Acyloxyacyl hydrolase, a leukocyte enzyme that acts on bacterial lipopolysaccharides (LPSs) and many glycerolipids, is synthesized as a precursor polypeptide that undergoes internal disulfide linkage before being proteolytically processed into two subunits. The larger subunit contains an amino acid sequence (Gly-X-Ser-X-Gly) that is found at the active sites of many lipases, while the smaller subunit has amino acid sequence similarity to saposins (sphingolipid activator proteins), cofactors for sphingolipid glycohydrolases. We show here that both acyloxyacyl hydrolase subunits are required for catalytic activity toward LPS and glycophosphatidylcholine. In addition, mutations that truncate or delete the small subunit have profound effects on the intracellular localization, proteolytic processing, and stability of the enzyme in baby hamster kidney cells. Remarkably, proteolytic cleavage of the precursor protein increases the activity of the enzyme toward LPS by 10-20-fold without altering its activity toward glycophosphatidylcholine. Proper orientation of the two subunits thus seems very important for the substrate specificity of this unusual enzyme.

Few lipases have more than one polypeptide chain. The two-subunit structure of acyloxyacyl hydrolase (AOAH),1 a phagocytic cell enzyme that cleaves acyl chains from bacterial lipopolysaccharides (LPSs) as well as glycerolipids, thus poses interesting structure-function issues. In particular, how might the enzyme's unusual structure explain its ability to act on LPS, a heterogeneous substrate with a wide range of amphiplicity? Clues are found in the amino acid sequence homologies of the two subunits (1): the large subunit contains the sequence Gly-X-Ser-X-Gly, found as part of the active site in many lipases (2), whereas the small subunit has sequence similarity with saposins, surfactant protein B, and sulfated glycoprotein. The similarity to the saposins is particularly intriguing, since these proteins are cofactors for various glycohydrolases that act on sphingolipids (like LPS, amphipathic molecules with complex carbohydrate chains) (3). It seemed possible that the small subunit could enable the enzyme to recognize LPS as a substrate, while the large subunit would play the major role in catalysis.

In the experiments described here, we studied the properties of wild-type and mutated AOAH using pulse-chase analysis of 35S-labeled AOAH to explore its biosynthesis, immunofluorescence to localize the enzyme intracellularly, and assays for enzymatic activity to measure its function. Because we were unable to establish stable rAOAH-expressing transfections in leukocyte cell lines, we used the BHK570 fibroblast line for these studies. The results indicate that while the large subunit, as expected, plays an important role in catalysis, the small subunit is essential for several properties of the enzyme, including its intracellular localization and its catalytic activity toward LPS and glycero phospholipid.

EXPERIMENTAL PROCEDURES

Materials—Enzymes were purchased from Promega Biotech Inc., Dulbecco's modified Eagle's medium (DMEM) from Fisher, DMEM without l-Met and l-Cys (M-C-DMEM) from Sigma, and fetal calf serum from HyClone Laboratories (Logan, UT). Methotrexate was from Sigma and Geneticin (G418) from Life Technologies, Inc. Oligonucleotides were synthesized in the Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX. Polymerase chain reactions were performed with a GeneAmp PCR DNA amplification kit from Perkin-Elmer.

AOAH cDNA—Human AOAH cDNA constructs in vectors pZEM229R (4) and pRS431 were provided by ZymoGenetics, Seattle, WA, as were BHK570 and BHK570 431A cells. Plasmid pZEM229R 4-33 contains the 2162-bp full-length AOAH cDNA cloned into the EcoRI site of pZEM229R (1). AOAH expressed from this plasmid has full enzymatic activity but differs from wild-type AOAH at 3 amino acids (due to 3-base transitions introduced during PCR). Plasmid pRS431 carries a 1744-bp AOAH cDNA fragment cloned into the EcoRI site of pZEM229R.2 This 1744-bp insert comprises the correct coding sequence of AOAH with very little upstream cDNA sequence and no downstream sequence. BHK570 431A cells are stably transfected with pRS431. Plasmid clone 2.1 is a partial cDNA clone that harbors a 96-bp deletion (from bp 400 to 495 in the wild-type sequence (Fig. 1) (1)).

Site-directed Mutagenesis—The 3-base transitions in the 2162-bp AOAH cDNA insert of pZEM229R 4-33 were corrected with AOAH DNA from pRS431. This wild-type 2162-bp AOAH cDNA, cloned in pSE-LECT-1 (Promega Biotech Inc.), was used as template for site-directed mutagenesis (Promega Biotech's Altered Sites in situ Mutagenesis System). The introduced base changes were confirmed by DNA sequencing. Mutagenized AOAH cDNA was cloned into mammalian expression vectors pZEM229R (harbors dihydrofolate reductase gene (4) and pJB2O (neo) (5), kindly provided by David Russell (this institution)). Selection was performed using methotrexate or G418, respectively.

Plasmid Constructs—Plasmid clone 2.1 was digested with BclI and BglII, and the 430-bp fragment (which contains the 96-bp deletion) was substituted for the corresponding BclI-BglII fragment (526 bp) in pZEM229R 4-33. The new plasmid, pZEM229R 4-33, harbors the 96-base pair deletion in the region encoding the small subunit of AOAH. The cDNA encoding the AOAH signal sequence and propeptide was cloned upstream of the large subunit as follows. A 116-bp region from...
Acylcoxyacyl Hydrolase Structure-Function

The shaded area within the small subunit represents the leader sequence for 5 h followed by a chase of 1, 3, 5, and 7 h with complete medium. The added fractions were quantitated using enzymatic and immunoblot (8) assays. Fractions containing the highest amounts of enzymatic activity and/or rAOAH protein were pooled and concentrated (Cenitron-30; Amicon, Beverly, MA). Partially purified rAOAH was stored at -70°C in 15% glycerol.

**Purification of the AOAH Large Subunit**—Stably transfected BHK570 cells harboring pZEM138 were lysed with 140 mM potassium phosphate (pH 7), 50 mM NaCl, 0.1% Triton X-100, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 0.5 μg/ml pepstatin, and 2 mM EDTA. The nuclei were pelleted and the supernatant was centrifuged at 100,000 x g for 1 h at 4°C. Glycerol (final volume, 20%) was added to the resulting supernatant, which was then diluted with an equal volume of 50 mM Hepes, pH 7, 0.9% NaCl, 0.1% Triton X-100, 15% glycerol (Mab buffer) and filtered (0.22 μm, Corning Inc., Corning, NY). The large subunit was adsorbed from the filtrate by immunosafinity chromatography, using an agaroseconjugated Mab to AOAH (12). The eluted fractions were then digested with 2 x 100 μl of Mab buffer, concentrated (Cenitron-10, Amicon), washed in the concentrating device with Mab buffer, and stored at -70°C.

**Quantitation of rAOAH**—Samples were subjected to SDS-PAGE and Western transfer onto Immobilon-P membrane (Millipore Corp., Bedford, MA). rAOAH was detected by using affinity-purified rabbit IgG raised to the COOH-terminal 19 amino acids of AOAH, or polyclonal rabbit IgG raised to intact rAOAH, followed by affinity-purified 125I-labeled goat anti-rabbit IgG (gift of Ellen Vitetta, this institution). Sections of the membrane containing 125I-immunolabeled rAOAH polypeptides were removed and their radioactivity was counted. The amount of murine AOAH present relative to the amount of 2 x 100 μl of Mab buffer was determined by densitometric scanning of the radioautograph. Low molecular size protein standards were from the Sigma MW-SDS-17s kit (2.5-17.0 kDa).

**RESULTS**

An AOAH Precursor Is Secreted by BHK570 Cells—Wild-type rAOAH biosynthesis in transfected BHK570 431A cells was studied using SDS-PAGE and autoradiography to characterize biosynthetically labeled 35S-labeled AOAH. As shown in Fig. 2, anti-AOAH antibodies immunoprecipitated two labeled proteins, of apparent M, = 70,000 and M, = 60,000, from cell lysates. In contrast, the cell medias contained only one immunoprecipitable protein, of M, = 70,000. When immune precipitates were treated with 2-ME prior to SDS-PAGE, a protein of M, = ~14,000 was detected in the cell lysates, and a protein of M, = 52,000 was found instead of the 60,000-Da peptide seen in the unreduced lysates. When reduced, the 70,000-Da protein in the cell lysates and media migrated as 65,000-Da proteins.

Two-dimensional gel analysis (first dimension, unreduced; second dimension, reduced) of the cell lysate showed that the small polypeptide found following reduction derived from the
Acylxoyacyl Hydrolase Structure-Function

Fig. 2. Synthesis of wild-type rAOAH in BHK570 cells. BHK570 431A cells were labeled for 5 h and the radiolabeled AOAH from cell lysates and media was immunoprecipitated with rabbit anti-AOAH IgG as described under "Experimental Procedures." The immune precipitates were analyzed by SDS-PAGE followed by autoradiography. Lanes 1 and 3, immunoprecipitated AOAH from cell lysates; lanes 2 and 4, immunoprecipitated AOAH from cell medium. The samples in lanes 3 and 4 were treated with 2-ME prior to electrophoresis. Molecular size markers are shown at the left in kilodaltons. Treatment with 2-ME was associated with the appearance of a 14-kDa band (the glycosylated small subunit) in the lysate (lane 3), but not the media. The nature of the faint, nonspecific ~40-kDa band that appears in lanes 3 and 4 is not known.

Fig. 3. Pulse-chase analysis of BHK570 cells transfected with wild-type AOAH cDNA. BHK570 431A cells were radiolabeled for 5 h and the rAOAHs in cell lysates and media were immunoprecipitated with rabbit anti-AOAH IgG as described under "Experimental Procedures." AOAH-containing immune precipitates were analyzed by SDS-PAGE, with and without exposure to 2-ME, followed by autoradiography. Lane designations indicate the chase time in (P = immune precipitate of cell lysate or media with preimmune rabbit IgG). Molecular size markers are shown at the left in kilodaltons. Top panels, immunoprecipitated AOAH from cell lysates, analyzed without (left) or with (right) exposure to 2-ME. Bottom panels, immunoprecipitated AOAH from the cell media. Note in the top left panel (lysate) that the 70-kDa band gradually disappears during the chase while the 60-kDa band gains intensity. In the bottom left panel (medium), the intensity of the 70-kDa band increases during the chase, consistent with secretion of the precursor. The 14-kDa band was seen only when cell lysates were treated with 2-ME (top right). Faint nonspecific bands were again noted following 2-ME treatment.

Mₐ = 60,000 peptide in the unreduced sample (data not shown); the increase in mobility of the 70,000-Da polypeptides in the presence of 2-ME was possibly due to the loss of intrachain disulfide bonds.

These data suggest that the larger protein (70 kDa) is the precursor form of AOAH; as shown schematically in Fig. 1, the two subunits of the protein are not separable by disulfide bond reduction unless the precursor has been proteolytically cleaved between the subunits. Some of the rAOAH produced in BHK570 cells is appropriately processed (mature rAOAH) and can be separated into its two subunits by disulfide bond reduction (Fig. 2, lane 3), and a large fraction of the unprocessed AOAH is secreted into the media.

Pulse-chase analysis (Fig. 3) confirmed that the precursor polypeptide is partly processed to mature intracellular AOAH and partly secreted into the media. Little mature AOAH was found in the media. When ammonium chloride (10 mM) was present during the pulse and chase, very little of the 60 kDa peptide was found (Fig. 4), in keeping with the conclusions that 1) this protein is derived from the 70-kDa protein and 2) proteolytic cleavage of the enzyme into its subunits may occur in an acidic intracellular compartment.

The fate of the ³⁵S-labeled 70-kDa precursor was quantitated after a 5-h chase in nonradioactive medium (Table I). A large fraction (44.8%) of the precursor was secreted during the 5-h period, whereas approximately one-fourth was processed to the 60-kDa peptide. The total recovery of labeled rAOAH was lower following ammonium chloride treatment and much less of the precursor was found in the media at 5 h (Table I).

Proteolytic Processing in Vitro Using Trypsin or Chymotrypsin—The rAOAH precursor was partially purified from serum-free medium. Chymotrypsin or trypsin could cleave it between the two subunits; when reduced with 2-ME prior to SDS-PAGE analysis, the trypsin- or chymotrypsin-generated large subunit had the size of the native large subunit (Fig. 5A). The NH₂-terminal amino acid sequence of the trypsin-generated large subunit was Ser-Gly-Ser-Asp-Ile... indicating that trypsin treatment resulted in a large subunit that was two amino acids longer than the native large subunit (N-Ser-Asp-Ile...). The AOAH propeptide sequence (Ser-Pro-Ala-Asn...) was found at the amino terminus of the small subunit after trypsin cleavage, indicating that the propeptide was retained in the secreted protein.

Proteolytic Processing of the Precursor Protein Increases Its Enzymatic Activity toward LPS without Altering Its Activity toward GPC—Trypsin or chymotrypsin treatment of the precursor greatly increased its ability to attack LPS (Fig. 6). Activation was associated with a large increase in Vₘₐₓ with no change in Kₘ (Fig. 6, inset). In contrast, the activity of the enzyme toward GPC was not increased (Fig. 6). The preference of the enzyme for removing palmitate, rather than oleate, from the sn-2 position of GPC (16) was also not changed by protease treatment (data not shown). Neutrophil-derived (native) AOAH also has greater in vitro activity toward LPS than GPC (16).

The fatty acids released from LPS after incubation with activated and non-activated rAOAH were examined by TLC. Before and after activation, the enzyme removed the two secondary fatty acids (myristate, laurate) at similar rates (data not shown); there was no preference for attacking a particular acyloxyacyl bond on the lipid A backbone.

Structure-Function Analysis: Enzymatic Activity and Intracellular Localization of Wild-type and Mutant Forms of rAOAH—Intracellular AOAH was localized in BHK570 cells by

| Lanes | Time (min) |
|-------|-----------|
| 2     | 0         |
| 3     | 5         |
| 4     | 30        |
| 4     | 120       |
indirect immunofluorescence. Whereas no background fluorescence was seen in cells transfected with vector DNA (pZEM229R), cells transfected with wild-type AOAH-expressing plasmids showed bright fluorescence in large vesicular structures (Fig. 7, Panel B). When added to the culture medium, fluorescein isothiocyanate-dextran accumulated in the same structures; anti-AOAH antibodies were found in large vacuoles when studied by immunogold electron microscopy (data not shown). We consider these structures lysosomes. Fluorescence was also noted in a more diffuse, punctate pattern that involved most of the cytoplasm. Ammonium chloride treatment of the cells did not change this distribution (Fig. 7, Panel C).

Analysis of mutated rAOAH derivatives suggested the following conclusions:

1) N-linked glycosylation of the small subunit is not essential for enzymatic activity or intracellular localization. The small subunit contains a single site for N-linked glycosylation. This site is glycosylated in wild-type rAOAH, as was shown by a decrease in the apparent size of the small subunit following treatment with N-glycanase (Fig. 5, Panel B). An essentially identical result was obtained previously using native neutrophil AOAH (1). Elimination of this site by replacing Thr with Ala did not alter the catalytic activity of the enzyme toward LPS (Table II), nor did it change the intracellular distribution of AOAH (Fig. 7, Panel D). The small subunit of the mutated enzyme had the same apparent size as N-glycanase-treated wild-type rAOAH small subunit, indicating that the mutagenesis had the desired effect (Fig. 7, Panel B).

2) The complete small subunit is not required for enzymatic activity toward LPS, but may be necessary for proteolytic processing, secretion, and intracellular targeting. rAOAH that lacked an internal 33-amino acid region of the small subunit had approximately 42% of the wild-type enzymatic activity (Table II); its activity toward LPS could be enhanced by treatment with chymotrypsin. This variant form of AOAH did not localize to the large vesicles (Fig. 7, Panel E), was poorly secreted, and was less stable than wild-type rAOAH (Table I). Immune precipitates of cell extracts and cell media contained one peptide of Mr = 60,000 that could not be reduced into two subunits by 2-ME (data not shown). The absence of this 33 amino acid component of the small subunit apparently prevents normal intracellular targeting, processing, and secretion of rAOAH by BHK570 cells.

3) The large subunit contains a component of the catalytic site. AOAH has the sequence Gly-X-Ser-X-Gly; the serine is known to participate with His and Asp in a catalytic triad in many lipases (17). When AOAH Ser was replaced by Leu, the activity of the enzyme toward LPS decreased by 90-fold (Table II), in keeping with a key role for this serine in the catalytic activity of AOAH.

4) The small subunit also contributes to catalysis. Expression of the large subunit by itself produced a polypeptide that had ≤1% of the wild-type activity toward LPS (Table II) and GPC (data not shown), suggesting that the small subunit in some way contributes to the ability of the enzyme to attack both substrates. Immune precipitates of cell extracts of BHK570 cells harboring pZEM138 contained one polypeptide of 57,000 Da that was poorly secreted and could not be reduced into smaller subunits (data not shown). Although this construct included the AOAH signal and propeptide sequences, the large subunit had the same localization pattern as the rAOAH that lacked 33 amino acids of the small subunit (Fig. 7, Panel F), again consistent with a role for the small subunit in the intracellular targeting of AOAH by these cells.

**DISCUSSION**

A lipase with a preference for removing saturated (or short) acyl chains from glycerol-based lipids (16) and LPS (15), AOAH

---

**Panel A**

**Panel B**

**Panel C**

**Panel D**

**Panel E**

**Panel F**

**Fig. 4. Effect of ammonium chloride on the processing of wild-type AOAH in BHK570 cells.** Wild-type AOAH in transfected BHK570 cells was radiolabeled with [35S]methionine and [35S]cysteine in the presence of 10 μM ammonium chloride. AOAH in cell lysates was immunoprecipitated at different chase times and analyzed by SDS-PAGE and autoradiography. The lane designations are as in Fig. 3. Molecular size markers are shown at the left. The processed AOAH seen (as a 60,000-Da band) in the reduced samples was less than 10% of the total immunoprecipitated AOAH, and this percentage did not change with increasing chase time (compare to untreated cells (Fig. 3)).

**Table I**

Fate of the 70,000-Da rAOAH after a 5-h chase

Phosphorimager analysis of immunoprecipitated [35S]-labeled proteins. Values for the wild type and wild type plus NH₄Cl are the averages of values from two gels; the values for the truncated small subunit were quantitated from one gel.

| rAOAH                          | Percent of recovered [35S]-labeled 70-kDa peptidea | Percent of total [35S] cpm recovered after 5 h b |
|-------------------------------|-----------------------------------------------|-----------------------------------------------|
|                               | (found in media)                             | (to 60-kDa protein)                           | (70-kDa protein)                                  |
| Wild type                     | 44                                            | 27                                            | 27                                            |
| Wild type + NH₄Cl             | 25                                            | 0                                             | 75                                            | 55                                            |
| Truncated small subunit       | 7                                             | 0                                             | 93                                            | 24                                            |

a Recovery of counts from 70,000-Da precursor. Total recovered counts = 100%.

b Recovery of total [35S] counts after 5-h chase.
has been found only in cells that are thought to interact with LPS or Gram-negative bacteria in vivo. These studies were carried out to explore the contributions of the protein's two subunit domains to its enzymatic activities (acyloxyacyl hydrolase and phospholipase), stability, and intracellular localization.

AOAH is synthesized in BHK570 cells as a 70-kDa precursor. This precursor has two fates: secretion, and proteolytic processing to yield mature AOAH, a protein that has two disulfide-linked subunits. Very little mature AOAH is found in the cell media, and only in cells that produced mature rAOAH was it possible to find intense immunofluorescent staining in lysosomes. Processing of AOAH to the 60-kDa form could be blocked almost completely by ammonium chloride. Taken together, these results suggest that proteolytic processing occurs in lysosomes; AOAH that bypasses these structures remains unprocessed and may be secreted.

The AOAH small subunit bears a striking sequence similarity to the proteins known as saposins (3). Saposins (sphingolipid activator proteins) are small, heat-stable glycoproteins that greatly enhance the hydrolysis of glycosphingolipids by specific lysosomal hydrolases. Given the structural resemblance of LPS to glycosphingolipids, we hypothesized that the small subunit of AOAH facilitates the recognition of its unique target, LPS. Several of our observations indicate that, in fact, the small subunit contributes not only to LPS recognition but also to the intracellular targeting and catalytic function of the enzyme.

Like the four saposins, the small subunit of AOAH has a site for N-linked glycosylation. Although a mutation that destroyed this site in saposin B was associated with a variant form of metachromatic leukodystrophy (18), the function of the saposin carbohydrate chain is poorly understood. It does not seem to be necessary for saposin activity or for resistance to proteolytic
attack, and a role in protein folding has been suggested (19). Removing this glycosylation site from AOAH did not alter either the enzymatic activity of the protein (Table II) or its intracellular location (Fig. 7), nor did the intracellular or extracellular protein become more unstable (data not shown). The significance of glycosylation at this site thus is uncertain. On the other hand, both the rAOAH large subunit and the AOAH variant that lacked a 33-amino acid region within the small subunit (including 2 of the 7 cysteines and the glycosylation site) could not be found in the lysosomes that contained wild-type rAOAH and they were poorly processed, suggesting that (this region of) the AOAH small subunit is critical for targeting the intracellular enzyme. The small subunit deletion mutant was also considerably less stable intracellularly than wild-type rAOAH (Table I). The extent to which the instability of the protein leads to artifactual estimates of its other properties is unknown; its secretion rate would be underestimated, for example, if the protein were more susceptible to proteolytic degradation in the culture medium.

Elimination of the presumed active site serine (Ser<sup>265</sup>) in the large subunit reduced enzymatic activity toward LPS by greater than 99%, in keeping with the conclusion that this serine plays a key role in catalysis. The large subunit itself had greatly reduced enzymatic activity when expressed alone, however, again suggesting that the small subunit is required for enzymatic activity. Although the possibility that the large subunit was synthesized in an inactive form cannot be excluded, its DNA sequence, size on SDS-PAGE gels, and presence in immune precipitates with specific anti-AOAH antibodies all suggest that the desired polypeptide was produced.

The amino acid sequence similarity between saposins, surfactant protein B, and the small subunit of AOAH was noted by Hagen et al. (1). These molecules may constitute a family of small proteins that function at lipid-water or lipid-air interfaces. There are some important differences, however. First, although the AOAH small subunit amino acid sequence, deduced from the cDNA, includes the 6 Cys residues that are found in the other proteins, if the migration of the mature AOAH small subunit on SDS-PAGE gives a true estimate of molecular size (8,100 Da), this subunit has only 4 or 5 cysteines. At least one of these residues must be involved in disulfide linkage to the large subunit. Second, unlike the saposins, which are independently synthesized cofactors for the glycolydrolases they serve and which must be present in excess for optimal enzymatic activity (20), the AOAH small subunit is covalently linked to the large subunit and both peptides must be present for catalysis to occur.

Proper orientation of the two subunits, as presumably occurs following proteolytic cleavage of the rAOAH precursor, greatly enhanced the activity of the enzyme toward LPS but not toward GPC. This selective activation for enhanced activity toward a particular substrate evidently has little precedent; it is possible that this mechanism is used in vivo to direct the activity of AOAH toward LPS when, for example, phagocytic cells are activated by bacterial stimuli. Against this notion is the observation that substantial amounts of the precursor form were not isolated from human leukocytes using a monoclonal antibody to the large subunit (12), although the use of relatively weak protease inhibitors may have allowed cleavage of the precursor during the purification procedure. We have also found that LPS and various cytokines elicit relatively little augmentation of AOAH activity toward LPS in human monocytes and neutrophils.<sup>3</sup>

The extent to which AOAH biosynthesis in phagocytic cells matches that observed here in BHK670 cells is uncertain. In cells that naturally produce it (monocytes, macrophages, neutrophils, and endothelial cells), AOAH is present in such low abundance that immunolocalization has not been possible. In human neutrophils, the enzyme is not in the (lysosome-like?) azurophilic granules, although the ability of ammonium chloride to inhibit LPS deacylation by these cells suggests that AOAH is in an acidic compartment (21). Only mature AOAH has been purified from HL-60 cells and human neutrophils (i.e. little precursor form has been found) and these cells secrete relatively little of the AOAH that they produce. On the other hand, proteolytic processing of the precursor produces small subunits of similar sizes in both BHK cells and human neutrophils. It seems possible that, when rAOAH is produced in large amounts, as in transfected BHK cells, the mechanism for targeting the precursor to the site of proteolytic processing is constrained, so that regardless of whether or not the biosynthesis of AOAH in all of these cell types is identical, these studies in fibroblasts have revealed valuable information about the structure-function relationships of this unusual enzyme.

Acknowledgments—We thank R. Anderson for helpful advice, C. Slaughter and C. Moomaw for protein sequencing, R. Seale for plasmid constructs, L. Eidels for critical comments on the manuscript, and L. Frey for performing some of the lipid analyses.

REFERENCES
1. Hagen, F. S., Grant, F. J., Kuiper, J. L., Slaughter, C. A., Moomaw, C. R., Orth, R., O'Hara, P. J., and Munford, R. S. (1991) Biochemistry 30, 8415–8423
2. Persson, B., Bengtsson-Olovsson, G., Eserback, S., Olovsona, T., and Jernvall, H. (1989) Eur. J. Biochem. 179, 39–45
3. Kishimoto, Y., Hiraishi, M., and O'Brien, J. S. (1992) J. Lipid Res. 33, 1255–1267
4. Foster, D. C., Holly, R. D., sprecher, C. A., Walker, K. M., and Kumar, A. A. (1991) Biochemistry 30, 367–372
5. Slinn, A. E., Scott, T. C., and Lehrman, M. A. (1993) J. Biol. Chem. 268, 6729–6733
6. McPhaul, M. J., Nobel, J. F., Simpson, E. R., Mendelson, C. R., and Wilson, J. D. (1988) J. Biol. Chem. 263, 16358–16363
7. Alt, F. W., Kellemes, R. E., Bertino, J. R., and Schimke, R. T. (1978) J. Biol. Chem. 253, 1367–1370
8. Harlow, E., and Lane, D. (1988) Antibodies; A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
9. Anderson, R. G. W. (1986) Methods Enzymol. 129, 201–216
10. Laemmli, U. K. (1970) Nature 227, 680–685
11. Fujibayashi, S., and Wenger, D. A. (1986) J. Biol. Chem. 261, 15339–15343
12. Munford, R. S., Eidels, L., and Hansen, E. J. (1990) in Cellular and Molecular Aspects of Endotan Reactions (Nowotny, A. H., Spitzer, J. J., and Ziegler, E.

<sup>3</sup> A. Erwin, M. Luchi, and R. S. Munford, unpublished results.
Acyloxyacyl Hydrolase Structure-Function

J., eds) pp. 305–313, Elsevier Science Publishers B.V., Amsterdam
13. Schäger, H., and von Jagow, G. (1987) Anal. Biochem. **166**, 368–379
14. Munford, R. S., and Erwin, A. L. (1992) *Methods Enzymol.* **209**, 485–492
15. Erwin, A. L., and Munford, R. S. (1990) *J. Biol. Chem.* **265**, 16444–16449
16. Munford, R. S., and Hunter, J. P. (1992) *J. Biol. Chem.* **267**, 10116–10121
17. van Tilbeurgh, H., Egloff, M.-P., Martinez, C., Rugani, N., Verger, R., and Cambillau, C. (1993) *Nature* **362**, 814–820
18. Kretz, K. A., Carson, G. S., Morimoto, S., Kishimoto, Y., Fluharty, A. L., and O'Brien, J. S. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 2541–2544
19. Hiraiwa, M., Soeda, S., Martin, B. M., Fluharty, A. L., Hirabayashi, Y., O'Brien, J. S., and Kishimoto, Y. (1993) *Arch. Biochem. Biophys.* **303**, 326–331
20. Morimoto, S., Kishimoto, Y., Tomich, J., Weiler, S., Ohashi, T., Barranger, J. A., Krets, K. A., and O'Brien, J. S. (1990) *J. Biol. Chem.* **265**, 2933–2937
21. Luchi, M., and Munford, R. S. (1993) *J. Immunol.* **151**, 959–969