Brain intracellular platelet-activating factor acetylhydrolase (PAF-AH) isoform I is a member of a family of complex enzymes composed of mutually homologous α₁ and α₂ subunits, both of which account for catalytic activity, and the β subunit. We previously demonstrated that the expression of one catalytic subunit, α₁, is developmentally regulated, resulting in a switching of the catalytic complex from α₁/α₂ to α₁/α₂ during brain development (Manya, H., Aoki, J., Watanabe, M., Adachi, T., Asou, H., Inoue, Y., Arai, H., and Inoue, K. (1998) J. Biol. Chem. 273, 18567–18572). In this study, we explored the biochemical differences in three possible catalytic dimers, α₁/α₁, α₁/α₂, and α₂/α₂. The α₁/α₁ homodimer exhibited different substrate specificity from the α₁/α₂ homodimer in which showed similar substrate specificity. The α₁/α₂ homodimer hydrolyzed PAF and 1-O-sn-alkyl-2-acetyl-sn-glycero-3-phosphorylethanolamine (AAGPE) most efficiently among 1-O-sn-alkyl-2-acetyl-sn-sn-glycero-3-phosphorylethanolamine (AAGPE) and 1-O-sn-alkyl-2-acetyl-sn-glycero-3-phosphoric acid more efficiently than PAF. AAGPE was the poorest substrate for these catalytic complexes from the α₁/α₂ homodimer when expressed individually in E. coli (3). Recently, the crystal structure of the α₁/α₂ homodimer was determined (4). Surprisingly, the α₁ subunit has a tertiary fold reminiscent of small GTPases such as p21ras and harbors a chymotrypsin-like Ser-Asp-His triad in its active site (4). Although the catalytic subunits of PAF acetylhydrolase were first identified as a α₁/α₂ heterodimer from young adult bovine brain (1), recent studies have revealed that the α₁/α₂ homodimers are present in vivo as well (5). We have demonstrated in a previous study that expression of the α₁ polypeptide is restricted to actively migrating neurons at the embryonic and early postnatal stages in rats and that switching of the catalytic dimer from the α₁/α₂ heterodimer to the α₁/α₁ homodimers occurs in these cells during brain development (5, 6). Although both the α₁/α₂ heterodimer and the α₁/α₂ homodimer hydrolyze PAF equally 

in vitro, it is essential to elucidate the differences between those catalytic dimers in order to understand the significance of the switching of catalytic subunits and the function of this enzyme. In the present study, we studied the biochemical properties of three possible catalytic dimers, α₁/α₁, α₁/α₂, and α₂/α₂, and their complexes with the β subunit. One striking feature of brain PAF-AH isoform I is that the gene for the β subunit is identical to a causative gene (LIS1) for Miller-Dieker lissencephaly (7). Miller-Dieker lissencephaly is a genetic brain malformation manifested as a smooth cerebral surface caused by abnormal neuronal migration at early developmental stages. Recent data demonstrated that deletions or mutations of the β/LIS1 gene accounts for approximately 40% of classic lissencephaly (8). Point mutations in the β subunit were recently reported in the LIS1 gene from isolated lissencephaly sequence patients (9). In one case of the mutant β, histidine 149 was replaced with arginine. Although it is assumed that this amino acid substitution may produce a radical change in steric hindrance due to the loss of an imidazolic ring, direct evidence for the functional abnormality of this mutation has not been presented so far. The effect of mutation in the β subunit on the interaction with α subunit was also examined.

Experimental Procedures

Baculoviruses—Recombinant α₁ and α₂ were expressed either by the E. coli system (3) or the baculovirus system. Reombinant β was expressed by the baculovirus system, since protein was not recovered from soluble fractions of E. coli. The production of recombinant baculoviruses was designed as follows. The coding regions of bovine α₁, α₂, and β enzyme consisting of two catalytic α₁ and α₂ subunits and a noncatalytic β subunit (1). The oligomeric brain PAF-AH contains a dimer of two highly homologous catalytic subunits, α₁ and α₂, that share 63% amino acid sequence identity but are not related to any other known proteins (2, 3). The α₁ and α₂ subunits form a heterodimer in the PAF-AH purified from the bovine brain cortex (1). They can also form a catalytically active homodimer when expressed individually in E. coli (3).
cDNA were inserted into the SalI/NotI sites of pFASTBAC (Life Technologies, Inc.). His_Tag → Arg mutant β was prepared as follows: two oligonucleotides, 5′-GGGGGAAATTTAGGCCTGCTTGGCCTTGGGGGGA CAA-3′ (nucleotide positions 124 of bovine β cDNA (7)) and 5′-GAG CCCCCAGAGCACACGACAAATG-3′ (nucleotide positions 485-508 of bovine β), were used as primers in a polymerase chain reaction (PCR) to introduce a mutation. The amplified PCR product and oligonucleotide, CCCCCGATTCCTACACAGGGCCTTTCAAG GTC (nucleotide positions 1348–1371 of bovine β), were then used as primers for a second PCR. The resulting PCR product was introduced into the SalI/NotI sites of the pFASTBAC plasmid. Recombinant baculoviruses were prepared according to the manufacturer’s protocol (Bac to Bac System, Life Technologies, Inc.). For infection, Sf9 cells were mixed with the recombinant viruses at a multiplicity of infection of 10 in various combinations of recombinant viruses and incubated at 27°C for 72 h.

Separation of Each Catalytic Dimer (α1/α1, α2/α2, and α1/α2) Expressed Using Baculovirus System—The Sf9 cells (4 × 10^6) infected with each baculovirus were homogenized in 10 ml of SET buffer (10 mM Tris-HCl, 250 mM sucrose, pH 7.4), and the cell supernatants were recovered by ultracentrifugation at 100,000 × g. Each recombinant protein was purified using DEAE ion exchange, hydroxyapatite, and anion exchange column chromatographies as follows. All column chromatographies were performed at 4°C using fast protein liquid chromatography system (Amersham Pharmacia Biotech). DEAE-ion-exchange chromatography was performed essentially as described previously (5). The cell supernatants obtained from the infected Sf9 cells were loaded onto a DEAE-Sepharose Fast Flow column (5 ml, Amersham Pharmacia Biotech) (flow rate 1 ml/min), which had been equilibrated with buffer A (0.1 M NaCl, 10 mM Tris-HCl, 10% glycerol, pH 7.4) and eluted with a linear gradient of 0.1–1 M NaCl in buffer A. The active fractions as judged by PAF-AH activity assay were further applied onto hydroxyapatite column (Econo-Pac CHT II, 5 × 50 mm, Bio-Rad) (flow rate 0.8 ml/min), which had been equilibrated with buffer B (1 mM KH2PO4, 10% glycerol, pH 6.8). The proteins were eluted with a linear gradient of 1–400 mM KH2PO4 in buffer B. Each catalytic dimer was separated by anion exchange column (POROS H-Q/M, 4.6 × 100 mm) (Perspective Biosystems, Inc. Framingham, MA.). After the column was first equilibrated with buffer C (10 mM Tris-HCl, 1 mM EDTA, 10% glycerol, pH 7.4), the active fractions from hydroxyapatite column chromatography which was dialyzed against buffer C were loaded onto the column and eluted with a linear gradient of 0–0.5 M NaCl in buffer C (flow rate 4 ml/min).

Preparation of the β Polypeptide—For the production of the β protein, the baculovirus (4 × 10^6) infected with β baculovirus were homogenized in 10 ml of SET buffer (10 mM Tris-HCl, 250 mM sucrose, pH 7.4), and the cell supernatants were recovered by ultracentrifugation at 100,000 × g. The β protein was purified by DEAE-Sepharose and hydroxyapatite column chromatographies, as described above.

PAF-AH Assays—PAF-AH assays were performed as described previously (5), except that the radioactivity of liberated [3H]acetate was counted by a β-counter. A kinetic study was performed using various concentrations of PAF as a substrate of an assay as described previously (2).

Cross-linking—Cross-linking was performed as described previously (5). Briefly, each sample was dialyzed against 100 mM sodium phosphate (pH 8.0), cross-linked by adding 10 mM BS3 (Pierce), and then subjected to SDS-PAGE and Western blotting. Polyclonal antibodies, α1, α2, and β were used to detect cross-linked products, since our monoclonal antibodies failed to react with the BS3-treated polypeptides.

Preparation of PAF Analogs—1-O-Alkyl-2-acyl-sn-glycero-3-phosphoryl ethanolamine (AAGPE) and 1-O-alkyl-2-acyl-sn-glycero-3-phosphoric acid (AAGPA) were prepared according to the method described by Satouchi et al. (10) using base exchange reaction by phospholipase D (from Actinomadura, Meito Sangyo, Tokyo, Japan). Briefly, nonradioactive PAF (C16, 5 mg) mixed with [3H]-PAF (87 μCi) was subjected to phospholipase D reaction in the presence of ethanolamine (200 mM for AAGPE). For AAGPA, synthesis nonradioactive PAF (C16, 5 mg) mixed with [3H]-PAF (87 μCi) was subjected to phospholipase D reaction in the absence of alcohol. The resulting products were isolated by preparative thin layer chromatography. Activity assays using these analogs were performed as described above, except that 0.05 N HCl was added when liberated acetic acid was separated from the substrate when AAGPA was used.

DFP Labeling—Chemical labeling of catalytic subunits was performed as described previously (3), except that [3H]DFP (NEN Life Science Products) was used in this study. Briefly, 2 μg of recombinant catalytic subunits was incubated with 0.1 μCi of [3H]DFP for 30 min at room temperature. The incorporation of radiolabeled DFP into the catalytic subunits was detected by SDS-PAGE and subsequent autoradiography.

results

Expression of Various Catalytic Complexes in the Sf9 Cells—In order to obtain various combinations of catalytic dimers, the α1 and α2 subunits of brain PAF-AH were expressed in the Sf9 cells by infecting the Sf9 cells with recombinant baculoviruses. The expression of catalytic dimers in the cytosol of Sf9 cells infected with both the α1 and α2 viruses was examined with POROS-HQ/M anion exchange column chromatography. Both α1 and α2 subunits were detected in a single peak when expressed individually in Sf9 cells (Figs. 1A and B). In contrast, when these two subunits were co-expressed in the cells, three peaks of activity were detected after the column chromatography (Fig. 1C). By immunoblot analysis (Fig. 1C, bottom), we found that the α1 polypeptide was detected in both the first and second peak fractions, and the α2 polypeptide was detected in the second and third peak fractions. These data...
Fig. 2. Cross-linking of α subunits. The α₁ or α₂ proteins in the three peaks (peaks I, II, and III) in Fig. 1C were cross-linked using BS₃ and detected by Western blotting using anti-α₁ (1st to 4th lanes) or anti-α₂ (5th to 8th lanes) antibodies. The results before (1st, 3rd, 5th, and 7th lanes) and after (2nd, 4th, 6th, and 8th lanes) cross-linking reaction are shown. Molecular size is shown at left.

suggested that the α₁/α₂ heterodimer and the α₁/α₁ and α₂/α₂ homodimers were formed in the Sf9 cells infected with both α₁ and α₂ viruses. The α₁/α₂ heterodimer was predominantly but not exclusively formed under the present conditions.

Dimer formation of the catalytic subunits in each fraction in Fig. 1C was examined by cross-linking studies. Incubation of each activity fraction with the cross-linking agent BS₃ yielded similar ~60-kDa bands. The 60-kDa bands obtained from the first and third peak fractions cross-reacted with the anti-α₁ and -α₂ antibodies, respectively, and the 60-kDa bands from the second peak fraction cross-reacted with both the anti-α₁ and -α₂ antibodies (Fig. 2). This indicates that these three peak fractions represent the α₁/α₁, α₁/α₂, and α₂/α₂ dimers.

Substrate Specificity—By using recombinant proteins, we first examined the substrate specificities of three different catalytic complexes. The substrates used in this study were 1-O-alkyl-2-acetyl-glycerophospholipids with different head groups. Each recombinant catalytic complex produced in the Sf9 cells was purified to near-homogeneity (see Fig. 4A) by chromatographies, as described under “Experimental Procedures.” As shown in Fig. 3, the α₂/α₂ homodimer exhibited different substrate specificity from the α₁/α₁ homodimer and the α₁/α₂ heterodimer, both of which showed similar substrate specificity to each other. The α₁/α₂ homodimer hydrolyzed PAF and 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylethanolamine (AAGPE) more efficiently than 1-O-alkyl-2-acetyl-sn-glycero-3-phosphoric acid (AAGPA). In contrast, both α₁/α₁ and α₁/α₂ hydrolyzed AAGPA more efficiently than PAF. AAGPE was the poorest substrate for these enzymes. The α₂/α₂ homodimer hydrolyzed PAF 3–4 times better than the α₁/α₁ heterodimer and the α₁/α₁ homodimer. When AAGPE was used as a substrate, change in composition of the catalytic dimer from α₁/α₂ to α₂/α₂ induced a dramatic increase in the catalytic efficiency. It was demonstrated from these observations that the catalytic complexes of PAF-AH isozyme I can hydrolyze PAF analogs with a different head group depending on the composition of the catalytic subunit. Moreover, it was shown that the α₁/α₂ heterodimer and the α₂/α₂ homodimer, both of which were detected in vivo, exhibited different substrate specificity.

The Active Site Labeling with [³H]DFP—As shown in Fig. 3, the α₁/α₂ heterodimer exhibited a substrate specificity similar to the α₁/α₁ homodimer rather than the α₂/α₂ homodimer, suggesting that catalysis by the α₁ subunit dominates in the α₁/α₂ heterodimer. To test this possibility, we performed an active site labeling study using [¹⁴C]DFP, which binds covalently to an active serine residue. When the recombinant α₁/α₁ and α₂/α₂ homodimers were incubated with [¹⁴C]DFP, both α₁ and α₂ polypeptides were labeled with [¹⁴C]DFP (Fig. 4). The α₁ subunit was preferentially labeled with this reagent when the α₁/α₂ heterodimer was used. These data are consistent with previous observations that the α₁ subunit is preferentially labeled by [³H]DFP in the purified PAF-AH (isoform Ib) from bovine brain (1). These data suggest that a serine residue of the α₂ subunit catalytic center is shielded to be inactive in the α₁/α₂ heterodimer, although the same residue in the α₁/α₂ homodimer is catalytically active.

Effect of the β Subunit on the Enzyme Activity of Each Catalytic Complex—Next, we examined whether each catalytic dimer can associate with the β subunit. Each catalytic dimer was prepared by Sf9 cells as described above. The recombinant β subunit also prepared by baculovirus system in Sf9 cells was purified to near-homogeneity by column chromatographies, as described under “Experimental Procedures.” Each catalytic dimer was incubated with the β subunit on ice for 60 min, and then the mixture was analyzed with hydroxyapatite column.
chromatography. When each purified catalytic dimer was applied to the column, the activity was eluted as a single peak (Fig. 5, A—C), respectively, whereas preincubation of the catalytic complexes with the β subunit generated new activity peaks. These peaks contained the respective catalytic subunit and the β subunit (Fig. 5, D—F), indicating the association of catalytic dimers with the β subunit.

We then examined the effect of the β subunit on the enzyme activity of each catalytic dimer. As shown in Fig. 6C, the enzyme activity of the α₂/α₂ homodimer increased significantly by adding the recombinant β. In the optimum β concentration (5 μg per tube), the enzyme activity of the α₂/α₂ homodimer increased approximately 4-fold. Increasing β to more than 5 μg per tube did not cause a further increase in enzyme activity (data not shown) under the present conditions. In contrast, β slightly suppressed the activity of the α₁/α₁ homodimer (Fig. 6A) and essentially had no effect on that of the α₁/α₂ heterodimer (Fig. 6B). We performed the same experiments using the native β purified from bovine brain instead of the recombinant β, obtaining essentially the same results (data not shown).

We also tested the effect of the β subunit on catalytic activity in SF9 cells. The SF9 cells were infected with either the α₁ baculovirus or the α₂ baculovirus with increasing amounts of β baculovirus, and the lysates of the infected cells were examined for protein expression and PAF-AH activity. The PAF-AH activity of the α₁/α₁ homodimer was decreased in proportion to the increase of the β expression, whereas that of the α₂/α₂ homodimer was rather increased (data not shown). The levels of α₁ and α₂ subunit expression were not affected appreciably by the co-expression of the β subunit. These findings also support the idea that the β subunit stimulates the activity of the α₂/α₂ homodimer but suppresses that of the α₁/α₁ homodimer.

Effect of His₁⁴⁹ to Arg Mutation in the β Subunit—Like wild-type β/LIS1 protein the mutant β could be expressed in SF9 cells and purified. The mutant β subunit exhibited no effect on the catalytic activity of all the catalytic dimers (data not shown). Unlike the native β subunit, as judged from the failure
Catalytic Complexes of PAF Acetylhydrolase

Fig. 6. Effect of β on the catalytic activity of α₁/α₁, α₁/α₂, and α₁/α₂ in vitro. Recombinant purified α₁/α₁, α₁/α₂, and α₁/α₂ (the specific activity is 0.45 nmol/min/μg (α₁/α₁), 0.55 nmol/min/μg (α₁/α₂), 1.76 nmol/min/μg (α₁/α₂), respectively), and β proteins were used in this study. To form complexes, each catalytic dimer (α₁/α₁ (A), α₁/α₂ (B), and α₁/α₂ (C), each 0.05 μg) was mixed on ice for 60 min with increasing amounts of β, and PAF acetylhydrolase activity was measured. PAF-hydrolyzing activities in the absence of β protein were indicated as 100%. The values are the means ± S.E. of three determinations.

Fig. 7. H1149R mutant β lost activity to bind to the catalytic dimers. The mutant β proteins were expressed in Sf9 cells and purified. α₁(2 μg) was mixed in vitro with the mutant β proteins (5 μg), and the mixtures were subjected to hydroxyapatite column chromatography. The elution profiles of PAF acetylhydrolase activity (A) and each subunit detected by Western blotting are shown (B).

Discussion

Brain PAF-AH (also PAF-AH isoform I) contains two mutually homologous α₁ and α₂ subunits, both of which can form a respective homodimer and a heterodimer, and in fact, the α₁/α₂ heterodimer and the α₂/α₂ homodimer have been detected in vivo (1, 5). In rats, α₁ expression is restricted in early developing neurons at embryonic stages at which the neural cell migration is most active, and switching of catalytic subunits from the α₁/α₂ heterodimer to the α₂/α₂ homodimer occurs during brain development (5). It is also well established that the other subunit of PAF-AH isoform I, β, is a product of the causative gene for Miller-Dieker syndrome, which has a defect in neuronal migration during brain development. It was thus essential to delineate the biochemical differences of each catalytic dimer in order to understand the biological role of PAF-AH. In the present study, we have demonstrated that each catalytic dimer exhibited distinct substrate specificity and that their enzyme activity is modulated by the β subunit in catalytic subunit composition-dependent manner.

Previously, we showed that PAF-AH isoform I has strict specificity for the acetyl group attached to the sn-2 position of phosphoglyceride as a substrate (11). The essential amino acid residue involved in the recognition of acetyl moiety has also been postulated from crystallographic studies (4). However, it was unknown whether the enzyme may recognize the phospholipid substrate with a different head group. In this study, we demonstrated that the enzyme hydrolyzed the phospholipid substrate with a head group other than choline moiety. 1-O-Alkyl-2-acetyl-sn-glycero-3-phosphatidylethanolamine (AAGPE) or alk-1-enlyacylglycerophosphoethanolamine (2-acetyl-plasmalogen) can also be synthesized in vivo. Lee et al. (15) demonstrated 2-acetyl-plasmalogen is produced in intact HL-60 cells by incubating with PAF after stimulating with calcium ionophore A-23187. They also reported the presence of a unique membrane-associated transacylase that transfers the acetate group from PAF to lysoplasmalogen with the formation of 2-acetyl-plasmalogen (15). Since the α₁/α₂ homodimer hydrolyzes AAGPE very efficiently, it is highly possible that 2-acetyl-plasmalogen can be hydrolyzed by the enzyme as well. It has not been reported so far that 2-acetyl-plasmalogen may have a role in the cells. However, if it is a new type of intracellular lipid messenger (14), the level of which is regulated by PAF-AH isoform I.

1-O-Alkyl-2-acetyl-sn-glycero-3-phosphatidyethanolamine (AAGPE) or alk-1-enlyacylglycerophosphoethanolamine (2-acetyl-plasmalogen) can also be synthesized in vivo. Lee et al. (15) demonstrated 2-acetyl-plasmalogen is produced in intact HL-60 cells by incubating with PAF after stimulating with calcium ionophore A-23187. They also reported the presence of a unique membrane-associated transacylase that transfers the acetate group from PAF to lysoplasmalogen with the formation of 2-acetyl-plasmalogen (15). Since the α₁/α₂ homodimer hydrolyzes AAGPE very efficiently, it is highly possible that 2-acetyl-plasmalogen can be hydrolyzed by the enzyme as well. It has not been reported so far that 2-acetyl-plasmalogen may have a role in the cells. However, if it is a new type of intracellular lipid messenger (14), the level of which is regulated by PAF-AH isoform I.
The substrate specificity and the specific activity of the \(a_1/a_2\) heterodimer were very similar to the \(a_1/a_1\) homodimer but not to the \(a_2/a_2\) homodimer. We previously reported that DFP, a reagent that specifically binds to an active serine residue, reacted preferentially with the \(a_1\) subunit in the isoform 1b, suggesting that the catalytic serine residue of the \(a_2\) subunit is shielded to be inactive. A crystallographic study of the \(a_1/a_1\) homodimer revealed that the two active sites are at the bottom of the catalytic gorge, only 12 Å from each other (4). This proximity suggests that these two active sites do not function independently, since this catalytic gorge is capable of accommodating only one PAF molecule. Thus we speculate that only one active serine residue is enough for catalysis. It is also possible that one active serine out of two in the catalytic gorge of all three dimers are actually shielded to be inactive. A substrate specificity study as well as DFP labeling experiments have demonstrated that catalysis by the \(a_2\) subunit may dominate in the \(a_1/a_2\) heterodimer. We have detected the \(a_1/a_2\) heterodimer and the \(a_2/a_2\) homodimer in vivo, but the \(a_1/a_1\) homodimer has not been detected yet. It is still possible that the \(a_1/a_1\) homodimer exists in vivo, but even the \(a_1/a_2\) heterodimer can be substituted for the \(a_1/a_1\) homodimer in terms of substrate specificity. Which amino acid residue(s) is critical for determining the substrate specificity in the catalytic subunits? According to the crystal structure of the \(a_1/a_1\) homodimer, the amino acid residues Tyr\(^{191}\) and Tyr\(^{193}\) are exposed to the catalytic gorge and possibly recognize a head group of PAF (4). Interestingly, these Tyr residues of the \(a_1\) subunit are substituted into Phe\(^{192}\) and Phe\(^{194}\) in the \(a_2\) subunit (4). Our preliminary mutation study demonstrated that the exchange of Tyr\(^{191}\) into Phe gives the \(a_1\) subunit a substrate specificity similar to that of \(a_2\).

We previously concluded that the \(\beta\) subunit does not possess a regulatory role on catalytic activity, based on the observation that the \(\beta\) subunit can be dissociated without loss of enzyme activity from the \(a_1\cdot a_2\cdot \beta\) complex purified from bovine brain (7). We demonstrated in the present study that this was true only for the \(a_1/a_2\) heterodimer. The rate of PAF hydrolysis by the \(a_2/a_2\) homodimer was, however, accelerated about 4 times by the addition of \(\beta\), whereas the rate of hydrolysis by the \(a_1/a_1\) was slightly suppressed by it. In the rat brain, composition of the catalytic subunit of PAF-AH changes drastically from the \(a_1/a_2\) heterodimer to the \(a_2/a_2\) homodimer during development. These changes in the catalytic subunit from \(a_1/a_2\) to \(a_2/a_2\) may result in a drastic increase in the PAF-AH activity, since (i) \(a_1/a_2\) hydrolyzes PAF four times better than \(a_1/a_2\) does, and (ii) only the enzyme activity of \(a_1/a_2\) is accelerated by \(\beta\). Our preliminary study shows that PAF-AH activity in the rat brain is much higher in adulthood than that in embryonic stages, although the expression levels of \(a_1\) and \(\beta\) were roughly the same during this period. Change in enzyme activity can be explained by the change in the composition of the catalytic subunit. The change in PAF-AH activity during brain development might regulate PAF content in neural cells. Consistent with this idea, Tokumura et al. (14) have reported that PAF content in the young rat brain is higher than that in the adult brain.

In conclusion, we have demonstrated that the enzyme activity of PAF-AH isoform I is regulated in multiple ways by switching the combination of the catalytic subunit and by manipulating the \(\beta\) subunit. We have also postulated the possibility that some 1-O-alkyl-2-acetyl-phospholipids other than PAF may be genuine substrates for the enzyme. It is still unclear at present which effect is relevant in vivo and what the in vivo substrate for this enzyme is. Our next challenge will be to identify the in vivo lipid substrate for this enzyme and its cellular function.

**REFERENCES**

1. Hattori, M., Arai, H., and Inoue, K. (1993) *J. Biol. Chem.* 268, 18748–18753
2. Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1994) *J. Biol. Chem.* 269, 23150–23155
3. Hattori, M., Adachi, H., Aoki, J., Tsujimoto, M., Arai, H., and Inoue, K. (1995) *J. Biol. Chem.* 270, 31345–31352
4. Ho, Y. S., Swenson, L., Derewenda, L. S., Wei, Y., Dauter, Z., Hattori, M., Adachi, T., Aoki, J., Arai, H., Inoue, K., and Derewenda, Z. S. (1997) *Nature* 385, 89–93
5. Manya, H., Aoki, J., Watanabe, M., Adachi, T., Asoo, H., Inoue, Y., Arai, H., and Inoue, K. (1998) *J. Biol. Chem.* 273, 18567–18572
6. Albrecht, U., Abu-Issa, R., Ratz, B., Hattori, M., Aoki, J., Arai, H., Inoue, K., and Eichele, G. (1996) *Dev. Biol.* 180, 579–593
7. Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1994) *Nature* 370, 216–218
8. Pilz, D. T., Matsumoto, N., Minnerath, S., Mills, P., Gleeson, J. G., Allen, K. M., Walsh, C. A., Barkovich, A. J., Dohyns, W. B., Ledbetter, D. H., and Ross, M. E. (1998) *Hum. Mol. Genet.* 7, 2029–2037
9. Lo, N. C., Chong, C. S., Smith, A. C., Dobyns, W. B., Carrozzi, R., and Ledbetter, D. H. (1997) *Hum. Mol. Genet.* 6, 157–164
10. Satouchi, K., Pinckard, R. N., McManus, L. M., and Hanahan, D. J. (1981) *J. Biol. Chem.* 256, 4425–4432
11. Hattori, K., Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., and Inoue, K. (1995) *J. Biol. Chem.* 270, 22308–22313
12. Snyder, F., Fitzgerald, V., and Blank, M. L. (1996) *Adv. Exp. Med. Biol.* 416, 5–10
13. Baker, R. R. (1995) *Neurochem. Res.* 20, 1345–1351
14. Tokumura, A., Yotsumoto, T., Hoshikawa, T., Tanaka, T., and Tsukatani, H. (1992) *Life Sci.* 51, 303–308
15. Lee, T. C., Uemura, Y., and Snyder, F. (1992) *J. Biol. Chem.* 267, 19992–20001