

Biosynthesis of the Unsaturated 14-Carbon Fatty Acids Found on the N Termini of Photoreceptor-specific Proteins*

(Received for publication, October 3, 1995, and in revised form, December 11, 1995)

James C. DeMar, Jr.‡§, Theodore G. Wensel‡, and Robert E. Anderson¶||

From the ‡Department of Biochemistry, Baylor College of Medicine, Houston, Texas, 77030 and the ¶Dean A. McGee Eye Institute, Oklahoma Center for Neurosciences and the Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, 73104

In the vertebrate retina, a number of proteins involved in signal transduction are known to be N-terminal acylated with the unusual 14 carbon fatty acids 14:1*n*-9 and 14:2*n*-6. We have explored possible pathways for producing these fatty acids in the frog retina by incubation *in vitro* with candidate precursor fatty acids bearing radiolabels, including [³H]14:0, [³H]18:1*n*-9, [³H]18:2*n*-6, and [³H]18:3*n*-3. Rod outer segments were prepared from the radiolabeled retinas for analysis of protein-linked fatty acids, and total lipids were extracted from the remaining retinal pellet. Following saponification of extracted lipids, fatty acid phenacyl esters were prepared and analyzed by high pressure liquid chromatography (HPLC) with detection by continuous scintillation counting. Transducin, whose α -subunit (G_{α}) is known to bear N-terminal acyl chains, was extracted from the rod outer segments and subjected to SDS-polyacrylamide gel electrophoresis and fluorography to detect radiolabeled proteins. G_{α} was also subjected to methanolysis, and the resulting fatty acyl methyl esters were analyzed by HPLC. The identities of HPLC peaks coinciding with unsaturated species of both phenacyl esters and methyl esters were confirmed by reanalyzing them after catalytic hydrogenation. The results showed that 14:1*n*-9 can be derived in the retina from 18:1*n*-9 and 14:2*n*-6 from 18:2*n*-6, most likely by two rounds of β -oxidation, but that neither is produced in detectable amounts from 14:0. Retroconversion of unsaturated 18 carbon fatty acids to the corresponding 14 carbon species showed specificity, in that 18:3*n*-3 was not converted to 14 carbon fatty acids in detectable amounts. Myristic acid (14:0), 14:1*n*-9, and 14:2*n*-6 were all incorporated into G_{α} . A much less efficient incorporation of 18:1*n*-9 into G_{α} was also observed, but no radiolabeling of G_{α} was observed in ret-

inas incubated with 18:3*n*-3. Thus, retroconversion by limited β -oxidation of longer chain unsaturated fatty acids appears to be the most likely metabolic source of the unusual fatty acids found on the N termini of signal transducing proteins in the retina.

A number of proteins of both eukaryotic and viral origin are modified by fatty acylation through an amide linkage to N-terminal glycine residues (reviewed by James and Olson (1990), McIlhinney (1990), Towler *et al.* (1988), Schlesinger (1993), and Gordon *et al.* (1991)). This modification, which in the great majority of cases studied involves the saturated 14 carbon fatty acid myristate (14:0), has been shown to play an important role in the function of these proteins (Jones *et al.*, 1990; Linder *et al.*, 1991; Yonemoto *et al.*, 1993; Wedegaertner *et al.*, 1995). In vertebrate retinas, the types of N-terminal fatty acids for proteins involved in signal transduction are strikingly different from those found in other tissues. These proteins, which include the α -subunit of the G protein transducin (G_{α})¹ (Johnson *et al.*, 1994; Kokame *et al.*, 1992; Neubert *et al.*, 1992; Yang and Wensel, 1992), guanylyl cyclase-activating protein (GCAP) (Palczewski *et al.*, 1994), recoverin (Dizhoor *et al.*, 1992; Johnson *et al.*, 1994), and the catalytic subunit of cAMP-dependent protein kinase (Johnson *et al.*, 1994), are heterogeneously acylated with frequent occurrence of 14:1*n*-9 and 14:2*n*-6 in addition to 14:0 and 12:0. This unusual pattern appears to reflect unusual pathways for synthesizing and utilizing fatty acids for N-terminal acylation in the retina rather than specific characteristics of the acylated proteins. The ubiquitous protein kinase is exclusively modified with 14:0 in the brain and heart (Carr *et al.*, 1982; Johnson *et al.*, 1994) but in the retina shows the same heterogeneous pattern of fatty acylation as G_{α} . Indeed, although 12:0 and 14:0 are found in animal tissues, 14:1*n*-9 and 14:2*n*-6 are quite rare and have been found in abundance only in marine mammals (14:1*n*-9) (Markley, 1960) and the Asian plant *Evodia rutaecarpa* (14:2*n*-6) (Kurono *et al.*, 1972).

It is conceivable that the two unsaturated fatty acids could arise from the desaturation of 14:0. However, previously described Δ^5 -desaturase enzymes prefer long chain polyunsaturated fatty acids as substrates (Numa *et al.*, 1984), and Δ^8 -desaturases have only been found in a few cell types, such as testicular Sertoli (Oulhaj *et al.*, 1992) and brain glioma cells (Cook *et al.*, 1991). Alternatively, these unusual 14-carbon fatty acids might arise by retroconversion from longer chain fatty

* This work was supported by NEI National Institutes of Health Grants EY07001, EY00871, EY04149, and EY02520 (to R. E. A.) and EY07981 (to T. G. W.), by grants from the Retina Research Foundation, by Research to Prevent Blindness Inc., by the National Retinitis Pigmentosa Foundation Inc., by the Samuel Roberts Nobel Foundation, Inc., and by the Presbyterian Health Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The work presented in this paper is based on platform presentations given by James C. DeMar, Jr., at the 86th American Oil Chemists Society 1995 Annual Meeting, San Antonio, TX and the Association for Research in Vision and Ophthalmology 1995 Annual Meeting, Ft. Lauderdale, FL.

§ Recipient of 1995 Honored Student and Outstanding Paper Presentation awards from the American Oil Chemists Society and a 1995 Association for Research in Vision and Ophthalmology/National Eye Institute Travel Fellowship.

|| To whom correspondence should be addressed: Dean A. McGee Eye Inst., 608 Stanton L. Young Blvd., Oklahoma City, OK 73104. Tel.: 405-271-8250; Fax: 405-271-8128.

¹ The abbreviations used are: G_{α} , transducin α -subunit; FAME, fatty acid methyl ester; FAPE, fatty acid phenacyl ester; G_{β} , transducin β -subunit; G_{γ} , transducin γ -subunit; HPLC, high pressure liquid chromatography; PAGE, polyacrylamide gel electrophoresis; ROS, rod outer segments; GTP- γ S, guanosine 5'-3'-O-(thio)triphosphate.

acids through partial β -oxidation. For example, 18:1*n*-9 (oleic acid) or 18:2*n*-6 (linoleic acid) might be converted by this route to 14:1*n*-9 and 14:2*n*-6, respectively. Indeed, it has been hypothesized that 14:2*n*-6 is generated within the photoreceptor cell in this manner (Hansen, 1993; Wang and Anderson, 1993). Such partial β -oxidation is characteristic of peroxisomal metabolism in contrast to mitochondrial β -oxidation, which favors complete degradation to acetyl-CoA (Schulz, 1991). Retroconversion pathways of this kind have been demonstrated to convert 13-hydroxy-9,11-octadecadienoic acid (13-OH, 18:2*n*-6) to 9-hydroxy-5,7-tetradecadienoic acid (13-OH, 14:2*n*-6) in lymphocytes (Hadjuagapiou *et al.*, 1990) and to 18:2*n*-6 to 14:2*n*-6 in rat liver peroxisomes (Baykousheva *et al.*, 1994). In the retina, a similar retroconversion pathway was found to convert 22:5*n*-3 to 20:5*n*-3 (Wang and Anderson, 1993). We describe here experiments designed to determine if retroconversion pathways can produce 14:1*n*-9 and 14:2*n*-6 in the frog retina and if they are used for N-terminal fatty acylation of $G_{\alpha\beta}$.

EXPERIMENTAL PROCEDURES

Animals—Adult frogs (*Rana pipiens*) were purchased from J. M. Hazen (Alburt, VT). Frogs were housed under a constant diurnal cycle of 10 h of dark and 14 h of light. All animal care tightly followed the guidelines set by the Association for Research in Vision and Ophthalmology Resolution for the use of Animals in Research and the NIH Guide for the Care and Use of Animals in Research. The experimental protocol was reviewed and approved by the Animal Protocol Review Committee of Baylor College of Medicine (Houston, TX).

[³H]Fatty Acids—[9,10-³H]14:0 (myristic acid) (31 Ci/mmol) and [9,10-³H]18:1*n*-9 (oleic acid) (10 Ci/mmol) were purchased from DuPont NEN. [9,10,12,13-³H]18:2*n*-6 (linoleic acid) (90 Ci/mmol) and [9,10,12,13,15,16-³H]18:3*n*-3 (linolenic acid) (120 Ci/mmol) were from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Each fatty acid was examined for purity as outlined in the section "Fatty Acid Phenacyl Ester Preparation and HPLC Analysis" and unless otherwise noted was found to be free of interfering contaminants. The [³H]fatty acid, 1 mCi total for [³H]-14:0 or [³H]-18:1*n*-9 and 120 μ Ci total for [³H]-18:2*n*-6 or [³H]-18:3*n*-3, was suspended by sonication in a 200- μ l aliquot of fatty acid-free (<0.003%) bovine serum albumin (Sigma) in 50 mM NaHCO₃, such that the final fatty acid/bovine serum albumin molar ratio was 2:1.

Preparation of Frog Retinas—All dissection procedures described here were performed under dim red lights. Dark-adapted frogs (16 h) were killed by decapitation followed by immediate pithing of the spinal column. Eyes were enucleated and placed in room temperature Krebs-Ringer buffer (118 mM NaCl, 4.7 mM KCl, 1.17 mM KH₂PO₄, 1.17 mM MgSO₄, 5.6 mM D-glucose, 35 mM NaHCO₃, and 1.0 mM EDTA, pH 7.4) and then converted to eyecups through removal of the cornea, lens, and vitreous humor. Retinas were detached by further incubation (15 min) of eyecups in room temperature Krebs-Ringer buffer. Retinas were removed and cleaned of adhering retinal pigmented epithelium using jewelers forceps and then stored in ice-cold Krebs-Ringer buffer.

Retina in Vitro Incubation—All ensuing incubation procedures were performed under dim room lighting (50–100 lux). Dissected frog retinas (24–32) were placed in a flask containing 3.8 ml of oxygenated (1 h with O₂/CO₂ (95:5)) Krebs-Ringer buffer supplemented with 2.5 mM CaCl₂ and antibiotic mixture (final concentrations, 20 units/ml penicillin G sodium, 20 μ g/ml streptomycin sulfate, and 50 ng/ml Fungizone (Squibb); purchased from Hazelton Research Products, Lenexa, KS). Retinas were preincubated for 20 min at 24 °C under constant humidified O₂/CO₂ (95:5) with mild agitation. Incubation was started by addition of the [³H]fatty acid/bovine serum albumin conjugate with final concentrations for each medium of 8 mM [³H]-14:0, 25 mM [³H]-18:1*n*-9, 0.34 mM [³H]-18:2*n*-6, and 0.25 mM [³H]-18:3*n*-3. Incubation was continued for 8 h under the same conditions as the preincubation step. Incubated retinas were gently washed by four sequential passages through dishes of ice-cold Ca²⁺-plus Krebs-Ringer buffer and then frozen at -20 °C in $d = 1.175$ g/ml sucrose buffer (7 mM Tris acetate, pH 7.4, 70 mM NaCl, and 0.7 mM MgCl₂).

Preparation of Rod Outer Segments and Retina Membranes—ROS were prepared by the method of Louie *et al.* (1988). Frozen retinas were thawed and immediately homogenized in additional $d = 1.175$ g/ml sucrose buffer containing 1 mM phenylmethylsulfonyl fluoride, which was sequentially overlaid with $d = 1.155$ g/ml, 1.135 g/ml, and 1.115 g/ml sucrose buffer (1 mM Tris acetate, pH 7.4, and 0.1 mM MgCl₂). After centrifugation at 75,000 $\times g$ for 90 min, ROS were collected from the d

$= 1.115/1.135$ interface. ROS were washed by 4-fold dilution in Tris acetate buffer (50 mM Tris acetate, pH 7.4, 5 mM MgCl₂, 0.1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride) with centrifugation at 27,000 $\times g$. Remaining gradient fractions were pooled, diluted, and pelleted in the same manner to yield retina membranes.

Lipid Extraction and Separation of Lipid Species—Membranes were homogenized in 2 ml of Tris acetate buffer and extracted twice with 2 ml of CHCl₃/MeOH/12 N HCl (100:100:1, v/v/v) and once with 1.5 ml of CHCl₃. Following centrifugation, the CHCl₃ layers were pooled, total lipid phosphorus was estimated by the method of Rouser *et al.* (1970), and total radioactivity was determined by scintillation counting. An equal portion of each extract was separated into phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine classes), free fatty acids, and triglycerides by TLC (Kupke and Zeugner, 1978) on a silica gel 60 TLC plate (EM Science), with development up one-third of the plate with CHCl₃/MeOH/H₂O (65:30:5, v/v/v) and complete development with hexane/Et₂O/HOAc (80:20:1.5, v/v/v). For analysis of total radioactivity in each class, positions were located by I₂ vapor and marked, and after I₂ sublimation, spots were scraped and counted in BCS mixture (Amersham Corp.) 1N HCl (15:1, v/v). For fatty acid analysis, spots corresponding to the lipid classes were located by spraying with 2,7-dichlorofluorescein and illuminating with UV and then scraped and analyzed as described below.

Fatty Acid Phenacyl Ester Preparation and HPLC Analysis—Total lipid extracts or isolated lipid classes were saponified for 45 min at 100 °C in 2% KOH/EtOH (w/v), diluted with H₂O, acidified with 12 N HCl, and extracted three times with hexane. Free fatty acid extracts were used to prepare FAPes according to the method of Wood and Lee (1983) as modified by Chen and Anderson (1993a). FAPes were separated on HPLC using a Supelco (Bellefonte, PA) Supelcosil[®] LC-18 column (25 cm \times 4.6 mm I.D.) with elution (2 ml/min) by a linear gradient of CH₃CN/H₂O starting at 80:20 (v/v), increasing to 92:8 in 45 min, holding at 92:8 for 10 min, and returning to 80:20 in 5 min. Elution of FAPes was monitored by UV absorbance at 242 nm. Radioactivity profiles were obtained with an on-line continuous scintillation counter (Flo-One: Radioactive Flow Detector A-200, Radiomatic, Tampa, FL) using Flo-scent A mixture (Packard Instrument Co., Inc., Meriden, CT) at a 2.5:1 (v/v) ratio with the column eluent. Preliminary identification of FAPe peaks was based upon retention times obtained for FAPes prepared from radiolabeled and nonradiolabeled fatty acid standards (Sigma). Fatty acid standards of 14:1*n*-9, 14:2*n*-6, and [1-¹⁴C]14:2*n*-6 were generously provided by Dr. Howard Sprecher (Department of Medical Biochemistry, Ohio State University).

Analysis of FAPes by Catalytic Hydrogenation—Individual FAPe peaks were collected and extracted three times with hexane, followed by saponification with 2% KOH/EtOH (w/v), as described above. Free fatty acids were solubilized in EtOH/hexane (2:1) and bubbled vigorously with hydrogen (20 min) in the presence of ~10 mg of Pt₂O (Matheson Coleman & Bell Manufacturing Chemists, Norwood, OH) catalyst. Spent Pt₂O was removed by centrifugation. Hydrogenated fatty acids in the supernatant were phenacylated as described above, and FAPes were chromatographed on HPLC with the modification of a 30-min elution with 100% acetonitrile immediately following the 98:2 acetonitrile/water, to elute saturated fatty acids larger than 18 carbon atoms.

Isolation of Transducin—Transducin was isolated using a modification of the procedures of Ohguro *et al.* (1990) and Umbarger *et al.* (1992). ROS were homogenized at 4 °C in isotonic wash buffer (100 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 1 mM EGTA, 2 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride) and centrifuged at 100,000 $\times g$ for 30 min. The isotonic buffer wash was repeated once, and the ROS pellet was homogenized at 4 °C in hypotonic wash buffer (5 mM Tris-HCl, pH 7.5, 0.5 mM MgCl₂, 2 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride) and centrifuged at 100,000 $\times g$ for 30 min. The ROS pellet was homogenized at 4 °C in hypotonic wash buffer containing 200 μ M GTP γ S and centrifuged at 100,000 $\times g$ for 30 min. The hypotonic buffer wash with 200 μ M GTP γ S was repeated once, and the transducin subunits ($G_{\alpha\beta\gamma}$) were recovered in the GTP γ S wash supernatants.

Electrophoresis and ³H-Fluorography of Transducin—Aliquots of the supernatants for the GTP γ S washes were made 16% (w/v) in trichloroacetic acid (Fisher) and centrifuged at 27,000 $\times g$ for 30 min. The protein pellet was solubilized in strongly reducing sample application buffer (20% (w/v) sucrose, 2% (w/v) SDS, 50 mM Na₂CO₃, pH 9, 50 mM dithiothreitol, 0.3 mM 2-mercaptoethanol, and 0.15 mM bromophenol blue) and incubated 16 h at 25 °C. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) using 12% (w/v) polyacrylamide gels. Gels were stained with Coomassie Blue R-250, destained for 2–3 days with MeOH/HOAc/H₂O (1:1:8, v/v/v),

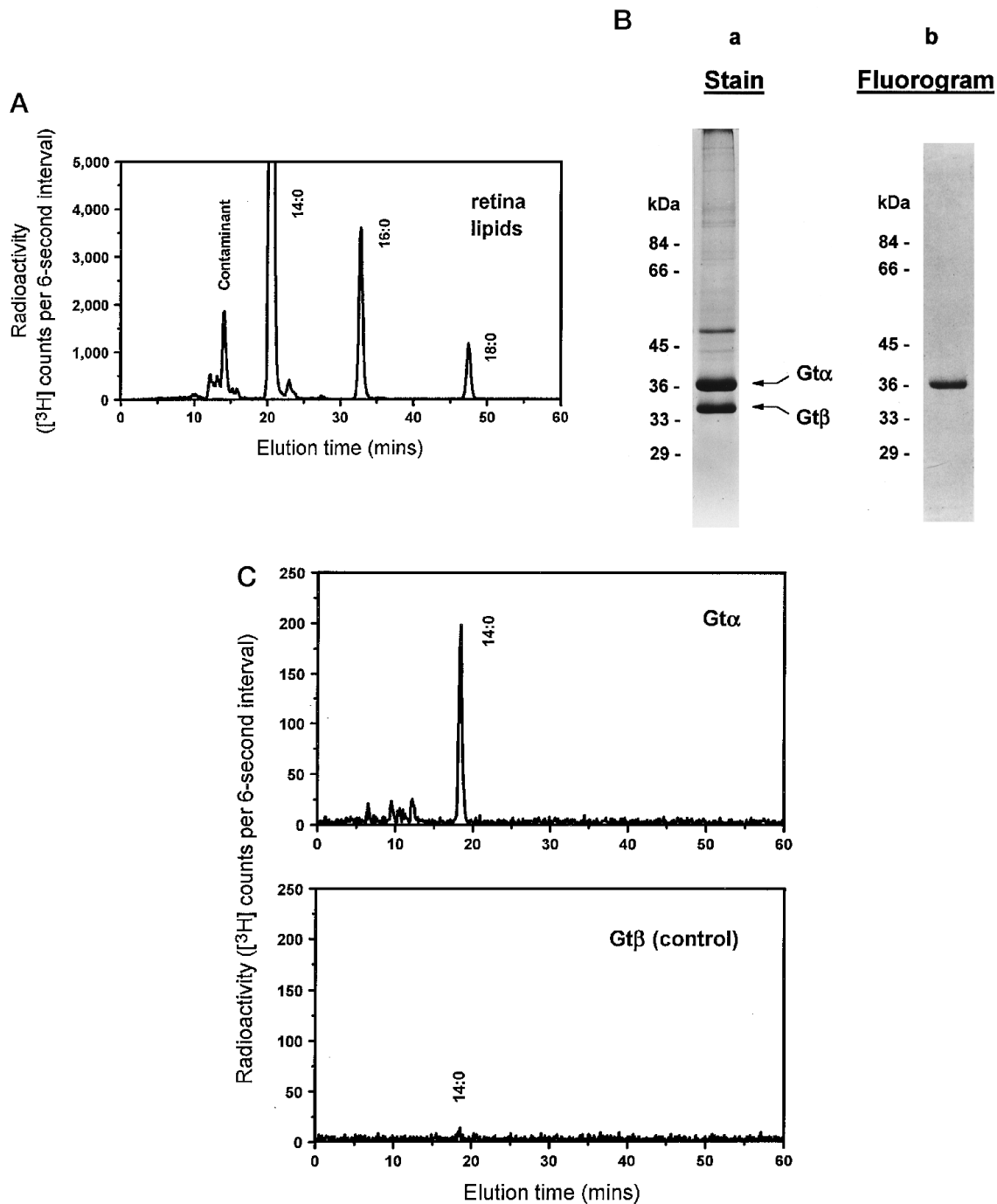


FIG. 1. **Incubation with [9,10- ^3H]14:0.** A, HPLC elution profile of FAPE radioactivity for total lipids extracted from frog retinas incubated with 1 mCi of [^3H]14:0. The y axis (^3H) counts/6-s interval) is shown at a 10 \times reduction of the original scale; the maximum for [^3H]14:0 peak was 41,900. B, Coomassie Blue-stained electrophoretic gel (a) and corresponding fluorogram (b) for transducin ($G_{\alpha\beta\gamma}$) isolated from frog retinas labeled with 1 mCi of [^3H]14:0. Fluorography was performed for 28 days. G_{α} and G_{β} are the α - and β -subunits of transducin, respectively. The G_{γ} (8 kDa) was run off the gel. C, HPLC elution profiles of FAME radioactivity for G_{α} and G_{β} (control) isolated from frog retinas labeled with 1 mCi of [^3H]14:0. FAMES were released from the SDS-PAGE-purified G_{α} and G_{β} by acidic methanolysis.

and photographed. After washing for 1–2 h in 10% (v/v) HOAc, the gels were treated with DuPont NEN EN 3 HANCE $^{\text{TM}}$ following the manufacturer's instructions. After vacuum drying onto filter paper, the treated gels were exposed to autoradiography x-ray film at -80°C for 4–6 weeks.

To test for resistance of radiolabel of proteins to hydrolysis by hydroxylamine (NH_2OH) as evidence of amide linkage, SDS-PAGE was repeated and gels were treated with NH_2OH using a modification of the methods of Buss *et al.* (1987) and Olson *et al.* (1985). The gels were fixed (30 min) in isopropanol/ H_2O /HOAc (25:65:10, v/v/v), washed (45 min) with H_2O , soaked (16 h) in 1 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ (pH 6.7), and washed (6 h) with isopropanol/ H_2O /HOAc (10:80:10, v/v/v). Gels were stained, destained, treated with EN 3 HANCE $^{\text{TM}}$, dried, and autoradiographed. To

determine the efficiency of thioester-linked fatty acid removal by NH_2OH , we performed the procedure on rhodopsin, a protein known to carry two thioester linked 16:0 groups (Papac *et al.*, 1992). Using previously described procedures, frog retinas were incubated *in vitro* with 150 μCi of [9,10(*N*)- ^3H]16:0 (American Radiolabeled Chemicals Inc., St. Louis, MO), and ROS were prepared, stripped of transducin, and subjected to SDS-PAGE using low reducing conditions (1 h of incubation in sample application buffer containing 2 mM dithiothreitol and 15 μM 2-mercaptoethanol). Gel slices ($n = 4$) containing rhodopsin (~ 50 μg , estimated by BCA* protein assay kit, Pierce) were saponified in 1 M NaOH at 37°C for 2 h and acidified with 12 N HCl, and radioactivity was determined by counting gel slice and hydrolysate together (BCS mixture/hydrolysate, 15:1, v/v). Identical gels were sub-

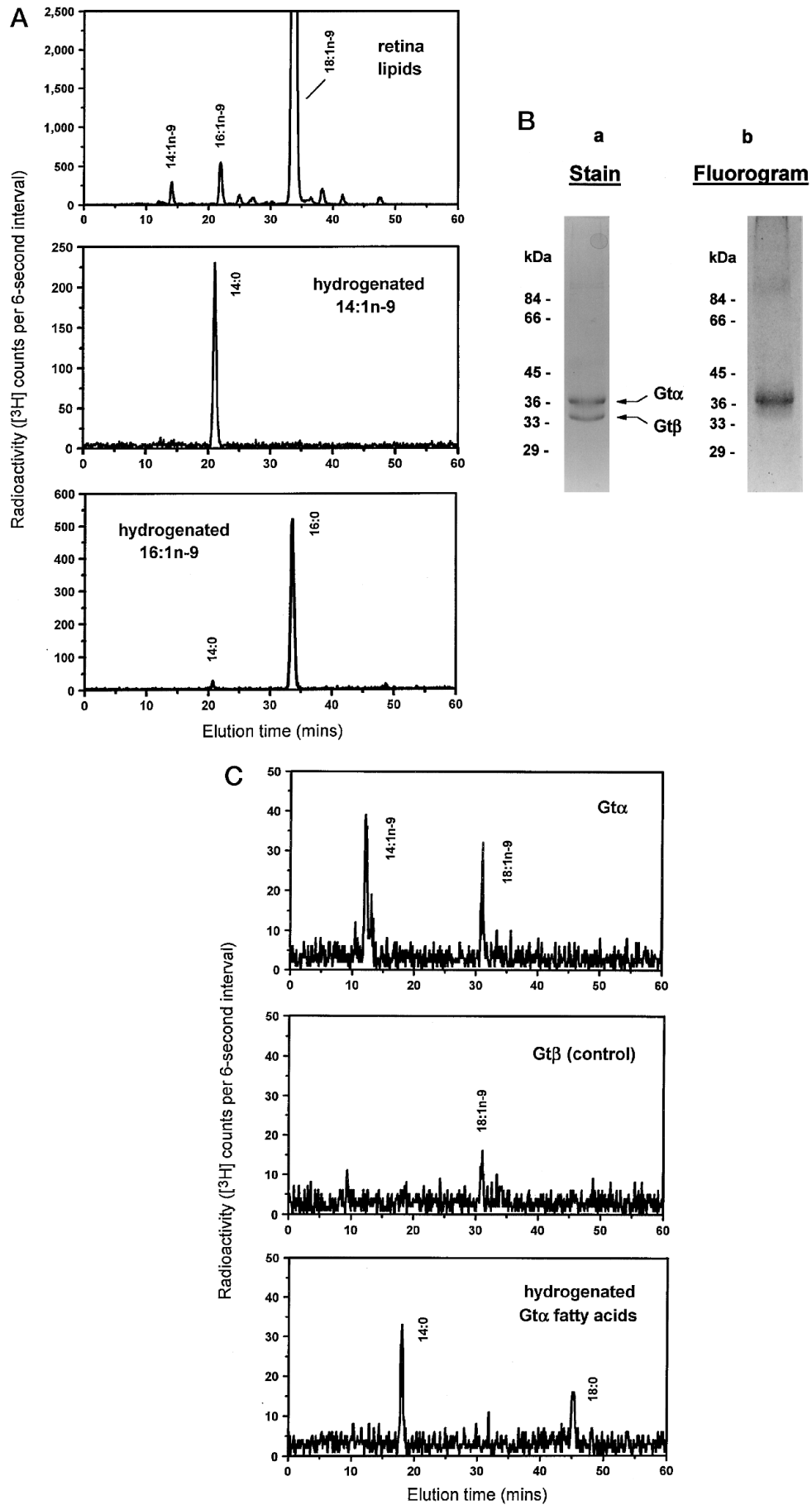


FIG. 2

jected to treatment with 1 M NH₂OH as described above; rhodopsin was excised, saponified, and counted. NH₂OH treatment removed 84 ± 2% of the radiolabel from this protein.

Fatty Acid Analysis of Transducin—Samples of transducin ($n = 3$) were subjected to SDS-PAGE and amounts of G_α and G_β in each were estimated by scanning the gel photographs with a laser densitometer (Personal Densitometer SI[®] with ImageQuant analysis software, Molecular Dynamics Inc., Sunnyvale, CA) using a linear proportion comparison with a known amount of carbonic anhydrase (29 kDa) run on the same gels. The amounts of G_α in each were estimated to be 18 ± 4 (14:0), 24 ± 4 (18:1*n*-9), 24 ± 2 (18:2*n*-6), and 8 ± 1 μg (18:3*n*-3), with corresponding G_β at 13 ± 2, 16 ± 3, 15 ± 2, and 5 ± 1 μg, respectively. Gel slices containing G_α and G_β were washed with MeOH/H₂O/HOAc (50:40:1, v/v/v) (24 h), 50% MeOH (24 h), and 100% MeOH (2 h). After the addition of 100 nmol 16:1*n*-9 and 17:0 as carrier, the gel slices were hydrolyzed in 2 N HCl/83% MeOH for 8 h at 98 °C under argon gas. The hydrolysate was extracted three times with hexane, and the fatty acid methyl esters (FAMES) were chromatographed on HPLC with UV absorbance being monitored at 192 nm (Avelldano *et al.*, 1983). The identities of unsaturated FAMES were confirmed by catalytic hydrogenation.

RESULTS

Incubation with [9,10-³H]14:0—HPLC of FAPes derived from the total retinal lipid pool after incubation with [³H]14:0 (Fig. 1A) revealed only chain elongation to [³H]16:0 (~11% of [³H]14:0) and [³H]18:0 (~7%). A minor peak eluting at 14 min had the identical retention time as a contaminant that was present in the commercial stock of [³H]14:0 and therefore is unlikely to represent a metabolic product of 14:0.

The Coomassie staining pattern (Fig. 1B, a) and fluorogram (Fig. 1B, b) showed intense radiolabeling at a migration position in SDS-PAGE aligning precisely with G_α (39 kDa), and there was no detectable radiolabeling of G_β (36 kDa) or any other proteins. HPLC of methanolysis products (Fig. 1C) confirmed that the radiolabel on G_α was 14:0 (481 ± 74 counts/6 s/10 μg), along with some minor peaks of uncertain identity. A comparatively insignificant amount of labeled 14:0 was associated with G_β (18 ± 11 counts/6 s/10 μg).

Incubation with [9,10-³H]18:1*n*-9—HPLC of FAPes derived from the total retinal lipid pool after incubation with [³H]18:1*n*-9 (Fig. 2A) revealed only retroconversion to [³H]14:1*n*-9 (~2% of [³H]18:1*n*-9) and [³H]16:1*n*-9 (~4%). HPLC of the retroconversion products after catalytic hydrogenation (Fig. 2A) confirmed their identities by showing an appropriate shift in retention time to that of 14:0 and 16:0, respectively. A trace amount of 14:0 was also produced during the hydrogenation of 16:1*n*-9.

The Coomassie staining pattern (Fig. 2B, a) and fluorogram (Fig. 2B, b) revealed faint radiolabeling concentrated at a migration position in SDS-PAGE aligning precisely with G_α (39 kDa), with some diffuse radiolabeling extending into the region for G_β (36 kDa). Another area of diffuse radiolabeling was observed to be focused at a migration position aligning with a faint doublet protein band above 84 kDa. HPLC of methanolysis products (Fig. 2C) showed that the radiolabel on G_α was a mixture of 14:1*n*-9 (108 ± 16 counts/6 s/10 μg) and 18:1*n*-9 (51 ± 6 counts/6 s/10 μg), whereas the radiolabel seen in the region for G_β was only 18:1*n*-9 (35 ± 9 counts/6 s/10 μg). HPLC of the G_α methanolysis products after simultaneous collection and catalytic hydrogenation (Fig. 2C) confirmed their identities by

showing only two peaks with appropriate retention times for 14:0 and 18:0 within the predicted proportions (14:0 (63%) and 18:0 (37%).)

Incubation with [9,10,12,13-³H]18:2*n*-6—HPLC of FAPes derived from the total retinal lipid pool after incubation with [³H]18:2*n*-6 (Fig. 3A) revealed retroconversion to [³H]14:2*n*-6 (~2% of [³H]18:2*n*-6) and [³H]16:2*n*-6 (~1%), along with desaturation and elongation to [³H]18:3*n*-6, [³H]20:3*n*-6, and [³H]20:4*n*-6 (~8, 3, and 7%, respectively). HPLC of the retroconversion products after catalytic hydrogenation (Fig. 3A) confirmed their identity by showing an appropriate shift in retention time to that of 14:0 and 16:0, respectively. A small amount of 18:0 was also produced during the hydrogenation of 16:2*n*-6, possibly arising from contaminating 18:3*n*-6. Identities of all desaturation and elongation products were also confirmed by catalytic hydrogenation (results not shown).

The Coomassie staining pattern (Fig. 3B, a) and fluorogram (Fig. 3B, b) revealed moderate radiolabeling at a migration position in SDS-PAGE aligning precisely with G_α (39 kDa), with faint radiolabeling also seen aligning with G_β (36 kDa). No other areas of radiolabeling were observed in the fluorogram. HPLC of methanolysis products (Fig. 3C) revealed that the radiolabel on G_α was only 14:2*n*-6 (101 ± 8 counts/6 s/10 μg), and no detectable radiolabel was associated with G_β. Catalytic hydrogenation and HPLC of the methanolysis product for G_α confirmed its identity by showing an appropriate shift in retention time to that of 14:0.

Incubation with [9,10,12,13,15,16-³H]18:3*n*-3—HPLC of FAPes derived from the total retinal lipid pool after incubation with [³H]18:3*n*-3 (Fig. 4A) revealed the absence of candidate peaks for the anticipated 14:3*n*-3 or 16:3*n*-3 retroconversion products. Desaturation and elongation products were observed in the form of [³H]18:4*n*-3, [³H]20:5*n*-3, and [³H]22:5*n*-3 (~12, 8, and 7% of [³H]18:3*n*-3, respectively). HPLC of the 18:4*n*-3 desaturation product after catalytic hydrogenation (Fig. 4A) confirmed its identity by showing an appropriate shift in retention time to that of 18:0. Likewise, identities of the other desaturation and elongation products were confirmed by catalytic hydrogenation (results not shown). Hydrogenation of the prominent peak at ~18 min produced a product with a retention time between that of 18:0 and 20:0 (result not shown), making its identification inconclusive.

The Coomassie staining pattern (Fig. 4B, a) and fluorogram (Fig. 4B, b) showed no radiolabeling corresponding with G_α (39 kDa), G_β (36 kDa), or any other protein observed on SDS-PAGE. HPLC of methanolysis products (Fig. 4C) revealed that no detectable radiolabel was associated with either G_α or G_β, consistent with the fluorogram results.

Hydroxylamine Treatment of Radiolabeled Transducin—Shown in Fig. 5 are the Coomassie staining patterns and fluorograms for gels containing transducin from the [³H]14:0, [³H]18:1*n*-9, and [³H]18:2*n*-6 incubations, after treatment with 1 M hydroxylamine. The radiolabelings on G_α (39 kDa), representative of [³H]14:0, [³H]14:1*n*-9/[³H]18:1*n*-9, and [³H]14:2*n*-6, respectively, all showed hydroxylamine resistance. Complete disappearance of radiolabeling was observed for G_β (36 kDa) and all other proteins.

FIG. 2. Incubation with [9,10-³H]18:1*n*-9. A, HPLC elution profile of FAPE radioactivity for total lipids from frog retinas incubated with 1 mCi of [³H]18:1*n*-9. The y axis ([³H] counts/6-s interval) is shown at a 10× reduction of the original scale; the maximum for the [³H]18:1*n*-9 peak was at 21.322. Also shown are the HPLC elution profiles of FAPE radioactivity for the 14:1*n*-9 and 16:1*n*-9 after being collected separately and subjected to catalytic hydrogenation. B, Coomassie Blue-stained electrophoretic gel (a) and corresponding fluorogram (b) for transducin (G_{αβ}) isolated from frog retinas labeled with 1 mCi of [³H]18:1*n*-9. Fluorography was performed for 41 days. G_α and G_β are the α- and β-subunits of transducin, respectively. G_γ (8 kDa) was run off the gel. C, HPLC elution profiles of FAME radioactivity for G_α and G_β (control) isolated from frog retinas labeled with 1 mCi of [³H]18:1*n*-9. FAMES were released from the SDS-PAGE-purified G_α and G_β by acidic methanolysis. Also shown is the HPLC elution profile of FAME radioactivity for the 14:1*n*-9 and 18:1*n*-9 from the methanolysis of G_α, after being collected simultaneously and subjected to catalytic hydrogenation.

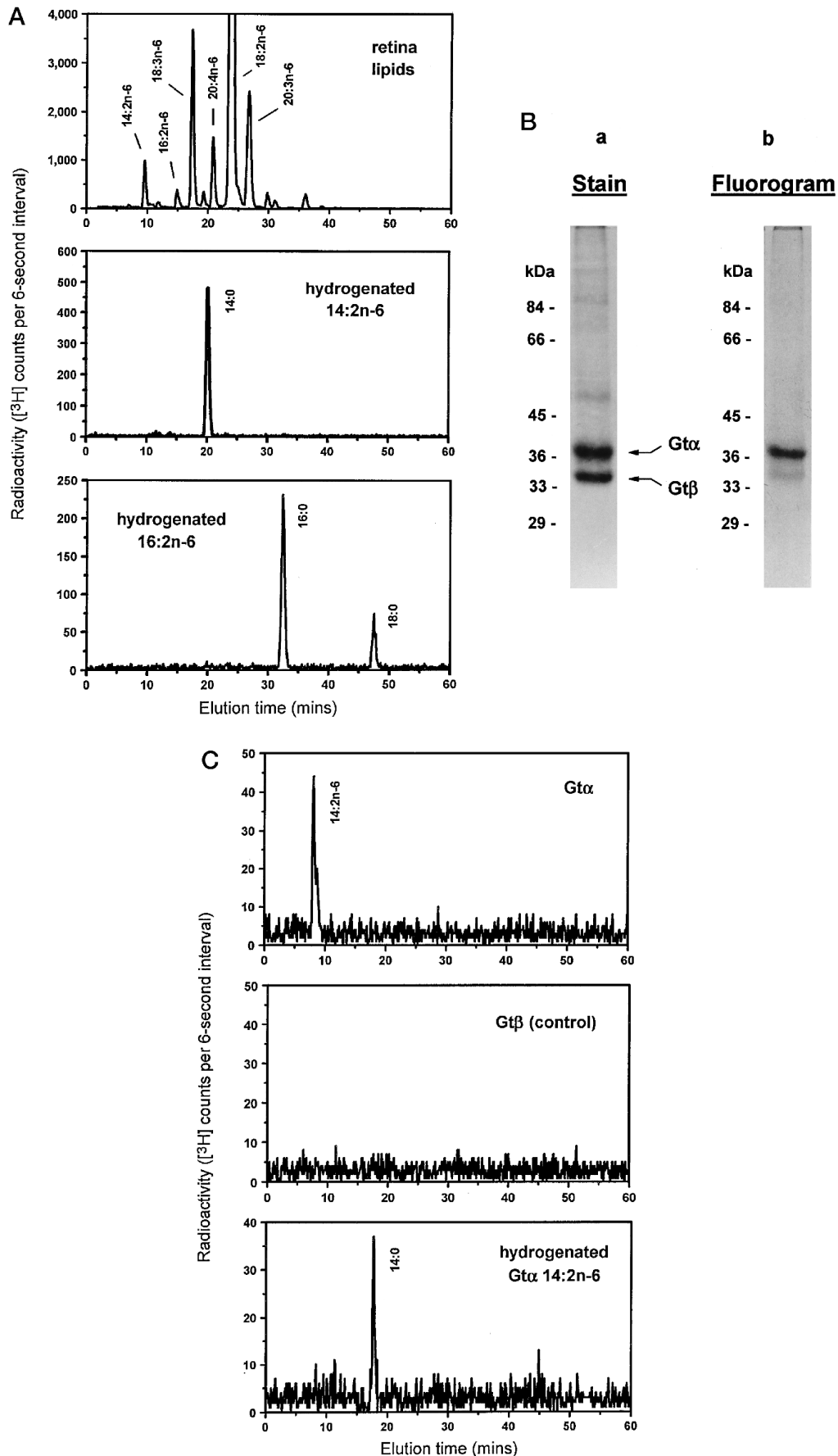


FIG. 3. Incubation with [9,10,12,13- ^3H]18:2n-6. A, HPLC elution profile of FFAE radioactivity for total lipids from frog retinas incubated with 120 μCi of [^3H]18:2n-6. The y axis (^3H) counts/6-s interval) of the radioactivity profile is shown at a 10 \times reduction of the original scale; the maximum for the [^3H]18:2n-6 peak was at 32,946. Also shown are the HPLC elution profiles of FFAE radioactivity for the 14:2n-6 and 16:2n-6 after

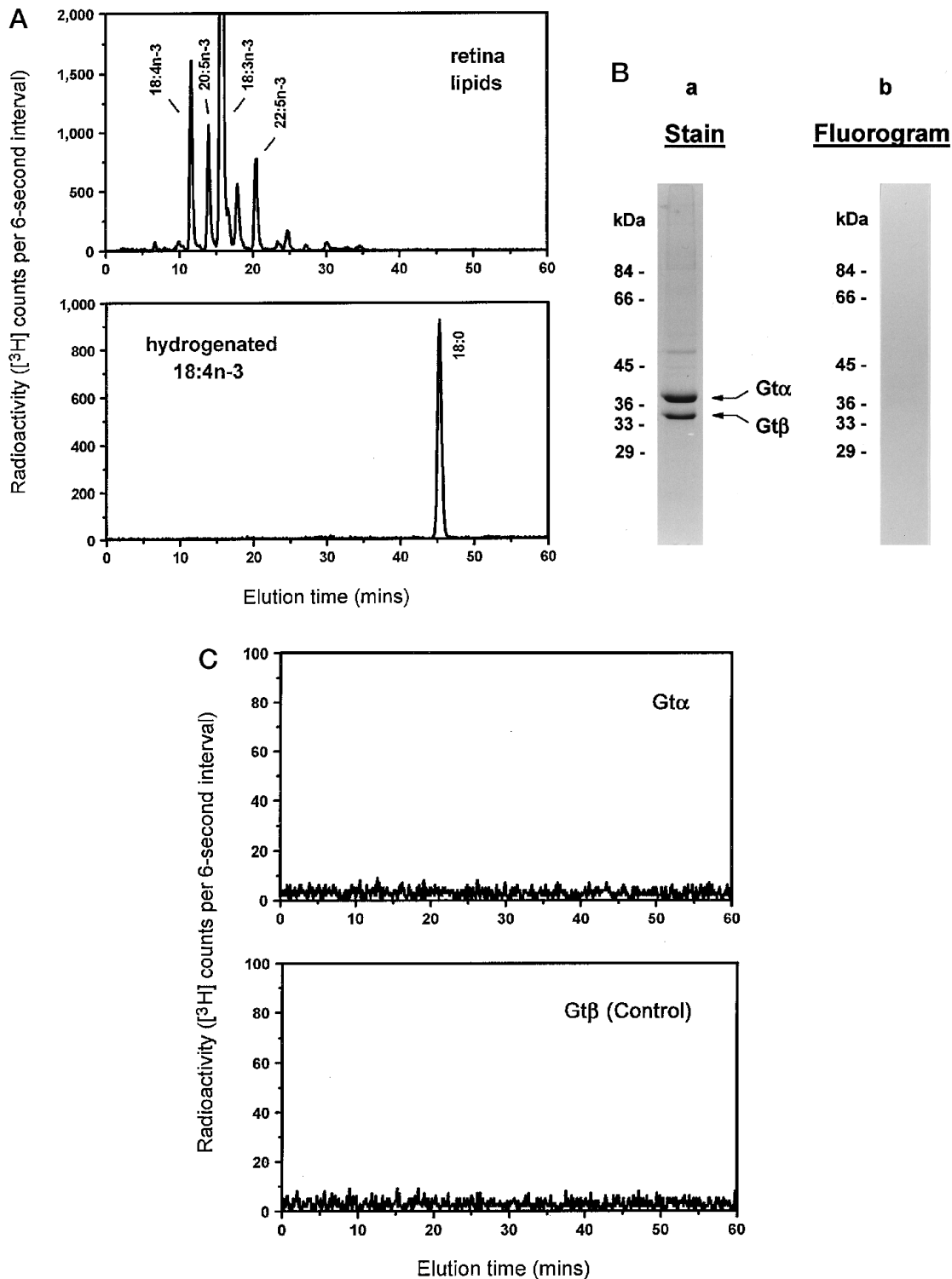


FIG. 4. **Incubation with [9,10,12,13,15,16- ^3H]18:3n-3.** *A*, HPLC elution profile of FAME radioactivity for total lipids from frog retinas incubated with 120 μCi of [^3H]18:3n-3. The y axis (^3H) counts/6-s interval) of the radioactivity profile is shown at a 4 \times reduction of the original scale; the maximum for the [^3H]18:3n-3 peak was at 7086. Also shown are the HPLC elution profiles of FAME radioactivity for the 18:4n-3 after being subjected to catalytic hydrogenation. *B*, Coomassie Blue-stained electrophoretic gel (*a*) and corresponding fluorogram (*b*) for transducin ($G_{t\alpha\beta}$) isolated from frog retinas labeled with 120 μCi of [^3H]18:3n-3. Fluorography was performed for 35 days. $G_{t\alpha}$ and $G_{t\beta}$ are the α - and β -subunits of transducin, respectively. $G_{t\gamma}$ (8 kDa) was run off the gel. *C*, HPLC elution profiles of FAME radioactivity for $G_{t\alpha}$ and $G_{t\beta}$ (control) isolated from frog retina labeled with 120 μCi of [^3H]18:3n-3. FAMES were released from SDS-PAGE-purified $G_{t\alpha}$ and $G_{t\beta}$ by acidic methanolysis.

being collected separately and subjected to catalytic hydrogenation. *B*, Coomassie Blue-stained electrophoretic gel (*a*) and corresponding fluorogram (*b*) for transducin ($G_{t\alpha\beta}$) isolated from frog retinas labeled with 120 μCi of [^3H]18:2n-6. Fluorography was performed for 42 days. $G_{t\alpha}$ and $G_{t\beta}$ are the α - and β -subunits of transducin, respectively. $G_{t\gamma}$ (8 kDa) was run off the gel. *C*, HPLC elution profiles of FAME radioactivity for $G_{t\alpha}$ and $G_{t\beta}$ (control) isolated from frog retina labeled with 120 μCi of [^3H]18:2n-6. FAMES were released from SDS-PAGE-purified $G_{t\alpha}$ and $G_{t\beta}$ by acidic methanolysis. Also shown is the HPLC elution profile of FAME radioactivity for the 14:2n-6 from the methanolysis of $G_{t\alpha}$, after being subjected to catalytic hydrogenation.

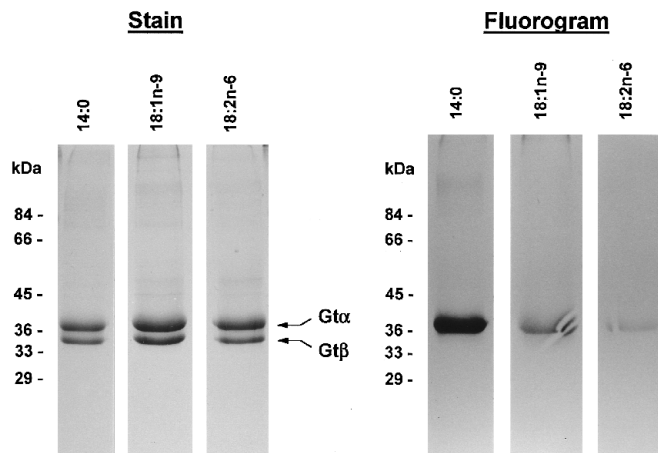


FIG. 5. Hydroxylamine treatment of radiolabeled transducin. Frog retinas were incubated with either 1 mCi of $[9,10\text{-}^3\text{H}]14:0$, 1 mCi of $[9,10\text{-}^3\text{H}]18:1n-9$, or 120 μCi of $[9,10,12,13\text{-}^3\text{H}]18:2n-6$. Radiolabeled retinas were used to prepare rod outer segments. Transducin was isolated and run on the same electrophoretic gel. The gel was treated with 1 M hydroxylamine and subsequently subjected to fluorography for 44 days. Shown here are the Coomassie Blue-stained electrophoretic gel sections after hydroxylamine treatment and the corresponding fluorograms. G_{α} and G_{β} are the α - and β -subunits of transducin, respectively. G_{γ} (8 kDa) was run off the gel.

Distribution of Desaturation, Elongation, or Retroconversion Products in Retina Lipid Classes—Fig. 6 shows the distribution of isotope in the retinal phospholipid, free fatty acid, and triglyceride pools from the $[^3\text{H}]14:0$, $[^3\text{H}]18:1n-9$, $[^3\text{H}]18:2n-6$, and $[^3\text{H}]18:3n-3$ incubations. The figure also shows the composition of radioactivity contained within the individual lipid classes. In all incubations the majority of the radiolabel was sequestered in the phospholipid pool after 8 h. We did not examine the distribution of radioactivity among the various phospholipid classes. For all incubations, fatty acid analysis of each lipid class revealed all the previously described desaturation, elongation, or retroconversion products, with percentages similar to that found when the total lipid pools were analyzed. There was not a significant enrichment of any metabolic product into a particular lipid class.

DISCUSSION

N-terminal fatty acylation of a protein is carried out cotranslationally by the enzyme myristoylCoA:protein *N*-myristoyltransferase (Towler *et al.*, 1988 and Schlesinger, 1993). Human and yeast myristoyltransferases have a high substrate specificity for 14:0-CoA (Kishore *et al.*, 1991, 1993; Lu *et al.*, 1994). Although 14:2(*n*-6)-CoA has not been tested, 14:1(*n*-9)-CoA is utilized by both myristoyltransferases at a ~ 3 -fold lower catalytic efficiency (V_{\max}/K_m) than 14:0-CoA (Kishore *et al.*, 1993). Despite extensive study, no evidence has been found for myristoyltransferase isozymes with drastically altered substrate specificity, and supporting this mRNA from the single copy human gene appears to be identical in all tissues studied (Duronio *et al.*, 1992). Consequently, to successfully compete for myristoyltransferase, levels of available 14:1(*n*-9) and 14:2(*n*-6) CoAs must be higher than that of 14:0. To attain these levels, the retina would need to possess special pathways for generating 14:1(*n*-9) and 14:2(*n*-6), which are apparently absent or less active in other tissues. However, this does not mean 14:1(*n*-9) and 14:2(*n*-6) would necessarily accumulate in the retina, because their CoAs might be taken up rapidly by the myristoyltransferase during active protein synthesis. This hypothesis is consistent with the lack of detectable ($<0.1\%$) 14:1(*n*-9) and 14:2(*n*-6) in the total lipid pool of the frog and bovine retina (Chen and Anderson, 1993a; Bartley *et al.*, 1962).

We first tested whether 14:1(*n*-9) and 14:2(*n*-6) might be generated by *de novo* synthesis, because the 14:1(*n*-5) isomer can be synthesized via a Δ^9 -desaturase (Hamosh and Bitman, 1992; Koletzko *et al.*, 1992), but we found that 14:0 does not serve as a desaturase substrate in the retina and only 16:0 and 18:0 are produced. We next considered that 14:1(*n*-9) and 14:2(*n*-6) might be produced during the β -oxidation (retroconversion) of long chain unsaturated fatty acids. Our experiments showed that retroconversion of 18:1(*n*-9) and 18:2(*n*-6) leads to the formation of 14:1(*n*-9) and 14:2(*n*-6), respectively, in the frog retina. Interestingly, 18:1(*n*-9) was only metabolized to 14:1(*n*-9) and 16:1(*n*-9), suggesting a restricted function for this fatty acid in the retina. Our results showed 18:2(*n*-6) has multiple roles in the retina; it was retroconverted to 14:2(*n*-6) and 16:2(*n*-6) and also underwent extensive elongation and desaturation, following the steps toward 20:4(*n*-6) as in liver (Sprecher, 1972).

The fatty acid composition of frog retinas (Chen and Anderson, 1993a) suggests that 18:1(*n*-9) and 16:1(*n*-9) are the likely precursors of 14:1(*n*-9), because they are the only *n*-9 fatty acids present in reasonable abundance (12 and 3% of total fatty acid, respectively). In contrast, multiple *n*-6 species are present, with 20:4(*n*-6) being the most abundant (7%) and 18:2(*n*-6) much less so (1%). Because rat liver peroxisomes are known to convert 20:4(*n*-6) to 14:2(*n*-6) with 18:3(*n*-6) and 16:3(*n*-6) intermediates (Luthria *et al.*, 1995), 20:4(*n*-6) is a more likely candidate for the major 14:2(*n*-6) precursor in the retina. Preliminary results from incubation of $[^3\text{H}]20:4n-6$ (DuPont NEN) with frog retinas ($n = 2$) showed production of $[^3\text{H}]16:3n-6$ (3%) and $[^3\text{H}]14:2n-6$ (2%) with no detectable $[^3\text{H}]18:3n-6$ (data not shown), supporting this hypothesis.

N-terminal acylated retina proteins do not contain 14:3(*n*-3) (Johnson *et al.*, 1994), even though *n*-3 polyunsaturated fatty acids are abundant in the vertebrate retina (Fliesler and Anderson, 1983). We found that metabolism of $[^3\text{H}]18:3n-3$ did not produce detectable $[^3\text{H}]16:3n-3$ or $[^3\text{H}]14:3n-3$ retroconversion products and radiolabel was not incorporated into G_{α} . This result shows that the retroconversion process in the frog retina has a selectivity dependent on double bond position, as well as on chain length. The 18:3(*n*-3) did undergo extensive elongation and desaturation, following the steps toward 22:6(*n*-3), as described for liver (Sprecher, 1972), consistent with previous observations in the frog retina (Wang and Anderson, 1993).

Under the *in vitro* incubation conditions we used, there was an active uptake and metabolism for all the radiolabeled fatty acids, as indicated by the amount of the original starting radioactivity that was incorporated into the retina glycerolipids ($[^3\text{H}]14:0$, 12%; $[^3\text{H}]18:1n-9$, 14%; $[^3\text{H}]18:2n-6$, 5%; and $[^3\text{H}]18:3n-3$, 7%). In all incubations, the phospholipids contained most of the radiolabel, whereas only a small percentage was incorporated into triglycerides, consistent with previous studies (Wang and Anderson, 1993; Chen and Anderson, 1993b). Incorporation into glycerolipids indicates effective conversion to precursor CoA ester derivatives. Acyl-CoAs are also the substrates for myristoyltransferase, and labeling of G_{α} with $[^3\text{H}]14:0$ suggested efficient conversion to the CoA ester.

In addition to incorporation of $[^3\text{H}]14:0$, $[^3\text{H}]14:1n-9$, and $[^3\text{H}]14:2n-6$ into G_{α} , we also noted some labeling with $[^3\text{H}]18:1n-9$. We have not determined whether this represents N-terminal acylation by myristoyltransferase, which in yeast can utilize 18:1(*n*-5) $\sim 2\%$ as effectively as 14:0 (Rudnick *et al.*, 1992), or trace labeling by thioesterification or some other means. Our ability to incorporate $[^3\text{H}]14:2n-6$ into frog G_{α} is consistent with the data of Johnson *et al.* (1994), which shows 100% modification with 14:2(*n*-6). The incorporation of $[^3\text{H}]14:0$ and $[^3\text{H}]14:1n-9$ into G_{α} indicates that at least some G_{α} in frog

FIG. 6. Distribution of desaturation, elongation, or retroconversion products in lipid classes derived from retina membrane pellets. Total lipids obtained from [^3H]14:0, [^3H]18:1*n*-9, [^3H]18:2*n*-6, and [^3H]18:3*n*-3 radiolabeled retina membranes were resolved into phospholipids (PL), free fatty acids (FFA), and triglycerides (TG) using one-dimensional two-step TLC. Lipid classes were directly counted to determine total radioactivity. Lipid classes were then saponified, and the resulting fatty acids were converted to phenacyl esters and chromatographed on HPLC. The data are represented as percentages calculated by taking the ratio of radioactivity for the individual lipid classes or fatty acid species to the total radioactivity in the original total lipid extract or separated lipid class.

	PL	FFA	TG		PL	FFA	TG		
14:0	% Total Lipid Radioactivity	81.3 ± 0.3	15.3 ± 0.3	2.6 ± 0.4	18:2<i>n</i>-6	% Total Lipid Radioactivity	68.5 ± 2.1	26.0 ± 1.1	5.2 ± 0.3
	% 14:0	66	74	81		% 14:2	2	1	3
	% 16:0	7	9	4		% 16:2	3	2	2
	% 18:0	3	2	2		% 18:2	68	80	82
18:1<i>n</i>-9	% Total Lipid Radioactivity	77.5 ± 0.2	18.8 ± 0.5	3.6 ± 0.3	18:3<i>n</i>-3	% Total Lipid Radioactivity	82.2 ± 4.5	12.2 ± 3.3	4.9 ± 1.4
	% 14:1	2	1	0.3		% 18:3	50	69	66
	% 16:1	4	3	3		% 18:4	12	8	11
	% 18:1	71	82	88		% 20:5	8	5	7

retina can be modified with these fatty acids, possibly at levels too low to be detected by mass spectrometry.

Amide linkage of the [^3H]14:0, [^3H]14:1*n*-9, and [^3H]14:2*n*-6 to G_{α} was supported by finding that the radiolabel was resistant to hydroxylamine, under conditions where 86% removal of rhodopsin's thioester-linked fatty acid was achieved. Taken together with the mass spectrometric results of Johnson *et al.* (1994) and the well established specificity of myristoyltransferase in yeast and mammals, this result strongly suggests that the most likely site for attachment of these fatty acids is the α -amino group of the N-terminal glycine of frog G_{α} .

Because frog G_{α} is reported to be modified exclusively by 14:2*n*-6, we investigated whether radiolabeled 14:2*n*-6 would directly incorporate into the protein. We performed *in vitro* incubations ($n = 2$) of frog retinas with 20 μCi of [^{14}C]14:2*n*-6 (data not shown). Analysis of the retina total lipids showed significant chain elongation of the [^{14}C]14:2*n*-6 to [^{14}C]16:2*n*-6 and [^{14}C]18:2*n*-6 (3 and 4% of [^{14}C]14:2*n*-6, respectively). Metabolism of nonradiolabeled 14:2*n*-6 to 16:2*n*-6 and 18:2*n*-6 has been previously noted in rat liver (Sprecher, 1967). Methanolysis of SDS-PAGE-purified G_{α} (19 ± 2 μg) and G_{β} (13 ± 1 μg) from the [^{14}C]14:2*n*-6 labeled retinas failed to release any detectable radiolabeled fatty acids. Because the [^{14}C]14:2*n*-6 used in our incubations was of very low specific activity (55–60 Ci/mol), it is possible that dilution with endogenously produced 14:2*n*-6 precluded our detecting radioactivity in protein product. However, it is also possible that 14:2*n*-6 supplied directly may not be readily available as a substrate for myristoyltransferase to incorporate into G_{α} . It is clearly converted into the necessary chemical form, CoA ester, as evidenced by chain elongation products and its incorporation into glycerolipids. However, subcellular compartmentalization may limit the access of myristoyltransferase and the nascent G_{α} polypeptide to 14:2*n*-6 when it is supplied directly, while allowing free access to the 14:2*n*-6 pool produced by retroconversion.

The fatty acid retroconversions seen in our experiments have the limited chain shortening characteristics associated with peroxisomal β -oxidation (Schulz, 1991), as shown by the lack of production of 12:1*n*-9 and 12:2*n*-6 or other short chain fatty acids. In support of this are electron microscopy studies showing that Müller and photoreceptor cells of frog retinas contain significant peroxisome-like organelles (St. Jules *et al.*, 1992). However, more experiments are necessary to determine where the observed β -oxidation occurs. Retina peroxisomes may prove to be more vigorous or less stringent in retroconverting these fatty acids compared with those from other tissues. Such differences may account for the absence of 14:1*n*-9 or 14:2*n*-6 on

myristoylated liver proteins such as cytochrome b_5 reductase (Ozols *et al.*, 1984), even though liver peroxisomes carry out fatty acid retroconversions.

Although we do not know the functional significance for N-terminal fatty acylation of photoreceptor proteins with unsaturated forms of myristate, the retroconversion pathways we have investigated could play a major role in both normal visual function and retinal disease states. Congenital defects such as adrenomyeloneuropathy (Moser *et al.*, 1987), neonatal adrenoleukodystrophy (Jaffe *et al.*, 1982), infantile Refsum's disease (Poll-the *et al.*, 1987), and Zellweger syndrome (Bowen *et al.*, 1964) are known afflictions where peroxisomal β -oxidation is impaired. The general phenotype of these diseases is deterioration of nervous system, often involving the retina. These symptoms may arise in part from impairment of the biosynthesis of docosahexaenoic acid (22:6*n*-3) (Martinez *et al.*, 1994), an essential component of neuronal cells including retina photoreceptors, which requires the peroxisomal based retroconversion of 24:6*n*-3 to 22:6*n*-3 (Voss *et al.*, 1991). Because N-terminal fatty acylation with 14:1*n*-9 and 14:2*n*-6 may be required for proper function of phototransduction proteins, impairment of 14:1*n*-9 or 14:2*n*-6 production could also have a devastating effect on normal visual function. It is known that many types of retinal degeneration involve lipidated phototransduction proteins, such as rhodopsin (autosomal dominant and autosomal recessive retinitis pigmentosa) (Dryja *et al.*, 1990; Rosenfeld *et al.*, 1992) and the β -subunit of cGMP phosphodiesterase (PDE β) (autosomal recessive retinitis pigmentosa) (McLaughlin *et al.*, 1993). A specific example of defective protein lipidation in retinal degeneration is choroideremia, whose basis is a mutation in geranylgeranyl transferase (Seabra *et al.*, 1993), the enzyme that isoprenylates PDE β and G proteins of the Rab family. Therefore, genes encoding proteins involved in the pathways required for heterogeneous fatty acylation of retina protein warrant further consideration as retinal degeneration candidates.

REFERENCES

- Aveldano, M. I., VanRollins, M., and Horrocks, L. A. (1983) *J. Lipid Res.* **24**, 83–93
 Bartley, W., Van Heyningen, R., Notton, B. M., and Renshaw, A. (1962) *Biochem. J.* **85**, 332–335
 Baykousheva, S. P., Luthria, D. L., and Sprecher, H. (1994) *J. Biol. Chem.* **269**, 18390–18394
 Bowen, P., Lee, C. M. S., Zellweger, H., and Lindenberg, R. (1964) *Bull. Johns Hopkins Hosp.* **114**, 402–414
 Buss, J. E., Mumby, S. M., Casey, P. J., Gilman, A. G., and Sefton, B. M. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 7493–7497
 Carr, S. A., Biemann, K., Shoji, S., Parmelee, D. C., Titani, K. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79**, 6128–6131
 Chen, H., and Anderson, R. E. (1993a) *J. Chromatogr.* **578**, 124–129
 Chen, H., and Anderson, R. E. (1993b) *Curr. Eye Res.* **12**, 851–860

- Cook, H. W., Byers, D. M., Palmer, F. B., Spence, M. W., Rakoff, H., Duval, S. M., and Emken, E. A. (1991) *J. Lipid Res.* **32**, 1265-1272
- Dizhoor, A. M., Ericsson, L. H., Johnson, R. S., Kumar, S., Olshevskaya, E., Zozulya, S., Neubert, T. A., Stryer, L., Hurley, J. B., and Walsh, K. A. (1992) *J. Biol. Chem.* **267**, 16033-16036
- Duronio, R. J., Reed, S. I., Gordon, J. I. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 4129-4133
- Dryja, T. P., Mcgee, T. L., Hahn, L. B., Cowley, G. S., Olsson, J. E., Reichel, E., Sandberg, M. A., and Berson, E. L. (1990) *N. Engl. J. Med.* **323**, 1302-1307
- Fliesler, S. J., and Anderson, R. E. (1983) *Prog. Lipid Res.* **22**, 79-131
- Gordon, J. I., Duronio, R. J., Rudnick, D. A., Adams, S. P., and Gokel, G. W. (1991) *J. Biol. Chem.* **266**, 8647-8650
- Hadjuagapiou, C., Travers, J., Fertel, R., and Sprecher, H. (1990) *Biochim. Biophys. Acta* **1046**, 167-172
- Hamosh, M., and Bitman, J. (1992) *Lipids* **27**, 848-857
- Hansen, H. S. (1993) *Trends Biochem. Sci.* **18**, 164
- Jaffe, R., Crumline, P., Hashida, Y., and Moser, H. W. (1982) *Am. J. Pathol.* **108**, 100-111
- James, G., and Olson, E. N. (1990) *Biochemistry* **29**, 2625-2635
- Johnson, R. S., Ohguro, H., Palczewski, K., Hurley, J. B., Walsh, K. A., and Neubert, T. A. (1994) *J. Biol. Chem.* **269**, 21067-21071
- Jones, T. L. Z., Simonds, W. F., Merendino, J. J., Jr., Brann, M. R., and Spiegel, A. M. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 568-572
- Kishore, N. S., Lu T., Knoll, L. J., Katoh, A., Rudnick, D. A., Mehta, P. P., Devadas, B., Huhn, M., Atwood, J. L., Adams, S. P., Gokel, G. W., and Gordon, J. I. (1991) *J. Biol. Chem.* **266**, 8835-8855
- Kishore, N. S., Wood, D. C., Mehta, P. P., Wade, A. C., Lu, T., Gokel, G. W., and Gordon, J. I. (1993) *J. Biol. Chem.* **268**, 4889-4902
- Kokame, K., Fukada, Y., Yoshizawa, T., Takao, T., and Shimonishi, Y. (1992) *Nature* **359**, 749-752
- Koletzko, B., Thiel, I., and Springer, S. (1992) *Eur. J. Clin. Nutr.* **46**, (Suppl. 4) 45-55
- Kupke, I. R., and Zeugner, S. (1978) *J. Chromatogr.* **146**, 261-271
- Kurono, G., Nishikawa, Y., Miyano, S., and Aburano, S. (1972) *Chem. & Pharm. Bull. (Tokyo)* **20**, 559-563
- Laemmli, U. K. (1970) *Nature* **227**, 680-685
- Linder, M. E., Pang, I. H., Duronio R. J., Gordon, J. I., Sternweis, P. C., and Gilman A. G. (1991) *J. Biol. Chem.* **266**, 4654-4659
- Louie, K., Wiegand, R. D., and Anderson, R. E. (1988) *Biochemistry* **27**, 9014-9020
- Lu, T., Li, Q., Katoh, A., Hernandez, J., Duffin, K., Jackson-Machelski, E., Knoll, L. J., Gokel, G. W., and Gordon, J. I. (1994) *J. Biol. Chem.* **269**, 5346-5357
- Luthria, D. L., Baykousheva, S. P., and Sprecher, H. (1995) *J. Biol. Chem.* **270**, 13771-13776
- Markley, K. S. (1960) *Fatty Acids: Their Chemistry, Properties, Production, and Uses*, Part 1, p. 12, Interscience Publishers, Inc., New York
- Martinez, M., Mougan, I., Roig, M., and Ballabriga, A. (1994) *Lipids* **29**, 273-280
- McIlhinney, R. A. J. (1990) *Trends Biochem. Sci.* **15**, 387-391
- McLaughlin, M. E., Sandberg, M. A., Berson, E. L., and Dryja, T. P. (1993) *Nat. Genet.* **4**, 130-134
- Moser, H. W., Naidu, S., Kumar, A. J., and Rosenbaum, A. E. (1987) *Crit. Rev. Neurobiol.* **21**, 240-249
- Neubert, T. A., Johnson, R. S., Hurley, J. B., and Walsh, K. A. (1992) *J. Biol. Chem.* **267**, 18274-18277
- Numa, S., Jeffcoat, R., and James, A. T. (1984) in *Fatty Acid Metabolism and Its Regulation* (Numa, S., ed) pp. 93-96, Elsevier Science Publishers B.V., Amsterdam
- Ohguro, H., Fukada, Y., Saito, T., and Akino, T. (1990) *Comp. Biochem. Physiol.* **4**, 763-765
- Olson, E. N., Towler, D. A., and Glaser, L. (1985) *J. Biol. Chem.* **260**, 3784-3790
- Oulhaj, H., Huynh, S., and Nouvelot, A. (1992) *Comp. Biochem. Physiol.* **4**, 897-904
- Ozols, J., Carr, S. A., and Strittmatter, P. (1984) *J. Biol. Chem.* **259**, 13349-13354
- Palczewski, K., Subbaraya, I., Gorczyca, W. A., Helekar, B. S., Ruiz, C. C., Ohguro, H., Huang, J., Zhao, X., Crabb, J. W., Johnson, R. S., Walsh, K. A., Gray-Keller, M. P., Detwiler, P. B., and Baehr, W. (1994) *Neuron* **13**, 395-404
- Papac, D. I., Thornburg, K. R., Bullesbach, E. E., Crouch, R. K., and Knapp, D. R. (1992) *J. Biol. Chem.* **267**, 16889-16894
- Poll-the, B. T., Saudubray, J. M., Ogier, H. A. M., Odievre, M., Scotto, J. M., Monnens, I., Govaerts, L. C. P., Roels, F., Cornelis A., Schutgens, R. B. H., Wanders, R. J. A., Scram, A. W., and Tager, J. M. (1987) *Eur. J. Pediatr.* **146**, 477-483
- Rudnick, D. A., Lu, T., Jackson-Machelski, E., Hernandez, J. C., Li, Q., Gokel, G. W., and Gordon, J. I. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 10507-10511
- Rosenfeld, P. J., Cowley, G. S., McGee, T. L., Sandberg, M. A., Berson, E. L., and Dryja, T. P. (1992) *Nat. Genet.* **1**, 209-213
- Rouser, G., Fliescher, S., and Yamamoto, A. (1970) *Lipids* **5**, 494-500
- Schlesinger, M. J. (1993) in *Lipid Modifications of Proteins*, pp. 23-25 and 62, CRC Press, Inc., Boca Raton, FL
- Schulz, H. (1991) *Biochim. Biophys. Acta* **1081**, 109-120
- Seabra, M. C., Brown, M. S., and Goldstein, J. L. (1993) *Science* **259**, 377-381
- Sprecher H. (1972) *Lipids* **3**, 14-20
- Sprecher H. (1972) *Fed. Proc.* **31**, 1451-1457
- St. Jules, R., Kennard, J., Setlik, W., and Holtzman, E. (1992) *Exp. Eye Res.* **54**, 1-8
- Towler, D. A., Gordon, J. I., Adams, S. P., and Glaser, L. (1988) *Annu. Rev. Biochem.* **57**, 69-99
- Umbarger, K. O., Yamazaki, M., Hutson, L. D., Hayashi, F., and Yamazaki, A. (1992) *J. Biol. Chem.* **267**, 19494-19502
- Voss, A., Reinhart, M., Sankarappa, S., and Sprecher, H. (1991) *J. Biol. Chem.* **266**, 19995-20000
- Wang, N., and Anderson, R. E. (1993) *Biochemistry* **32**, 13703-13709
- Wedegaertner, P. B., Wilson, P. T., and Bourne, H. R. (1995) *J. Biol. Chem.* **270**, 503-506
- Wood, R., and Lee, T. (1983) *J. Chromatogr.* **254**, 237-243
- Yang, Z., and Wensel, T. G. (1992) *J. Biol. Chem.* **267**, 23197-23201
- Yonemoto, W., McGlone, M. L., and Taylor, S. S. (1993) *J. Biol. Chem.* **268**, 2348-2352