Characterization of a Plasma Membrane-associated Prenylcysteine-directed $\alpha$ Carboxyl Methyltransferase in Human Neutrophils*

(Received for publication, July 23, 1993)

Michael H. Pillinger†, Craig Volker‡, Jeffry B. Stock§, Gerald Weissmann**, and Mark R. Philips‡‡

From the Department of Medicine, New York University School of Medicine, New York, New York 10016 and Departments of Molecular Biology and Chemistry, Princeton University, Princeton, New Jersey 08540

Signal transduction in human neutrophils requires prenylcysteine-directed carboxyl methylation of ras-related low molecular weight GTP-binding proteins. We now report the subcellular localization and characterized a neutrophil prenylcysteine $\alpha$ carboxyl methyltransferase. The highest carboxyl methyltransferase activity copurified with biotinylated neutrophil surface membranes, supporting a plasma membrane localization of the enzyme. Neutrophil nuclear fractions contained little or no methyltransferase activity. Methyltransferase activity was detergent-sensitive but could be reconstituted by removal of detergent in the presence of phosphatidyl choline and an anionic phospholipid. $N$-Acetyl-$S$-trans,trans-farnesyl-$L$-cysteine (AFC) and $N$-acetyl-$S$-all-trans-geranylgeranyl-$L$-cysteine (AGGC) were effective substrates for neutrophil prenylcysteine-directed methyltransferase; $V_{\text{max}}$ values for AFC and AGGC (18.4 and 22.1 pmol of methylated/mg protein/min, respectively) are among the highest yet reported. Although both GTP-$\gamma$S and the chemotactant fMet-Leu-Phe stimulated methylation of ras-related proteins, neither affected methylation of AFC. These data suggest that neutrophil plasma membranes contain a phospholipid-dependent, prenylcysteine-directed carboxyl methyltransferase of relatively high specific activity that modifies ras-related protein substrates in the GTP-bound, activated state.

Eukaryotic cells possess a set of enzymes that modify the C termini of proteins ending in the consensus sequence CAAX, where C is cysteine, A is usually an aliphatic amino acid, and X is another amino acid (1). Several cytosolic prenyltransferases recognize these sequences and catalyze the

attachment of a 15-carbon farnesyl or 20-carbon geranylgeranyl polyisoprene chain via a thioether linkage to the cysteine (2–4). The prenylated C terminus is recognized by a membrane-associated protease that removes the AAX amino acids (5, 6). The newly COOH-terminal prenylcysteine then becomes a substrate for a membrane-associated carboxyl methyltransferase, which methylates the $\alpha$ carboxyl group (7, 8). Prenylation has not been shown to be reversible, whereas turnover of the $\alpha$ carboxyl methyl ester has been demonstrated (9).

Among the CAAX-containing proteins processed in this fashion are two classes of proteins in the GTPase superfamily that play critical roles in signal transduction, the ras-related GTP-binding proteins (9) and the subunit of heterotrimeric G proteins (G, (11). Both classes of GTP-binding regulatory proteins are biologically active when associated with membranes. CAAX processing is essential for membrane targeting of these otherwise intrinsically hydrophilic proteins (12). Blocking prenylation with metabolic inhibitors or by mutation of the COOH-terminal cysteine renders p21ras (13) and G, (11, 14) soluble and inactive. Because prenylation and AAX proteolysis are prerequisites for carboxyl methylation it has proven difficult to determine the role of methyl esterification in membrane targeting. However, genetic studies in yeast (15) and in vitro analysis of p21K-ras6 (5) indicate that carboxyl methylation augments the membrane association of processed CAAX-containing proteins, presumably by neutralizing a negative charge. It is unclear whether the COOH-terminal prenylcysteine methyl ester inserts nonspecifically into lipid bilayers or binds to specific membrane targets. The reversibility of carboxyl methylation and the central role of GTP-binding proteins in signaling suggest that carboxyl methylation may regulate signal transduction.

Unlike bacterial glutamyl or bacterial or eukaryotic isoaspartyl carboxyl methyltransferases, which are soluble, prenylcysteine-directed $\alpha$ carboxyl methyltransferase is membrane-associated in yeast and mammalian cells (1). G, and ras-related proteins are synthesized in the cytosol, prenylated by a family of soluble prenyltransferases (1), and subsequently associated with the plasma membrane (13, 16, 17), suggesting a plasma membrane localization of the prenylcysteine-directed carboxyl methyltransferase. Indeed, were carboxyl methylation to play a role in signal transduction, it would be expected that the enzyme would reside in or at the plasma membrane. Surprisingly, Stephenson and Clarke (18) have recently reported that a rat liver prenylcysteine-directed carboxyl methyltransferase is associated with microsomes (18).

Neutrophils are terminally differentiated, short-lived cells with no proliferative capacity but with relatively well characterized pathways of signal transduction (19). Chemotactant-mediated signaling involves coupling of surface receptors...
to a pertussis-sensitive G protein, probably G_{i2} (20). Downstream events such as assembly of the plasma membrane NADPH oxidase appear to be regulated by ras-related proteins (21, 22). Based on the findings that carboxyl methylation of ras-related proteins followed neutrophil activation and that specifiers of prenylcysteine-directed carboxyl methyltransferase inhibited neutrophil function (23), we tentatively suggested a role for carboxyl methylation of GTP-binding proteins in signal transduction. We now report the subcellular localization and characterization of a human neutrophyl prenylcysteine-directed carboxyl methyltransferase. The enzyme is present in the plasmalemma, requires anionic phospholipid for reconstitution after detergent solubilization, and is regulated at the level of its GTP-binding protein substrates.

EXPERIMENTAL PROCEDURES

Materials—Except where otherwise noted, reagents were purchased from Sigma. Hapycase was purchased from Winthrop Pharmaceuticals, Ficol 400, dextran T500, and Sephadex G-25 were from Pharmacia LKB Biotechnology Inc. Sulfo-NHS-biotin, Extractigel, and BCA protein assay were from Pierce Chemical Co. Streptavidin was from Life Technologies, Inc. Na_{152} and S-adenosyl-[methyl-3H] methionine (AdoMet) were from DuPont-NEN. Fornylmethionine-lysine-phenylalanine (FMLP) was purchased from Vega Biochemicals.

Preparation of Prenylcysteine Analogs—N-Acetyl-S-trans-geranyl-L-cysteine (AGC), N-acetyl-S-trans-farnesyl-L-cysteine (AF), and N-acetyl-S-all-trans-geranylgeranyl-l-cysteine (AGGC) were prepared from the prenyl bromide as previously described (24). The products were characterized by NMR, mass spectroscopy, and high pressure liquid chromatography.

Neutrophil Isolation and Subcellular Fractionation—Neutrophils were isolated from heparinized blood by Ficoll/Hypaque density ultracentrifugation (76, 25) and then resuspended in relaxation buffer and subjected to nitrogen cavitation and subcellular fractionation as described above. Samples of subcellular fractions (200 µl) were blotted onto nitrocellulose, which was then blocked for 1 h at room temperature in TGG buffer (PBS with 0.5% Tween 20, 1 mM glucose, 10% glycerol) containing 3% bovine serum albumin and 2% nonfat dry milk. The nitrocellulose was then washed in PBS, 0.5% Tween 20 and 0.5% Triton X-100 for 2 h in TGG buffer containing 0.3% bovine serum albumin and 30 Ci/mmol [3H]-streptavidin (500,000 cpm/mmol). The nitrocellulose was washed three times with PBS, 0.5% Tween and once with water and air dried. Biotinylated proteins were visualized and quantitated by phosphorimage analysis (Molecular Dynamics model 400A, Sunnyvale, California).

To enrich for biotinylated surface membranes from among the light membrane fraction, streptavidin- or cellulose- (control) conjugated-agarose beads were washed with TE buffer. Biotinylated light membranes (40 µg) were incubated in the presence of 25 µl of streptavidin- or cellulose-conjugated-agarose beads in 50 µl of TE buffer at 4 °C with vigorous mixing. After 30 min the beads were allowed to sediment, and samples were separated into pellet and supernatant. 50 µl of TE was added to each pellet, and 25 µl of cellulose-conjugated beads were added to each supernatant. Methyltransferase activity was assayed as described below.

Enzymatic Markers of Neutrophil Subcellular Fractions—Alkaline phosphatase (28) and glucose 6-phosphatase (29) were assayed as described.

Methyltransferase Assay—Methyltransferase activity was assayed according to the method of Volker et al. (24). Neutrophil subcellular fractions, 100 µm AIC, and, unless otherwise indicated, 720 nm [H] AdoMet (76 Ci/mmol) were incubated in 50 µl of TE buffer (50 mM Tris HCl, pH 8.0) for 1 h at 37 °C. Reactions were terminated by the addition of 50 µl of 20% trichloroacetic acid and vortexed for 10 s. Heptane (400 µl) was added to each sample and vortexed for 10 s. Samples were centrifuged at 16,000 × g, and 500 µl of the organic phases (supernatants) were recovered. The supernatants were dried by vacuum centrifugation, and the residues were hydrolyzed with 100 µl of 1 N NaOH and analyzed for vapor phase [H]methanol.

Detergent Solubilization and Reconstitution of Carboxyl Methyltransferase Activity—Neutrophil light membranes (20 µg) were incubated in 50 µl of TE buffer in the presence of detergent for 30 min at 4 °C, followed by incubation for 30 min at room temperature in the presence of phosphatidyl choline (PC) (5.0 mg/ml) with or without 0.5 mg/ml of phosphatidic acid (PA), phosphatidyl serine (PS), phosphatidyl ethanolamine, or phosphatidyl ethanolamine. Samples were incubated with 50 µl of Extractigel detergent removing gel for 1 h at 4 °C with frequent mixing. Extractigel was removed by centrifugation (500 × g), and supernatants were assayed for methyltransferase activity as described.

Cell-free Carboxyl Methylation of Neutrophil ras-related Proteins—Neutrophil light membranes (20 µg) and cytosol were resuspended in 50 µl of 100 mM NaFPO_4/NaHPO_4, pH 6.8, containing 1 µM EDTA (methylating buffer) and 85 µCi/µl [3H]AdoMet and incubated at 37 °C in the presence or absence of iMLP (100 nM) and/ or GTP-S (100 µM). After 1–60 min the reaction was stopped by the addition of 25 µl of 3% electrophoresis sample buffer, and the proteins were analyzed by 12% SDS-PAGE. Labeled proteins were visualized by fluorography (3–10 day exposures). Carboxyl methylation of labeled proteins was confirmed by excising bands from the dried gels, hydrolyzing methyl esters, and quantitating [H]methanol as described above.

RESULTS

Subcellular Localization of Neutrophil Prenylcysteine-directed Carboxyl Methyltransferase Activity—Neutrophil carboxyl methyltransferase was localized by means of nitrogen cavitation and discontinuous sucrose density centrifugation. Human neutrophils were processed as described (26) and separated into cytosol, light membranes, specific granules, azurophilic granules, and a nuclear pellet. In some experiments, intact neutrophils were biotinylated with a cell-impermeant reagent before cavitation and subcellular fractionation to label neutrophil surface membranes. The highest activity of neutrophil carboxyl methyltransferase was localized to the light membrane fraction. This fraction also contained the highest level of biotinylation (Fig. 1), consistent with the

1 The abbreviations used are: AdoMet, S-adenosyl-L-[methyl-3H] methionine; AF, N-acetyl-S-trans-farnesyl-L-cysteine; AGGC, N-acetyl-S-all-trans-geranylgeranyl-L-cysteine; IMLP, formyl-methionine-lysine-phenylalanine; AGC, N-acetyl-S-trans-geranyl-L-cysteine; KIU, kallikrein-inactivating unit(s); PBS, phosphate-buffered saline; PC, phosphatidyl choline; PA, phosphatidic acid; PS, phosphatidyl serine; AdoHcy, N-acetyl-S-adenosyl homocysteine; S- Me-S-Ado, S'-deoxy methylthiocoladeneosine; ER, endoplasmic reticulum.
observation that the neutrophil light membrane fraction consists predominantly of plasma membrane (30). A proportional amount of alkaline phosphatase activity, a commonly used marker for neutrophil plasma membrane, also segregated with the light membrane fraction. Neutrophil specific granules contained one-sixth as much carboxyl methyltransferase activity as that found in the light membrane fraction but also contained proportional levels of biotinylation and alkaline phosphatase activity, suggesting contamination of the specific granule fraction with plasma membranes. Azurophilic granules and cytosol were devoid of methyltransferase activity. In contrast to all other cell types reported (31–33), the nuclear fractions of neutrophils contained very little methyltransferase activity after detergent extraction alone, incubation with PC/PA in the absence of detergent extraction, or incubation with PC alone followed by detergent removal with Extractigel (DR) to promote liposome formation and assayed for methyltransferase activity as described under "Experimental Procedures." Results are expressed as percent of total methyltransferase activity for each condition. Values presented are the means of three experiments ± S.E. and the pellet/supernatant ratio for each condition.

### Table 1

<table>
<thead>
<tr>
<th>Membrane fraction</th>
<th>Agarose bead conjugate</th>
<th>Streptavidin</th>
<th>Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellet</td>
<td>56 ± 11</td>
<td>47 ± 11</td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>14 ± 15</td>
<td>53 ± 11</td>
<td></td>
</tr>
<tr>
<td>Pellet/Supernatant</td>
<td>3.3</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Subcellular localization of neutrophil carboxyl methyltransferase activity and surface biotinylation of neutrophil plasma membranes. For methyltransferase activity, neutrophil subcellular fractions were prepared as described under "Experimental Procedures." Aliquots of 0.4 mg/ml individual fractions were incubated for 60 min in TE buffer with 100 μM ΔFDR and 720 nM [3H]AdoMet (76 Ci/mmol). The reaction was stopped and assayed as described under "Experimental Procedures." For distribution of surface membranes, neutrophils were biotinylated and fractionated, and 200 ng of each fraction were dot blotted against 125I-conjugated streptavidin, as described under "Experimental Procedures." Biotinylation was quantitated by phosphor imaging. Values presented are the means of at least three experiments.

**Figure 2.** Reconstitution of prenylcysteine-directed carboxyl methyltransferase activity after detergent extraction. Neutrophil light membranes (20 μg) were solubilized in TE buffer containing 0.25% CHAPS, followed by incubation in the absence or presence of PC (0 mg/ml) or 0.5 mg/ml PA, PS, phosphatidyl inositol (PI), or phosphatidyl ethanolamine (PE) with or without detergent removal with Extractigel (DR) to promote liposome formation and assayed for methyltransferase activity as described under "Experimental Procedures." Results are expressed as percent methyltransferase activity of control samples not incubated with CHAPS. Data shown are representative of three experiments.

**Reconstitution of Detergent-solubilized Neutrophil Prenyl-

cysteine-directed Carboxyl Methyltransferase Activity—Exposure of neutrophil light membranes to CHAPS, octyl-β-D-glucoside, sodium deoxycholate, or a variety of other detergents at varying concentrations resulted in complete loss of prenylcysteine-directed carboxyl methyltransferase activity. We successfully reconstituted carboxyl methyltransferase activity from CHAPS-solubilized membranes by removing detergent in the presence of sufficient phospholipid to form liposomes (Fig. 2). Solubilization of neutrophil light membranes by CHAPS (0.25%) resulted in 98.6% loss of carboxyl methyltransferase activity. Incubation of CHAPS-solubilized membranes in PC/PA (10:1, w/w) for 30 min at room temperature followed by detergent removal with Extractigel resulted in 23.2% recovery of methyltransferase activity. Substitution of PS, phosphatidyl inositol, or phosphatidyl ethanolamine (all with PC, 10:1, w/w) for PA resulted in 16.0, 8.7, and 5.2% recovery of methyltransferase activity, respectively. Incubation with PC alone in the absence of detergent extraction resulted in no recovery of methyltransferase activity. Detergent extraction alone, incubation with PC/PA in the absence of detergent extraction, or incubation with PC alone followed by detergent extraction resulted in recovery of only 4–6% of...
methyltransferase activity. Incubation in PC ± PA subsequent to detergent extraction resulted in no more recovery of activity than detergent extraction alone. Similar results were obtained using deoxycholate-solubilized membranes followed by dialysis to remove detergent.

Specific Activity and Substrate Specificity of the Neutrophil Prenylcysteine-directed Carboxyl Methyltransferase—We have previously shown that membrane fractions from rat brain and liver retain the capacity to carboxylate both farnesylated and geranyl-geranylated analogs of the carboxy-terminal cysteine of prenylated ras-related proteins (32). To determine the specific activity and prenyl chain specificity of neutrophil carboxyl methyltransferase we incubated neutrophil light membranes with [3H]AdoMet in the presence or absence of varying concentrations of AGC, AFC, or AGGC and assayed for prenylcysteine methylesterification. Both AFC and AGGC were efficiently methylated by neutrophil light membranes, whereas AGC, at concentrations up to 100 μM, was not modified. The apparent KN for AFC and AGGC were 11.6 ± 4.6 and 1.4 ± 0.1 μM, respectively. The Vmax for AFC and AGGC were 16.3 ± 3.4 and 22.1 ± 5.2 pmol/mg of membrane/min, respectively (Fig. 3).

Inhibition of Carboxyl Methyltransferase Activity by Analogs of AdoMet—Agents that inhibit prenylcysteine-directed carboxyl methyltransferase activity may prove to be pharmacologically useful. We examined the ability of three AdoMet analogs to inhibit carboxyl methyl transferase of AFC (Fig. 4). N-acetyl-S-adenosyl-homocysteine (AdoHcy), the reaction product of the demethylation of AdoMet, competitively inhibited carboxyl methylation (Ki = 1.57 μM). Sinefungin was an even more potent inhibitor of methylation and also demonstrated kinetics consistent with competitive inhibition (Ki = 0.07 μM). Previous data has been inconsistent with regard to the capacity of 5′-deoxymethylthioadenosine (5′-Me-S-Ado) to inhibit carboxyl methylation in a variety of systems (18, 35). We observed only a modest competitive inhibition of carboxyl methylation of AFC by 5′-Me-S-Ado (Ki = 3.95 mM). Vmax and KN for AdoMet in the absence of inhibitors were 14.2 pmol/mg of light membrane/min and 1.3 μM, respectively.

Effect of fMLP and GTPyS on Carboxyl Methylolation of AFC—GTPyS stimulates the carboxyl methylation of ras-related proteins in broken cell preparations (23, 36–38). In contrast, carboxyl methylation of AFC by neutrophil light membrane was independent of GTPyS, suggesting regulation at the level of the GTP-binding protein substrates (Fig. 5). We have previously demonstrated that fMLP stimulates carboxyl methylation of neutrophil ras-related proteins in both intact cells and in a cell-free system containing light membranes and cytosol (23). We therefore tested the effect of fMLP on the ability of neutrophil light membranes to methylate AFC. At secretory doses (100 nM) fMLP had no effect on the kinetics of AFC methyl esterification by neutrophil light membranes. Finally, fMLP and GTPyS together failed to stimulate carboxyl methylation of AFC. These data suggest that fMLP stimulates carboxyl methylation of GTP-binding proteins through a substrate-dependent mechanism, possibly through promotion of GDP/GTP exchange.

**DISCUSSION**

Prenylcysteine-directed carboxyl methyltransferase activity has been identified in yeast and mammalian cells, but the enzyme has been only partially characterized. Methylation of ras-related proteins increases their affinity for membranes (5); whether this effect is due to increased hydrophobicity alone or to specific targeting by the prenylcy steine methylester remains unresolved. In neutrophils, a pool of cytosolic ras-related proteins is prenylated but not methylated, whereas the majority of carboxyl-methylated ras-related proteins is associated with membranes (23). Activation of neutrophils stimulates carboxyl methylation of these proteins. Because ras-related proteins appear to translocate from cytosol to membranes of activated cells where they are carboxyl-methylated and remain membrane-associated, the subcellular localization and character of prenylcysteine-directed carboxyl methyltransferase is of potential importance in neutrophil signaling.

Our data indicate that the highest level of neutrophil car-
those described in Fig. 1, in the absence of methylation of AFC by neutrophil light membrane. Described under "Experimental Procedures." Data shown are representative of three experiments. Inset, effect of fMLP and/or GTP\(_\gamma\)S on carboxyl methylation of ras-related proteins. Neutrophil light membrane (25 \(\mu\)g), cytosol (100 \(\mu\)g), and [\(\text{H}\)]AdoMet (85 \(\mu\)Ci/ml) were incubated at 37 °C for 1 min in the absence or presence of 100 \(\mu\)M fMLP and/or 100 \(\mu\)M GTP\(_\gamma\)S as described under "Experimental Procedures." The proteins were analyzed by SDS-PAGE and visualized by fluorography. Lane 1, control. Lane 2, fMLP only. Lane 3, GTP\(_\gamma\)S only. Lane 4, fMLP plus GTP\(_\gamma\)S. Similar results were obtained after a 1-h incubation.

Carboxyl methyltransferase activity resides in the light membrane fraction. A much lower level of activity was recovered in the specific granule fraction, but the colocalization of a number of intrinsic proteins with the plasma membrane and function as a mobilizable pool of latent plasma membrane. Indeed, human neutrophil specific granule membranes share a number of intrinsic proteins with the plasma membrane and function as a mobilizable pool of latent plasma membrane. Localization of methyltransferase activity to the light membrane fraction is consistent with previous data in rat brain and liver. Like rat brain and liver cytosol, human neutrophil cytosol was devoid of prenylcysteine-directed carboxyl methyltransferase activity. However, whereas the nuclear pellet was a major source of carboxyl methyltransferase in rat tissues, we detected no such activity in neutrophils. Rat liver and brain homogenates contain nuclei from cells capable of proliferation (e.g., hepatocytes and glia). In contrast, neutrophils are terminally differentiated and have no proliferative capacity. Because methylation and demethylation of nuclear lamins are associated with dissolution and reformation of the nuclear envelope during mitosis, these data suggest that during myelopoesis carboxyl methyltransferase activity is lost concomitantly with the ability to divide.

Circulating neutrophils contain little endoplasmic reticulum (ER) and Golgi. Neutrophil light membranes consist predominantly of plasma membrane with small amounts of Golgi and ER. In contrast, light membrane preparations from rat liver consist predominantly of ER in the form of microsomes with contaminating plasma membranes, Golgi, and other vesicles. In a recent study of rat liver, continuous sucrose density centrifugation was used to separate plasma membrane and Golgi from ER, and the peak of prenylcysteine-directed carboxyl methyltransferase activity was found to be associated with the microsomal (ER) fraction (18). In contrast, using continuous sucrose density centrifugation of neutrophil light membranes we failed to separate methyltransferase activity from biotinylated membrane or alkaline phosphatase activity, suggesting localization to the plasma membrane. As evidence for a paucity of ER, we were unable to detect glucose-6-phosphatase activity in neutrophil light membranes or any other subcellular fraction of human neutrophils. Moreover, streptavidin bead enrichment of biotinylated surface membranes copurified the bulk of light membrane methyltransferase activity. Finally, highly purified neutrophil plasma membranes isolated by free-flow electrophoresis contained methyltransferase activity equivalent to that of neutrophil light membranes. We therefore conclude that, in contrast to rat liver methyltransferase, neutrophil prenylcysteine-specific methyltransferase activity is located predominantly in the plasma membrane.

To characterize the bilayer membrane-dependence of neutrophil prenylcysteine-dependent carboxyl methyltransferase we solubilized light membranes in detergent and sought to establish the conditions necessary for reconstitution of activity. Our data confirmed previous observations in rat liver microsomes (18) that prenylcysteine-directed carboxyl methyltransferase activity is lost following extraction by a variety of detergents. Removal of detergent alone or addition of phospholipids after, or in the absence of, detergent removal failed to result in significant recovery of methyltransferase activity, suggesting the importance of an intact lipid bilayer for enzyme activity. Formation of PC liposomes also failed to result in significant recovery of methyltransferase activity. In contrast, the formation of liposomes containing anionic phospholipid by addition of phosphatidyl choline together with phosphatidic acid followed by detergent removal resulted in 23.2% recovery of methyltransferase activity. Because the orientation of reconstituted enzyme with respect to liposome membrane is presumably random whereas neutrophil plasma membrane vesicles are predominantly right-side out, maximal reconstitution would be predicted to be substantially less than 100%. Therefore, our results suggest substantial reconstitution. Substitution of other phospholipids for PA resulted in lesser degrees of methyltransferase reconstitution; recovery of activity appeared to be proportional, at least in part, to the net and/or total anionic charge of the phospholipid. These data suggest that neutrophil light membrane carboxyl methyltransferase activity is not only detergent-sensitive but dependent on the phospholipid composition of the membrane with which it is associated. Moreover, because resting neutrophils contain little if any PA but fMLP stimulation causes its accumulation (42, 43), the data provide a possible mechanism for PA as a second messenger in signal transduction.

In a variety of mouse tissues, the maximal methyltransferase activity, using AFC as an artificial substrate, has been measured at 8.4 pmol/mg/min (31). In rats, the maximal reported activity of 18 pmol/mg/min was identified in crude brain light membrane fractions (32), consistent with a role of carboxyl methyltransferase in signal-transducing cells. The maximal rate of AFC methylation by the neutrophil light membrane fraction was 16.4 pmol/mg/min, confirming that neutrophil plasma membranes possess a relatively high degree of methyltransferase activity as might be expected were the enzyme to play a role in signal transduction. The neutrophil light membrane \(K_m\) for AFC was lower than that for rat brain.
binding proteins (both ras-related and heterotrimeric), which are themselves implicated in a variety of signaling processes, steine-containing substrates for this enzyme consist of GTP-nuclear membrane vesiculation and reformation involves the role in signal transduction. First, a major subset of prenylcysteine-directed carboxyl methylation transiently in whole cells and concentrations up to microsomes plasma membrane-associated methyitransferases are distinct from nuclear- and heterotrimeric GTPyS does not, however, stimulate methylation of DNA and arginine residues but is a poor inhibitor of ras-related protein methylation by GTPyS is mediated by release of activated cytosolic ras-related protein substrates. We have demonstrated that increased levels of intra- cytosolic ras-related protein substrates. We have demonstrated that nuclear lamin B methylation by rat liver nuclei is also a poor inhibitor of neutrophil prenylcysteine-directed carboxyl methylation. Finally, we have demonstrated that prenyl- carboxyl methylation of ras-related proteins follows activation of host defense in part by their capacity to rapidly transduce a variety of signals via immune receptors, contain at least as much prenylcy steine-directed carboxyl methyltransferase activity as brain. Finally, we have demonstrated that prenylcy steine-directed carboxyl methyltransferase activity is absolutely dependent on anionic phospholipid of the type formed in the course of signal transduction. Our successful reconstitution of carboxyl methyltransferase activity after detergent solubilization may prove useful in purifying and further characterizing this regulatory enzyme.

Acknowledgments—We are grateful to N. Borregaard and H. Sengel for supplying free-flow electrophoresis-purified subcellular fractions. We thank R. Staud, A. Feoktistov, and S. Muscat for technical assistance.

REFERENCES

Prenylcysteine-directed Carboxyl Methyltransferase
Prenylcysteine-directed Carboxyl Methyltransferase