluoride; WGA, wheat-germ agglutinin.

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Millipore Corp. (Bedford, MA, U.S.A.). [^{3}H]PAF (90 Ci/mmol) was purchased from Amersham Canada Ltd. (Oakville, Ont., Canada).

Preparation of rabbit platelets

Platelets were prepared from rabbit blood by the method of Pinckard *et al.* (1979). Washed platelets were resuspended in binding buffer without BSA (10 mM-Tris/HCl, pH 7.5, 150 mM-KCl and 5 mM-$MgCl_2$) and an aliquot was taken for a platelet count and for protein determination by the method of Lowry *et al.* (1951), with BSA as the standard. BSA was then added to a final concentration of 0.25% and the platelets were frozen at −80 °C.

Binding assay

Platelets were pelleted at 3300 *g* for 15 min in a Beckman GPR centrifuge and then resuspended in Tris/HCl and KCl with protease inhibitors (see below) to a protein concentration of 10 mg/ml. An equal volume of 2-fold-concentrated solubilizing buffer was added so that the final concentration of components in the solubilizing mixture was as follows: 50 mM-Tris/HCl, pH 7.5, 150 mM-KCl, 5 mM-$MgCl_2$, 2 mM-EGTA, 10% glycerol, 2 mM-dithiothreitol, 20 μg of phenylmethanesulphonyl fluoride (PMSF)/ml, 20 μg of leupeptin/ml, 10 μg of soybean trypsin inhibitor/ml, 1 μg of pepstatin/ml, the appropriate concentration of various detergents, and platelets at a protein concentration of 5 mg/ml. The mixture was stirred gently for 30 min at 4 °C, then diluted 4-fold in cold binding buffer (see above) with 5 mM-$CaCl_2$ and the protease inhibitors as in solubilizing buffer. The insoluble portion was removed by centrifugation at 160000 *g* for 20 min, and the supernatant assayed for PAF-binding activity. Unless otherwise indicated in the Figure legends, samples were incubated with 1 nM-[^{3}H]PAF in the presence or absence of 1 μM unlabelled PAF for 30 min at room temperature. Samples contained 200 μg of platelet protein (based on the amount of protein in the mixture before solubilization) in a total binding volume of 200 μl. Incubation was followed by the addition of 0.5 ml of 0.05% γ-globulin and 0.5 ml of 24% PEG, and samples were placed on ice for a minimum of 30 min to allow precipitation of protein. Samples were then filtered through cellulose acetate filters, washed with 5 ml of cold binding buffer containing 10% PEG and counted for radioactivity in a liquid scintillation counter. Specifically bound radioactivity was determined by subtracting background d.p.m. non-specifically bound in the presence of excess unlabelled PAF (1.0 μM) from the total d.p.m. Background radioactivity due to non-specific binding of PAF to filters was determined in samples containing only detergent and [^{3}H]PAF; this background was generally less than 25% of specific binding. In the case of experiments where platelets were pre-bound with [^{3}H]PAF before solubilization, half of the protein was incubated with [^{3}H]PAF and the other half with [^{3}H]PAF plus 1 μM unlabelled PAF. Platelets were then washed twice with cold binding buffer, solubilized and precipitated with PEG as described above. During pre-binding, $CaCl_2$ was excluded from the binding buffer to prevent platelet aggregation.

Gel filtration

Solubilized samples (1–2 ml) were applied to a Bio-Gel A-0.5 m column (1 cm × 120 cm) equilibrated with 25 mM-Tris/HCl, pH 7.5, 150 mM-KCl, 5 mM-$MgCl_2$, 2 mM-EGTA and 0.05% BSA (necessary to keep free [^{3}H]PAF in solution). The column was eluted with this same buffer, and 1 ml fractions were collected either for direct scintillation counting (in the case of samples bound with [^{3}H]PAF before gel filtration) or for binding assays.

Trypsin treatment

Whole platelets or solubilized extracts were treated with 0–5.0 mg of trypsin/ml in the presence of 1 mM-EDTA for 5 min at 22 °C with continuous shaking. A 3-fold excess of soybean trypsin inhibitor was added, then samples were washed once with binding buffer and assayed for PAF-binding activity (whole platelets), or assayed immediately (solubilized extracts).

Lectin affinity chromatography

Solubilized samples were applied to 0.5 ml columns of Con A–agarose, WGA–agarose or ricin–agarose. Columns had been equilibrated at room temperature with their respective running buffers (50 mM-Tris/HCl, pH 7.5, 0.15 M-KCl and 0.02% CHAPS or digitonin for WGA and ricin columns, and 50 mM-Tris/HCl, pH 7.5, 2 mM-$MgCl_2$, 2 mM-$CaCl_2$ and 0.02% detergent for Con A columns). Samples were applied to columns and the flow-through re-applied twice, then columns were washed with 15 ml of running buffer. Elution was carried out by applying five 300 μl volumes of elution buffer (50 mM-Tris/HCl, pH 7.5, 0.15 M-KCl and protease inhibitors, with 0.5 M-α-methyl D-mannopyranoside for Con A, 0.3 M-*N*-acetyl-D-glucosamine for WGA and 0.5 M-D-galactose for ricin), and flow-through and eluates were either counted directly for radioactivity in the case of pre-bound samples, or assayed for [^{3}H]PAF binding activity in the case of unbound samples.

RESULTS

Detergent solubilization after pre-binding of [^{3}H]PAF

A series of detergents was tested for their ability to solubilize the PAF receptor from rabbit platelets that were pre-incubated with [^{3}H]PAF in the presence or absence of excess unlabelled PAF. The detergents used were CHAPS, digitonin, octyl glucoside, sodium cholate and Nonidet P-40. A representative

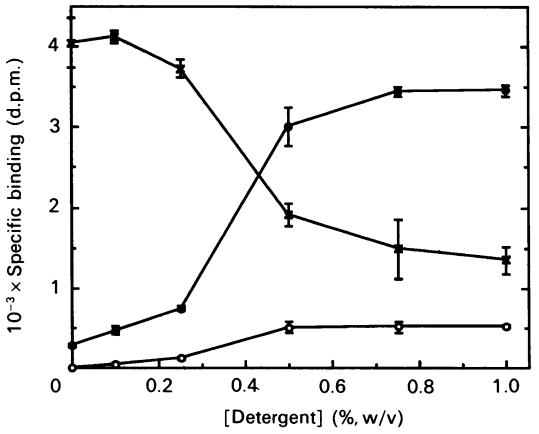

Fig. 1. Solubilization of pre-bound platelets with CHAPS

Platelets were incubated with 1 nM-[^{3}H]PAF in the presence or absence of 1 μM unlabelled PAF, washed and solubilized for 60 min at 4 °C with the various concentrations of CHAPS. The platelet protein concentration in the solubilizing mixture was 5 mg/ml. After 4-fold dilution in binding buffer and centrifugation at 160000 *g* for 20 min, portions of the resuspended insoluble pellet were filtered on cellulose acetate, washed, and counted (×) for radioactivity. Aliquots of the soluble supernatant were either counted direct (●) or PEG-precipitated, filtered, washed and counted (○). Samples contained 100 μg of platelet protein, based on the protein concentration in the solubilizing mixture. Specific binding was determined by subtracting d.p.m. non-specifically bound in the presence of 1.0 μM unlabelled PAF from total d.p.m. Each point is the mean ± S.D. of triplicate determinations.

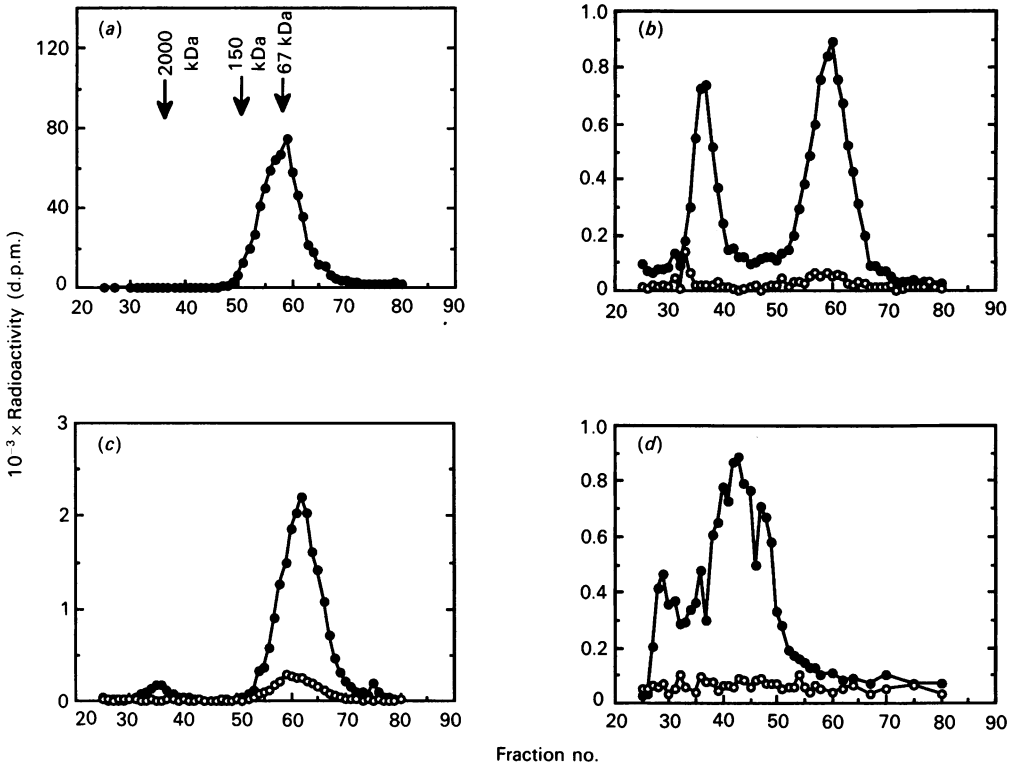

Fig. 2. Fractionation of solubilized platelets with pre-bound [^{3}H]PAF

Gel filtration on a Bio-Gel A-0.5 m column was carried out as in the Materials and methods section. (*a*) [^{3}H]PAF (0.3 μCi) in 0.5 ml of binding buffer (with 0.25% BSA) was applied. Arrows indicate the positions of the following molecular mass standards: BSA (67 kDa), human IgG (150 kDa) and Blue Dextran (2000 kDa). In (*b*)–(*d*), platelets pre-bound with 1.0 nM-[^{3}H]PAF in the presence (○) or absence (●) of 1.0 μM unlabelled PAF were solubilized with 0.5% CHAPS (*b*), 0.5% octyl glucoside (*c*) or 0.25% digitonin (*d*), and samples containing 1500 μg of protein were applied to the column. Fractions of 1 ml were collected and counted for radioactivity.

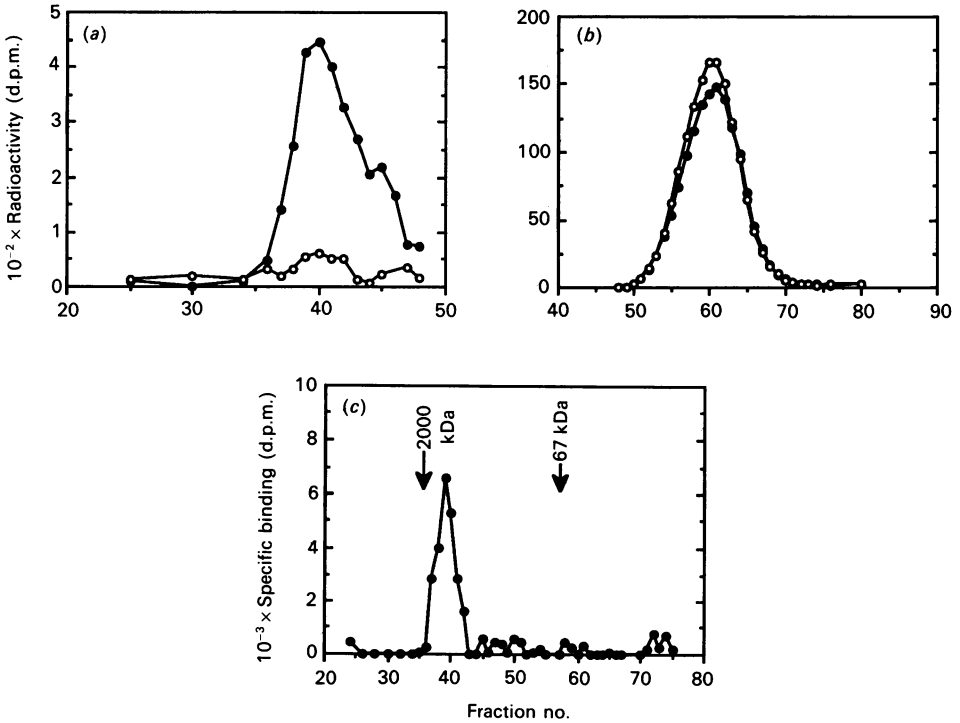

Fig. 3. Fractionation of solubilized platelets without pre-binding

(*a*) and (*b*), platelets were solubilized with 0.5% CHAPS and then incubated with 1 nM-[^{3}H]PAF in the presence (○) or the absence (●) of 1 μM unlabelled PAF and fractionated on a Bio-Gel A-0.5 m column (*a* for fractions 25–48 and *b* for fractions 48–80 of the same column runs). For (*c*), the solubilized platelet sample was fractionated by gel filtration and fractions were assayed using the PEG precipitation assay. Specific binding in each sample was determined from the difference in radioactivity bound in the presence or the absence of 1.0 μM unlabelled PAF.

solubilization experiment using CHAPS is shown in Fig. 1. The optimum concentration of CHAPS for release of [^{3}H]PAF into the supernatant was 0.75 %. Since solubilization by this criterion does not indicate whether the ligand remains bound to the receptor, several methods were tested to separate free from receptor-bound PAF. The procedures tested were precipitation of receptors using $(NH_4)_2SO_4$ or PEG, adsorption of free ligand to activated charcoal or silica, and gel filtration. Gel filtration was used for characterization of the receptor complex, as will be shown below, but a more rapid procedure that could be carried out on multiple samples was necessary in order to develop an assay that would be useful for receptor purification. Of the methods tested, the most reproducible results and the lowest backgrounds were obtained with precipitation by PEG (results not shown). Fig. 1 shows that increasing the CHAPS concentration beyond 0.5 % did not result in a greater proportion of counts that could be precipitated by PEG, so this was the concentration chosen for further experiments. This is very close to the critical micelle concentration of 0.48 %. Only 15–20 % of the pre-bound counts could be precipitated by PEG, which suggested that detergent solubilization was causing dissociation of [^{3}H]PAF from its receptor. This was more evident from gel filtration results (Fig. 2). Fig. 2(*b*) shows the profile obtained from CHAPS solubilization of platelets pre-bound with [^{3}H]PAF. Only 30 % of the radioactivity was recovered in a high-molecular mass fraction that presumably contained the PAF receptor complex (Nishihara *et al.*, 1985; Chau & Jii, 1988). The remaining radioactivity migrated at the position expected for [^{3}H]PAF alone, which associated with the BSA present in the column buffer (Fig. 2*a*). Sodium cholate produced similar results to those seen with CHAPS (results not shown). Fig. 2(*c*) shows that solubilization using octyl glucoside resulted in virtually complete dissociation of PAF from its receptor, which explained the inability of PEG to precipitate a significant portion of the radioactivity solubilized by octyl glucoside (results not shown). Nonidet P-40 gave similar results to those seen with octyl glucoside. On the other hand, very few of the counts migrated at the position expected for free PAF following digitonin solubilization, but this detergent always gave a poorly resolved peak in gel filtration, as shown in Fig. 2(*d*). Because of the distinct high-molecular-mass receptor complex (approx. molecular mass 350–500 kDa) obtained by gel filtration after CHAPS solubilization, this detergent was chosen for further experiments.

Binding of [^{3}H]PAF to solubilized receptors

Having verified that the PAF receptor with bound ligand could be solubilized by detergent, we next attempted to solubilize whole platelets without pre-binding, then demonstrate the presence of active receptors that could still bind PAF. Solubilization was carried out using 0.5 % CHAPS, after which the samples were incubated with [^{3}H]PAF as described in the Materials and methods section. When the incubation mixture was applied to a gel filtration column, the majority of the radioactivity migrated at the position expected for free PAF, since in this experiment there is no wash step to remove unbound PAF. Representative gel filtration profiles of the solubilized samples are shown in Figs. 3(*a*) and 3(*b*). At the position expected for the high-molecular-mass receptor complex, there was a distinct peak of [^{3}H]PAF binding that was displaced in the sample incubated with excess unlabelled PAF. The binding of PAF to CHAPS-solubilized receptors was further indicated by another experiment, in which solubilized platelets were fractionated by gel filtration and the fractions assayed for binding activity using the PEG precipitation procedure. As shown in Fig. 3(*c*), the only peak of binding activity migrated at the position expected for the receptor complex.

Heat and trypsin inactivation of binding activity

Since PAF is a lipid, there was some possibility that it could be associated with a lipid complex and that the receptor being assayed was not actually a protein. The involvement of a protein was suggested by heat inactivation experiments. When detergent-solubilized platelets were heated to 50 °C for 10 min, then assayed for binding activity, there was a 63 % decrease in activity. Heating to 60 °C for 10 min completely abolished PAF-binding activity.

Sensitivity of binding activity to proteases was tested by trypsin treatment of solubilized extracts and whole cells. When CHAPS-solubilized platelets were treated at 22 °C for 5 min with trypsin (1.0 mg/ml or 5.0 mg/ml), binding activity was decreased by 48 % and 64 % respectively compared with untreated controls. Interestingly, similar treatment of whole platelets with a range of trypsin concentrations from 0.1 mg/ml to 5.0 mg/ml caused no significant change in binding activity.

Lectin affinity columns

The potential identification of the PAF receptor as a membrane glycoprotein was tested using several lectin affinity columns. Numerous experiments in which solubilized platelets were applied

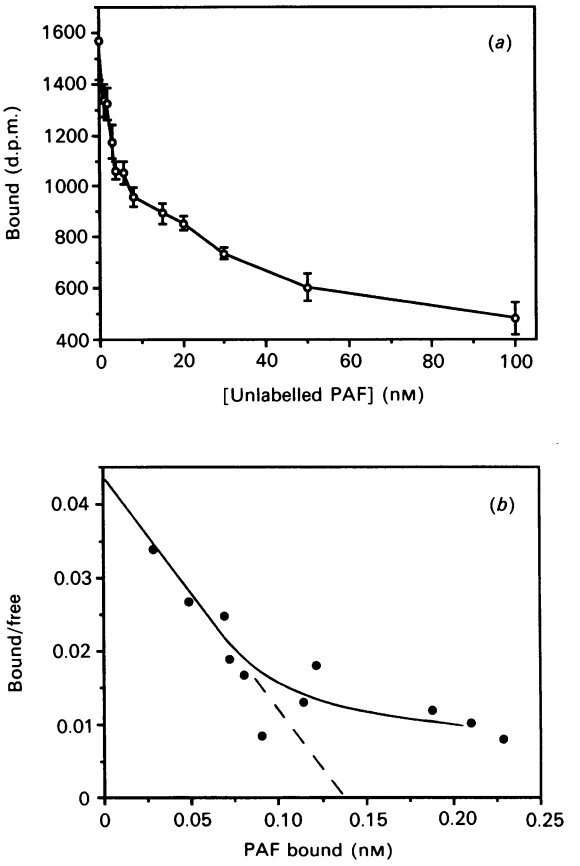

Fig. 4. PAF binding to solubilized receptors

Platelets were solubilized with 0.5 % CHAPS and incubated with 1.0 nM-[^{3}H]PAF and 0–1.0 μM unlabelled PAF for 30 min at room temperature (*a*). Results are the means of two experiments done in triplicate. Non-specific binding in the presence of 1.0 μM-PAF was 448 ± 38 d.p.m. Total radioactivity added per sample was 30000 d.p.m. In (*b*), results from a representative experiment are presented in a Scatchard plot. Samples contained 200 μg of protein in a volume of 200 μl. PEG precipitation was performed as described in the Materials and methods section. Each point is the average of triplicate determinations.

Table 1. Competition by PAF and antagonists

The binding assay with solubilized platelet extracts was carried out as described in the Materials and methods section.

Incubation conditions	Total radioactivity bound (d.p.m.)	Relative inhibition (%)
1.0 nM-[^{3}H]PAF	1302±84	–
1.0 nM-[^{3}H]PAF+PAF (1.0 μM)	664±57	100
1.0 nM-[^{3}H]PAF+WEB2086 (10 μM)	642±75	103
1.0 nM-[^{3}H]PAF+CV3988 (10 μM)	817±29	76

to columns of WGA, Con A or ricin linked to agarose demonstrated that there was no specific elution of the receptor complex from the lectins. This was true both for solubilized samples that were pre-bound with [^{3}H]PAF and for solubilized samples assayed for binding after passing through the lectin column. In some cases, a portion of the pre-bound [^{3}H]PAF was bound to the lectin column, but this was not eluted by the specific sugar, so it was attributed to non-specific association of PAF on the column.

Characterization of binding activity

The receptors' binding activity was further characterized using the solubilized receptor assay. Binding was found to reach an equilibrium at room temperature (22 °C) within 5 min, while at 15 °C and at 0 °C maximal binding required 15 min and 60 min respectively. Solubilized platelets were bound with [^{3}H]PAF at room temperature in the presence of various amounts of unlabelled PAF, and the results of a typical experiment are shown in Fig. 4(*a*). The binding of [^{3}H]PAF was significantly decreased by low concentrations of unlabelled PAF, as expected for high-affinity binding. A representative Scatchard plot is shown in Fig. 4(*b*). From the results of five experiments, the high-affinity portion of the binding gave a K_d of 2.5±1.4 nM, with (7.1±4.7)×10^{10} receptors/mg of platelet protein, which corresponds to about 100 receptor sites per cell.

Another result demonstrating that the binding being measured was to the PAF receptor is shown in Table 1. In this experiment, binding of [^{3}H]PAF was inhibited either by unlabelled PAF, which was normalized to 100% inhibition, or by two PAF antagonists, WEB2086 and CV3988. At a concentration of 10 μM, WEB2086 completely inhibited binding, and CV3988 decreased binding by approx. 76%.

DISCUSSION

The solubilization of functionally active PAF receptors for the purpose of characterization and eventual purification has not previously been documented. Therefore this receptor system is poorly understood, with only a minimum of attention being given to the nature of the receptor's components (Nishihara *et al.*, 1985; Chau & Jii, 1988; Chau *et al.*, 1989). In this report we have described our attempts to systematically examine the ability of a series of detergents to solubilize the PAF receptor in a form that allows it to retain its binding activity. The specific binding activity was assayed using a PEG precipitation procedure that has been used as an assay for numerous receptors (El-Refai, 1984). While our initial studies repeated the findings of others showing that the receptor with bound ligand could be solubilized and separated by gel filtration (Nishihara *et al.*, 1985; Chau & Jii, 1988), we also showed that CHAPS-solubilized receptors could bind PAF *in vitro*. Furthermore, this binding activity could be demonstrated after fractionation of the solubilized extract by gel filtration, showing that pre-binding of PAF to platelets was not necessary to stabilize the receptor complex. Therefore we have shown for the first time that the PAF receptor can be assayed after solubilization. This is an essential first step in designing procedures for purification of the receptor.

This study has also provided some important information on the initial characterization of the PAF receptor. First of all, the presence of protein in the solubilized receptor was verified by heat and trypsin inactivation experiments using solubilized extracts. This is an important finding, since PAF is a lipid and as such could conceivably be binding tightly to lipid molecules in membranes. Indeed, Braquet *et al.* (1987) have proposed that the binding site for PAF in membranes must accommodate the acyl chain in a hydrophobic environment. It will be interesting to know more about the receptor's membrane orientation, since binding studies suggest that the binding site for PAF could be on the inner side of the plasma membrane. This suggestion was based on the finding that although whole cells show no binding for PAF at 0 °C, there is measurable binding activity with membranes at cold temperatures (Valone *et al.*, 1982; Duronio *et al.*, 1990). There are two sets of results shown here that are consistent with this suggestion. The PAF receptor is not a common glycoprotein containing *N*-linked oligosaccharides, since it is unable to bind to WGA, Con A or ricin, and trypsin treatment of whole cells has no effect on PAF-binding activity. While certainly not conclusive, these results support the suggestion that the binding site of PAF may be at the inner surface of the plasma membrane.

Since completion of this study, a report by Honda *et al.* (1991) has described the cloning of a cDNA encoding the PAF receptor from guinea pig lung. This was accomplished by expression cloning in frog oocytes using RNA from an unspecified cellular source. Therefore it will be of great interest to determine whether this cloned receptor represents the only PAF receptor, or whether multiple receptor types exist, as has been suggested previously (Hwang, 1988). The molecular mass of the PAF receptor predicted from the cloning study is approx. 39 kDa. Of course, this is in sharp contrast with the size of the solubilized receptor observed by gel filtration in our study, as well as in previous reports (Chau & Jii, 1988; Nishihara *et al.*, 1985). The study by Honda *et al.* (1991) showed no characterization of the expressed protein, so it is difficult to know whether the solubilized receptor may have a higher molecular mass than expected due to either detergent and lipid micelles, or due to other associated proteins that remain tightly bound. Another aspect of PAF receptor function that remains unexplained is the involvement of tyrosine phosphorylation in signalling. We demonstrated that PAF stimulates tyrosine phosphorylation of a number of proteins in platelets (Salari *et al.*, 1990*b*), but the G-protein-coupled receptors containing the characteristic seven transmembrane domains are not known to stimulate tyrosine phosphorylation.

The binding activity of the solubilized preparation was further characterized and shown to retain properties of the PAF receptor in whole cells. The affinity of binding was high, with a K_d of 2.5±1.4 nM, and there were (7.1±4.7)×10^{10} receptors/mg of solubilized platelet protein, corresponding to approx. 100 receptors per cell. In comparison, for whole rabbit platelets the K_d has been reported by our group to be 0.7±0.1 nM, with 689±229 receptor sites/cell (Duronio *et al.*, 1990) and by Hwang *et al.* (1986) to be 1–2 nM, with 150–300 receptor sites per cell. The lower number of receptor sites and the slightly lower affinity in solubilized platelets is presumably a reflection of loss of activity during the solubilization procedure. Both high- and low-affinity binding components were present with the solubilized receptor.

This low-affinity component is also seen with PAF binding to whole platelets or membranes and has previously been attributed to association of PAF with hydrophobic membrane components. As mentioned above, the binding of PAF to its receptor may require an intimate association of the hydrophobic component of the molecule with membrane lipids (Braquet *et al.*, 1987), and it would be expected that the solubilized receptor complex retains some associated lipid. Therefore one could speculate that the low-affinity PAF binding involves interaction of PAF with lipid molecules that are tightly associated with the receptor. Of course, we cannot rule out other possibilities such as co-operative interactions that confer high-affinity binding following the low-affinity binding event. The other major characteristic of the binding activity was that it was displaced not only by unlabelled PAF, but also by two PAF antagonists, WEB2086 and CV3988, that have been shown to compete for binding to the PAF receptor. In addition, no specific binding was observed when [^{3}H]lyso-PAF was used in place of [^{3}H]PAF in the binding assay (results not shown).

In summary, this study demonstrates for the first time that the PAF receptor can be solubilized from rabbit platelets using CHAPS, and that the receptor retains its ability to bind PAF. The affinity of this binding correlates well with the binding to whole cells, and competition for binding by PAF antagonists is consistent with the characteristics of the intact receptor. These results provide good evidence that we are solubilizing the functional PAF receptor. Heat lability of the receptor suggests the involvement of a protein, as does trypsin sensitivity. The negative results with lectin columns suggest that the receptor does not contain a common glycoprotein. Numerous studies have demonstrated PAF binding to purified membranes, showing that the PAF receptor is clearly tightly associated with the plasma membrane, so it will be very interesting to determine the orientation of the receptor in the plasma membrane. Now that an assay procedure is available for the solubilized receptor, we will be able to attempt larger-scale preparations of platelets to be used for receptor purification by conventional methods. Molecular characterization of the PAF receptor protein in platelets and other cell types will be critical for a better understanding of the numerous activities of PAF and its role in certain pathological conditions, such as endotoxic shock (Salari *et al.*, 1990*a*) and asthma (Pretolani *et al.*, 1989).

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