Stereochemistry of the acyl dihydroxyacetone phosphate acyl exchange reaction

Samuel J. Friedberg,* Neera Satsangi,* and Susan T. Weintraub†

Department of Medicine,* Division of Endocrinology and Metabolism, and Department of Pathology,† The University of Texas Health Science Center, San Antonio, TX 78284

Abstract The fatty acid of acyl dihydroxyacetone phosphate can be exchanged enzymatically for another fatty acid. It has been shown that this reaction proceeds by cleavage of the oxygen bound to C-1 of the dihydroxyacetone phosphate (DHAP) moiety rather than by the more common cleavage at the acyl to oxygen bond. In the present study, the stereochemistry of this reaction was defined further; using deuterated substrates and fast atom bombardment-mass spectrometry, it was shown that the fatty acid exchange involves the stereospecific labilization of the pro-R hydrogen at C-1 of the DHAP moiety of acyl DHAP. The mechanism of ether bond formation, in which acyl DHAP is converted to *O*-alkyl DHAP, also proceeds via labilization of the pro-R hydrogen and cleavage of the fatty acid at the C-1 to oxygen bond. In addition, other workers have provided evidence that the enzyme responsible for the exchange reaction is *O*-alkyl DHAP synthetase. Therefore, the present results support the hypothesis that the acyl exchange is the reverse reaction of the first step in *O*-alkyl DHAP synthesis; in both of these reactions the pro-R hydrogen of C-1 of the DHAP moiety of acyl DHAP and the fatty acid moiety are labilized with cleavage of the fatty acid at the DHAP C-1 to oxygen bond. — **Friedberg, S. J., N. Satsangi, and S. T. Weintraub.** Stereochemistry of the acyl dihydroxyacetone phosphate acyl exchange reaction. *J. Lipid Res.* 1991. **32:** 259–266.

Supplementary key words *O*-alkyl DHAP • ether bond formation • ether lipids • glyceryl ethers • stereochemistry • enzymology • fast atom bombardment-mass spectrometry

The biosynthesis of the ether bond of ether lipids takes place by the replacement of the acyl moiety of acyl dihydroxyacetone phosphate (acyl DHAP) (1–3) by a long chain fatty alcohol, leading to the formation of *O*-alkyl DHAP (4–15). This reaction proceeds via an unusual mechanism that involves the stereospecific exchange of the pro-R[1] hydrogen of acyl DHAP and the intermediacy of an acyl enediol (16–25). In addition, it has been shown that the fatty acid moiety that is lost retains both carboxyl oxygens and that the fatty alcohol is added with retention of its oxygen (26–28). It has also been demonstrated by Davis and Hajra (29) and by Brown and Snyder (25, 28) that acyl DHAP can exchange its acyl group with a fatty acid from the medium, a reaction that is presumably mediated by the enzyme *O*-alkyl dihydroxyacetone P synthetase (25, 29). In this reaction, as in the fatty alcohol exchange reaction, both the leaving fatty acid and the fatty acid that is substituted have been shown to retain both carboxyl oxygens (30). Other known enzymatic acyl cleavage reactions, however, proceed via a different mechanism that involves cleavage of the fatty acid at the acyl to oxygen bond rather than at the DHAP C-1 to oxygen bond (31). In addition, chemical cleavage of acyl DHAP occurs at the acyl to oxygen bond (27). It has also been shown that there are enzymes that cleave acyl DHAP by the usual mechanism, that is, at the acyl to oxygen bond (21, 27).

In this report, data are provided to show that the acyl DHAP/fatty acid exchange reaction involves the same stereospecific hydrogen exchange observed in the reaction in which the fatty acid of acyl DHAP is replaced by a long chain fatty alcohol. Previous data provided by Brown and Snyder (25) indicated that tritium from acyl-[1-R-^{3}H]DHAP is lost in this acyl exchange reaction. The data presented here extend these observations to show that the reaction is stereospecific for the pro-R hydrogen and does not involve the pro-S hydrogen.

Abbreviations: DHAP, dihydroxyacetone phosphate; FAB, fast atom bombardment-mass spectrometry; GC-MS, gas chromatography-mass spectrometry.

[1]"[1-R-D]" designates a deuterium atom bound to C-1 of DHAP that has a spatial configuration such that the three largest substituents attached to C-1 of DHAP have a clockwise spatial arrangement, in decreasing priority order (i.e., OH>C-2>D), when the substituent of lowest priority at C-1 (H) is pointed away from the observer. "[1-S-D]" designates a deuterium atom that has a configuration such that the three largest substituents attached to C-1 of DHAP have a counterclockwise spatial arrangement in decreasing priority order (i.e., OH>C-2>D) when observed with the H at C-1 pointed away from the observer. When two of the substituents at C-1 are hydrogen atoms and, therefore, a center of asymmetry (four different substituents) does not exist at C-1, the two hydrogens are designated pro-R and pro-S, respectively (44). For definition of prochirality, see reference 44.

MATERIALS AND METHODS

Materials

Dihydroxyacetone phosphate (lithium salt), triosephosphate isomerase (yeast, coated on beads), fructose-1,6-diphosphate aldolase (rabbit), deuterium oxide (99.8%), 4-dimethylaminopyridine, and heptadecanoic anhydride were obtained from Sigma Chemical Co. (St. Louis, MO). Dry pyridine and BSTFA (N,O-*bis*(trimethylsilyl)-trifluoroacetamide) containing 1% trimethylchlorosilane were obtained from Pierce (Rockford, IL). Methoxyamine hydrochloride was obtained from Alltech Associates (Houston, TX). Prepared plates for thin-layer chromatography manufactured by Macherey-Nagle (MN) and by E. Merck (EM) were obtained from Brinkmann (Westbury, NY).

Methods

Mass spectrometry. Electron impact mass spectra were acquired on a Finnigan-MAT model 4615 quadrupole mass spectrometer in combination with an INCOS data system. The ion source temperature was 180°C and the electron energy was 70 eV. Gas chromatographic separation was accomplished by means of a 12 m × 0.32 mm BP-1 fused silica capillary column (SGE, Austin, TX) that was connected directly to the mass spectrometer. Helium was used as the carrier gas at a linear velocity of approximately 70 cm/sec. Both the injector and the interface to the mass spectrometer were maintained at 250°C. An initial GC oven temperature of 170°C was held for 2 min and was then increased at 20°C/min to a final temperature of 210°C. Split injections (20 ml/min splitter flow) were utilized.

Fast atom bombardment–mass spectrometry (FAB–MS) using negative ion detection was performed on a Finnigan-MAT model 212 mass spectrometer in combination with an INCOS data system. An Ion Tech saddle field atom gun was used with xenon at 8 kV. The accelerating voltage of the mass spectrometer was 3 kV and the ion source temperature was approximately 70°C. Samples were applied to the copper probe tip as a solution in chloroform–methanol 8:2 (v/v). Thioglycerol (approximately 2 μl) was then added and mixed with the sample. The contributions of the thioglycerol background were subtracted from all FAB spectra.

Synthesis of heptadecanoyl-[1-R-D]DHAP. The pro-R hydrogen at C-1 of DHAP was exchanged with deuterium by means of triosephosphate isomerase (32–35). The DHAP was subsequently acylated by the method of Gupta, Radhakrishnan, and Khorana (36). Twenty-five mg of DHAP, lithium salt, was dissolved in 8 ml of deuterium oxide. The pH was adjusted to 7.3 with concentrated ammonium hydroxide. In order to minimize dilution of deuterium with hydrogen, and in order to facilitate the recovery of DHAP, all buffers were avoided. Twenty-five units of solid triosephosphate isomerase was added, and the reaction was allowed to proceed at room temperature for 20 min. Twenty-five mg of carrier bovine serum albumin was then added, followed immediately by the addition of 12 ml of absolute ethanol to inactivate the isomerase (32). The reaction mixture was centrifuged at 2000 *g* for 10 min, and the supernatant was removed. An aliquot containing 100 μg of DHAP was dried under nitrogen and then under high vacuum for 20 min in a 1-ml conical glass vial provided with a Teflon-lined screw-cap closure (Reactivial, Pierce, Rockford, IL). The residue was treated with 50 μl of saturated methoxyamine hydrochloride in dry pyridine for 20 min at 60°C, with stirring provided by a small wedge-shaped magnetic stirring bar. Fifty μl of BSTFA was then added, and the reaction was continued for another 20 min. The resulting solution was used directly for mass spectrometric analysis. GC–MS analysis showed that approximately 3% of the DHAP remained undeuterated, and roughly 5% contained two atoms of deuterium. These percentages were deduced from the relative intensities of the $[M\text{-}15]^+$ ions for the variously labeled components after correction for natural isotopic abundances.

For acylation by the method of Gupta et al. (36), the ethanol–water solution of [1-R-D]DHAP was converted to the pyridinium salt by passage through a 5-g column of Dowex 50-4X, 200–400 mesh, in the pyridinium form. The latter was prepared by passing five volumes of 5 M pyridinium acetate through Dowex 50-4X in the hydrogen form, followed by thorough washing with water. The resulting solution of pyridinium DHAP was dried by means of reduced pressure on a rotary evaporator at 37°C and then by high vacuum overnight. To the gummy residue was added 25 ml of ethanol-free dry chloroform containing 3 equivalents of 4-dimethylaminopyridine and 3 equivalents of heptadecanoic anhydride. The reaction mixture was flushed with nitrogen, stirred in the dark for 3 days and then partitioned by extraction by the method of Bligh and Dyer (37) using 2 M formic acid instead of water to form the aqueous phase. 4-Dimethylaminopyridine was removed by washing the chloroform phase twice with blank upper phase made 2 M in formic acid.

The chloroform phase was dried under nitrogen, and the residue was applied to four 20 × 20 cm, 1-mm-thick silica gel plates (MN, from Brinkmann) that were then developed in chloroform–methanol–acetic acid 80:20:20 (v/v/v). The major product was heptadecanoyl DHAP (R_f 0.45). In addition, approximately 10 to 20% of a phosphorus-containing compound ran ahead of heptadecanoyl DHAP (R_f 0.60). By fast atom bombardment–mass spectrometry, this compound had an apparent molecular weight that was 14 units higher than that of heptadecanoyl DHAP. It seems likely that this material represented the methyl ester of heptadecanoyl DHAP, which might have formed during chloroform–methanol

extraction under acidic conditions. An additional phosphorus-containing compound was seen near the solvent front. This material, possibly a mixed anhydride of the phosphate of acyl DHAP and fatty acid (25), was not present in large amounts in our preparations and was discarded. Attempts to isolate this compound and analyze it by FAB yielded a spectrum identical to that of acyl DHAP.

Heptadecanoyl-[1-R-D]DHAP was extracted from the silica gel as follows. The silica gel was scraped from the plates, ground in a mortar, and transferred to a tube. Four volumes of chloroform–methanol–2 M formic acid 2:1:0.8 (v/v/v) was added to the silica gel, and the mixture was agitated for 15 min. After centrifugation, the supernatant was removed and the silica gel was extracted again with two volumes of the same mixture. To the combined extracts, sufficient chloroform and 2 M formic acid was added in the proportions required by the method of Bligh and Dyer (37) to separate the phases. The upper phase was discarded, and the lower phase was washed twice with blank upper phase using 2 M formic acid instead of water. Although some acyl DHAP is lost in the aqueous phase, this procedure eliminated many impurities that interfered with mass spectrometry.

The FAB mass spectrum of the product (**Fig. 1A**) indicated that the major component was mono-deuterated heptadecanoyl DHAP. The intense ion at *m/z* 422 represents $[M-H]^-$, the anion formed in the mass spectrometer by loss of a proton from heptadecanoyl-[1-R-D]DHAP (mol wt 423). In order to assess the degree of deuterium labeling, a comparison was made with the FAB mass spectrum of nondeuterated hexadecanoyl DHAP (Fig. 1B). By determining alterations in relative intensities of corresponding ions in the spectrum of the deuterated analog with those in the unlabeled compound, it was shown that the ratio of mono-deuterated to di-deuterated heptadecanoyl-DHAP remained the same as in the starting [1-R-D]DHAP; however, additional unlabeled material was formed by exchange with the medium during isolation. The composition of the final product was: 18% 0-D, 76% 1-D, 5% 2-D. These calculations take into account the natural relative abundance of ^{13}C of 1.1%, as illustrated by the peak at *m/z* 408 in Fig. 1B. Thus, the peak at *m/z* 423 is of appropriate relative intensity for the 5% di-deuterated heptadecanoyl DHAP in addition to the contribution from ^{13}C present in heptadecanoyl DHAP containing one deuterium.

Synthesis of heptadecanoyl [1-S-D]DHAP. The pro-S hydrogen of DHAP was exchanged with deuterium in a manner similar to that used for the exchange of the pro-R hydrogen except that fructose-1,6-diphosphate aldolase was used instead of isomerase (32, 33). Accordingly, 25 mg of the lithium salt of DHAP was dissolved in 8 ml of deuterium oxide. The pH was adjusted to 7.3 with concentrated ammonium hydroxide when necessary. Twenty-five units of

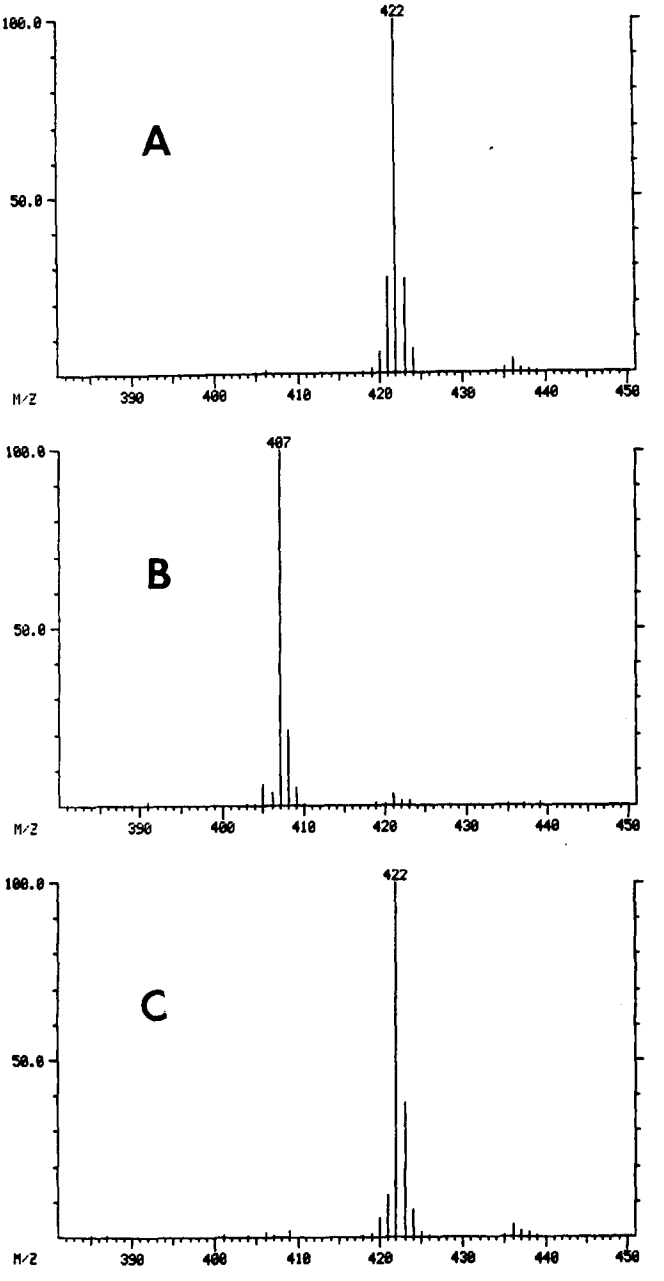

Fig. 1. FAB mass spectra (negative ion detection) of acyl DHAP: A, heptadecanoyl-[1-R-D]DHAP; B, unlabeled hexadecanoyl-DHAP; C, heptadecanoyl-[1-S-D]DHAP. The contributions from the thioglycerol matrix have been subtracted from the spectra.

lyophilized fructose-1,6-diphosphate aldolase was added, and incubation was carried out for 20 min. The procedure was otherwise identical to the one used for the synthesis of heptadecanoyl-[1-R-D]DHAP. After formation of the methoxime, trimethylsilyl ether derivative, GC–MS was used to characterize the product formed by aldolase-catalyzed deuterium exchange of DHAP. The resulting mass spectrum revealed that essentially none of the DHAP remained unlabeled, but that a significant portion

had acquired two deuterium atoms (approximately 17%). It was concluded that the second deuterium was attached to C-3 of DHAP because the C-1 fragment at *m/z* 104 ([CHD-O-TMS]$^+$) was present as expected, and because there was no unaccounted for increase in ion intensity at *m/z* 105 ([CD_2-O-TMS]$^+$). From additional experiments it was learned that the degree of labeling at C-3 depended on the duration of incubation with aldolase. This finding had been previously reported by Lowe and Pratt (38) who noted that aldolase exchanged the hydrogen at C-3 of DHAP as well as at C-1, but at a rate 30 times more slowly than at C-1. However, in our experiments, the rate of labeling at C-3 was found to be considerably more rapid than the rate reported by these workers. This necessitated stopping the reaction when maximal labeling at C-1 occurred before excessive labeling took place at C-3. Thus, it was not feasible to obtain an adequately labeled product which contained only 1-S mono-deuterated DHAP. The FAB mass spectrum of heptadecanoyl-[1-S-D]DHAP, shown in Fig. 1C, indicates that 6% of the product had no deuterium and that 14% contained two atoms of deuterium.

Other synthetic procedures. Tetradecanoyl and hexadecanoyl DHAP were synthesized by the method of Schlenk, Lamp, and De Haas (39) as modified by Hajra (6), Hajra, Saraswathi, and Das (40), and Peterson et al. (41).

Enzyme preparation. The enzyme used in these studies was isolated from Ehrlich ascites tumor cells obtained from IRC Swiss mice and was solubilized, partially purified, and delipidated by the method of Brown and Snyder (24). This preparation contained very little free fatty acid, a condition that was important for demonstrating exchange of the fatty acid of heptadecanoyl DHAP with added hexadecanoic acid without competition from endogenous fatty acids present in the enzyme preparation. Ehrlich ascites tumor cell microsomes were prepared from 20 to 25 mice as previously described (16). The microsomes were solubilized in 0.05 M Tris buffer, pH 7.3, containing 1% Triton X-100, 0.05 M sodium fluoride, and 0.25 M sucrose. The preparation was then centrifuged at 100,000 *g* for 40 min. The supernatant was rapidly added dropwise to 10 volumes of ice-cold acetone and stirred until a gummy precipitate was formed. The acetone was decanted, and the gummy mass was dried with a strong stream of nitrogen until the odor of acetone was no longer detectable. Excess water was blotted away with paper towels. The precipitate was rapidly transferred by scraping into a glass homogenizer containing 0.05 M Tris buffer, pH 9.0, 20% ethylene glycol, 0.05 M sodium fluoride, and 0.2% Triton X-100, and immediately dispersed with a Teflon pestle. Insoluble material was removed by centrifugation at 100,000 *g* for 45 min. The final preparation contained 2.6 mg protein per ml, a 3.1-fold purification over the microsomal suspension.

Incubation. In the complete system, 1.2 ml of delipidated, solubilized enzyme, adjusted to pH 8.3, was incubated in a final volume of 2.4 ml of 0.05 M Tris buffer, pH 8.3, containing 150 nmol of heptadecanoyl-[1-R-D] or heptadecanoyl-[1-S-D]DHAP, 150 nmol of hexadecanoic acid, 0.05 M sodium fluoride, 0.2% Triton X-100, and 20% ethylene glycol. Lipid substrates were first dried in the tubes to be used for incubation and then dispersed in the buffer containing Triton X-100 by brief heating and vortexing. Incubation was carried out for 2 h at 37°C.

Extraction and recovery of acyl DHAP from the reaction mixture. The following procedure for extraction and purification of acyl DHAP was devised to provide a preparation satisfactory for mass spectrometric analysis. At the end of incubation, the reaction was stopped by extraction by the method of Bligh and Dyer (37) using 2 M formic acid instead of water. An internal standard, 100 nmol of tetradecanoyl DHAP, was added at the beginning of the extraction procedure after the addition of methanol. The lipid extracts were applied in benzene in 2.5-cm bands to 20 × 20 cm silica gel plates (EM), 0.25 mm thick. In our hands, these plates were the only ones that did not produce excessive trailing of acyl DHAP. Development was carried out without wicks for 8 cm in a solvent system consisting of chloroform–methanol–acetic acid 90:15:10 (v/v/v). In this system, phosphatidylcholine, lysophosphatidylcholine, phosphatidylserine, sphingomyelin, phosphatidic acid, and lysophosphatidic acid had an R_f of 0.2 or less, while acyl DHAP and phosphatidylethanolamine exhibited R_f values of 0.3 and 0.35, respectively. After the first development, a 2-mm horizontal trench was cut across the plate to remove silica gel just below acyl DHAP and above slower running phospholipids. Acyl DHAP was located by means of standards, using the spray of Dittmer and Lester (42). After thorough drying, the plates were developed a second time with chloroform–methanol–acetic acid 80:20:20 (v/v/v) to 4 cm below the top of the plate. In this second development, enough solvent was added to the tank to reach just beyond the upper edge of the trench. This procedure effects good separation between acyl DHAP and phosphatidylethanolamine. The area containing acyl DHAP was scraped from the plate, pulverized with an agate mortar and pestle, and transferred to a screw-cap tube. Chloroform–methanol–2 M formic acid 6:3:2.4 (v/v/v), 11.4 ml, was added. The tube was vortexed for 2 min and centrifuged. The supernatant was removed to a second tube. More of the same solvent mixture (5.7 ml) was added to the silica gel and vortexing and centrifugation were repeated. To the combined supernatants were added 4.5 ml of chloroform and 4.5 ml of 2 M formic acid. After vortexing, the upper layer was discarded and the lower layer was washed twice with blank upper phase prepared with 2 M formic acid instead of water. The lower layer was dried and dissolved in 50 μl of chloroform–methanol 8:2 (v/v).

RESULTS

Experiments with heptadecanoyl-[1-R-D]DHAP

These experiments were carried out to determine whether the acyl DHAP/fatty acid exchange reaction, like the reaction leading to the formation of *O*-alkyl DHAP, takes place by a mechanism involving exchange of the pro-R hydrogen at C-1 of the DHAP moiety of acyl DHAP. After incubation of heptadecanoyl-[1-R-D]DHAP with hexadecanoic acid in a system containing partially delipidated, solubilized enzyme capable of synthesizing *O*-alkyl DHAP, the appearance of an ion at *m/z* 407, representing $[M-H]^-$ for hexadecanoyl DHAP in the negative ion FAB mass spectrum, demonstrated the formation of hexadecanoyl DHAP that had lost deuterium (**Fig. 2A**). Since the relative intensity at *m/z* 408 was appropriate for the normal isotopic distribution of ^{13}C, the results indicate that no hexadecanoyl-[1-R-D]DHAP was formed under these conditions. Control samples were generated by stopping the reaction immediately after addition of the enzyme preparation (Fig. 2B) or by incubating heptadecanoyl-[1-R-D]DHAP for 2 h without enzyme (Fig. 2C). Neither in these control experiments nor in the starting material (Fig. 1A) was there any appreciable ion intensity at *m/z* 407 or *m/z* 408.

In interpreting the results of these experiments, the following considerations must be taken into account. The [1-R-D]DHAP that was used as a starting material for synthesis of heptadecanoyl-[1-R-D]DHAP was shown to be essentially completely deuterated. However, because of a small amount of chemically induced enolization that occurs during purification, the substrate used in these experiments, heptadecanoyl-[1-R-D]DHAP, unavoidably contained some nondeuterated heptadecanoyl DHAP. An additional amount of nondeuterated heptadecanoyl DHAP was also formed during the isolation procedure following incubation. This is evident by comparing Fig. 1A with Fig. 2A–2C. All of the spectra in Fig. 2 exhibited increased ion intensity at *m/z* 421 as compared to the spectrum of substrate that was not subjected to additional manipulation (Fig. 1A). This includes substrate that was extracted, isolated, and purified without a 2-h incubation (Fig. 2C). Spontaneous chemical enolization of acyl DHAP appears to be unavoidable in an aqueous environment, but this problem did not interfere with interpretation of the results.

Experiments with heptadecanoyl-[1-S-D]DHAP

The substrate used in these experiments contained predominantly heptadecanoyl-[1-S-D]DHAP. Approximately 14% of the substrate consisted of material containing deuterium at both C-1 and C-3 of DHAP (Fig. 1C). As indicated in Methods, the rate of deuterium labeling at C-3 of DHAP was such that it was not practical to obtain material exclusively labeled at C-1.

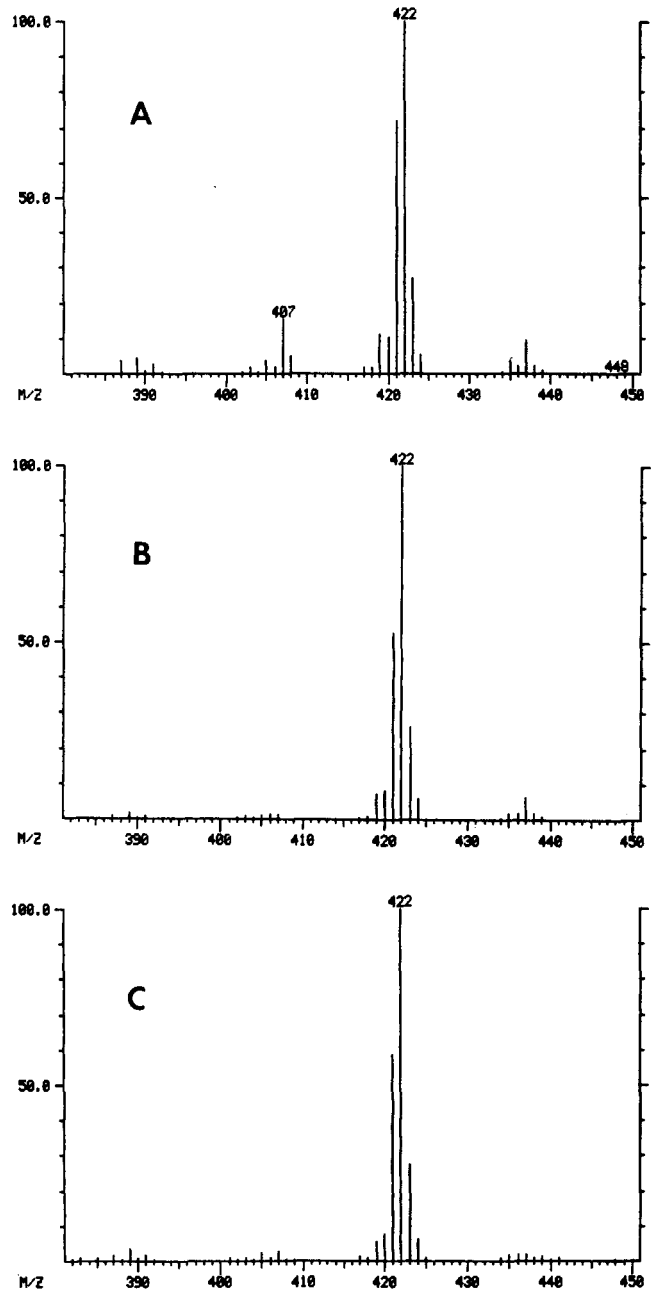

Fig. 2. FAB mass spectra (negative ion detection) of acyl DHAP fractions isolated after incubation of heptadecanoyl-[1-R-D]DHAP in an enzyme system containing *O*-alkyl DHAP synthetase and excess hexadecanoic acid: A, 2-h incubation; B, extraction immediately after addition of enzyme; C, 2-h incubation without enzyme. The contributions from the thioglycerol matrix have been subtracted from the spectra.

On incubation of this substrate for 2 h in the enzyme system described above, increased intensity was observed at both *m/z* 407 and *m/z* 408 (**Fig. 3A**). These ions represent hexadecanoyl DHAP formed by exchange of the acyl moiety of heptadecanoyl DHAP with hexadecanoic acid. The ion at *m/z* 408 is derived from acyl exchange with mono-deuterated heptadecanoyl-[1-S-D]DHAP (*m/z*

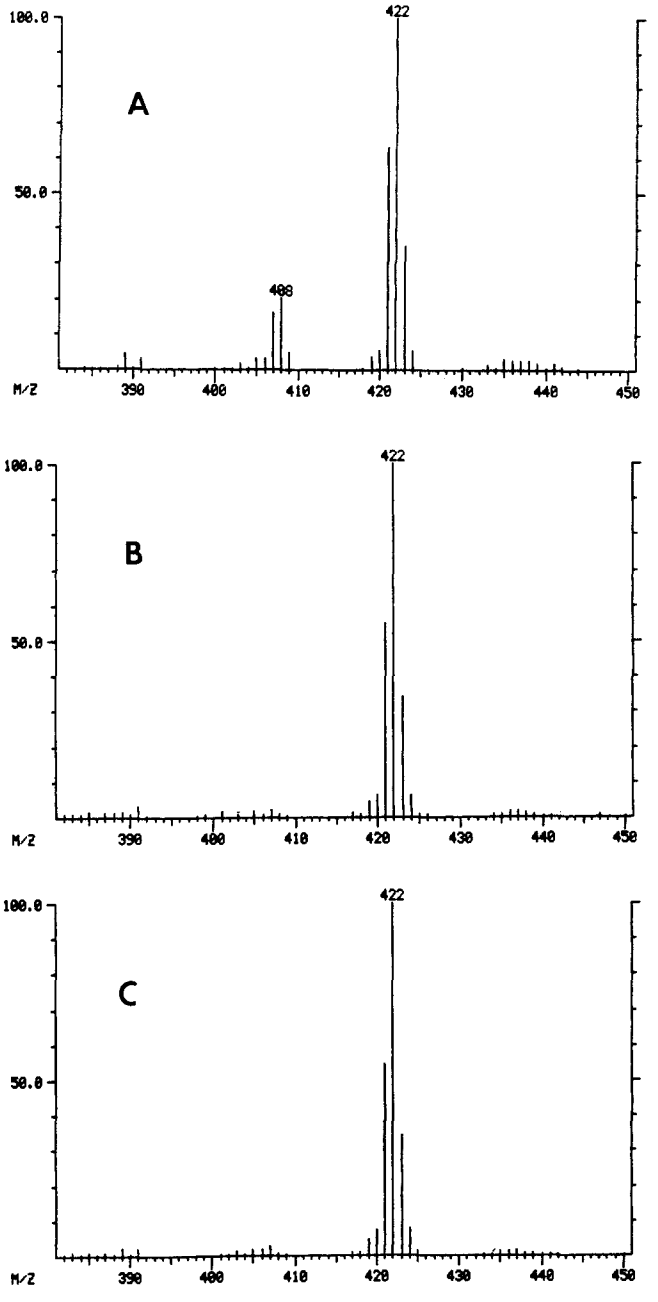

Fig. 3. FAB mass spectra (negative ion detection) of acyl DHAP isolated after incubation of heptadecanoyl-[1-S-D]DHAP in an enzyme system containing *O*-alkyl DHAP synthetase and excess hexadecanoic acid: A, 2-h incubation; B, extraction immediately after addition of enzyme; C, 2-h incubation without enzyme. The contributions from the thioglycerol matrix have been subtracted from the spectra.

422). The ion at *m/z* 407 is generated in part by acyl exchange with heptadecanoyl DHAP containing no deuterium (*m/z* 421) and in part from chemical enolization of deuterated hexadecanoyl DHAP (*m/z* 408) formed enzymatically from mono-deuterated heptadecanoyl-[1-S-D]DHAP. As in the results obtained with heptadecanoyl-[1-R-D]DHAP described above, the increase in intensity at *m/z* 421 (Fig. 3A–3C) is the result of nonspecific chemical enolization that occurred during extraction and purification after incubation. This conclusion is supported by the fact that the relative intensity at *m/z* 421, representative of nondeuterated heptadecanoyl DHAP, was approximately the same in the zero-time sample and in the samples incubated with and without enzyme (Figs. 3A–3C), while the unincubated substrate (Fig. 1C), which had not been subjected to the isolation procedure, contained a far lower relative intensity at *m/z* 421.

In the spectrum displayed in Fig. 3A, the small peak at *m/z* 409 is predominantly representative of ^{13}C associated with mono-deuterated hexadecanoyl DHAP. There is also a minor contribution from di-deuterated hexadecanoyl DHAP formed by enzymatic conversion of heptadecanoyl DHAP that had deuterium at both C-1 and C-3 of DHAP.

Control experiments for the studies with heptadecanoyl-[1-S-D]DHAP were performed in a manner analogous to those described above for heptadecanoyl-[1-R-D]DHAP. Once again, no acyl exchange was observed in the absence of active enzyme. Fig. 3B shows that no appreciable ion intensities were generated at *m/z* 407 or at *m/z* 408 when the reaction was stopped immediately after the addition of enzyme. Likewise, Fig. 3C shows that there were insignificant changes in the intensities of ions at *m/z* 407 or *m/z* 408 after 2 h of incubation when enzyme was omitted from the complete system.

DISCUSSION

These experiments were designed to determine whether the acyl DHAP/fatty acid exchange reaction involves a stereochemical exchange of the pro-R hydrogen at C-1 of the DHAP moiety. In order to perform these experiments, heptadecanoyl-[1-R-D]DHAP was prepared enzymatically by replacing the pro-R hydrogen at C-1 of DHAP with deuterium by means of triosephosphate isomerase in the presence of 99.8% deuterium oxide. The DHAP, stereospecifically labeled with deuterium, was then chemically acylated by the method of Gupta et al. (36). Fast atom bombardment–mass spectrometry with negative ion detection showed that this material exhibited an intense ion at *m/z* 422, the $[M-H]^-$ produced in the mass spectrometer by loss of a proton from mono-deuterated heptadecanoyl DHAP (mol wt 423). When heptadecanoyl-[1-R-D]DHAP was incubated in a lipid-poor *O*-alkyl DHAP generating system with added excess hexadecanoic acid, the result was the formation of hexadecanoyl DHAP which had lost deuterium. This was evident from the appearance of increased ion intensity at *m/z* 407, the $[M-H]^-$ of unlabeled hexadecanoyl DHAP (mol wt 408).

Subsequently, heptadecanoyl-[1-S-D]DHAP was synthesized by replacing the pro-S hydrogen of DHAP enzymatically with deuterium by means of fructose-1,6-

diphosphate aldolase, followed by acylation. When heptadecanoyl-[1-S-D]DHAP was incubated in the same enzyme system with added excess hexadecanoic acid, a significant amount of hexadecanoyl DHAP was generated which had not lost deuterium, as evidenced by the appearance of an ion at *m/z* 408, the $[M-H]^-$ of monodeuterated hexadecanoyl DHAP.

These experiments indicate that the acyl exchange reaction described by Davis and Hajra (29), in which the fatty acid of acyl DHAP is exchanged for another fatty acid in the medium, involves a stereospecific exchange of the pro-R hydrogen of the DHAP moiety of acyl DHAP with retention of the pro-S hydrogen. In a previous investigation of the exchange reaction it was shown that the newly acquired fatty acid retains both carboxyl oxygens (30). Since both the exchange reaction and the synthetase reaction involve the same unusual cleavage at the DHAP C-1 to oxygen bond (26–28), labilization of the pro-R hydrogen of DHAP (16–26), and utilization of chromatographically co-purified enzyme fractions, it has been postulated that this reaction is catalyzed by *O*-alkyl DHAP synthetase (24, 25, 28, 29). These findings provide additional evidence that the acyl exchange reaction represents the reverse reaction in the first step of the mechanism of ether bond formation in which the pro-R hydrogen and the fatty acid are lost. To date, however, no data have come to our attention that support the hypothesis (24) that steps following the binding of the fatty alcohol are reversible. To the contrary, Davis and Hajra have shown that labeled hexadecanoic acid does not appear to exchange enzymatically with the fatty alcohol of *O*-alkyl DHAP (43).

We gratefully acknowledge the technical assistance of Linda Chadwell.

Manuscript received 13 July 1990 and in revised form 9 October 1990.

REFERENCES

1. Hajra, A. K., and B. W. Agranoff. 1967. Acyl dihydroxyacetone phosphate. *J. Biol. Chem.* **242:** 1074–1078.
2. Hajra, A. K., and B. W. Agranoff. 1968. Acyl dihydroxyacetone phosphate. Characterization of a ^{32}P-labeled lipid from guinea pig liver mitochondria. *J. Biol. Chem.* **243:** 1617–1622.
3. Hajra, A. K. 1968. Biosynthesis of acyl dihydroxyacetone phosphate in guinea pig liver mitochondria. *J. Biol. Chem.* **243:** 3458–3465.
4. Hajra, A. K. 1970. Acyl dihydroxyacetone phosphate: precursor of alkyl ethers. *Biochem. Biophys. Res. Commun.* **39:** 1037–1044.
5. Snyder, F. 1972. Ether Lipids. Academic Press, New York, NY.
6. Hajra, A. K. 1983. Biosynthesis of *O*-alkylglycerol ether lipids. *In* Ether Lipids. Biochemical and Biomedical Aspects. H. K. Mangold and F. Paltauf, editors. Academic Press, New York, NY. 85–106.
7. Friedberg, S. J., and R. C. Greene. 1967. Glyceryl ether synthesis from long chain alcohols in elasmobranch stomach. *J. Biol. Chem.* **242:** 5709–5714.
8. Friedberg, S. J., and R. C. Greene. 1968. The synthesis of glyceryl ether glycerol in *Tetrahymena pyriformis*. *Biochim. Biophys. Acta.* **164:** 602–603.
9. Snyder, F., B. Malone, and R. L. Wykle. 1969. The biosynthesis of alkyl ether bonds in lipids by a cell-free system. *Biochem. Biophys. Res. Commun.* **34:** 40–47.
10. Wykle, R. L., and F. Snyder. 1969. The glycerol source for the biosynthesis of alkyl glyceryl ethers. *Biochem. Biophys. Res. Commun.* **27:** 658–662.
11. Hajra, A. K. 1969. Biosynthesis of alkyl-ether containing lipid from dihydroxyacetone phosphate. *Biochem. Biophys. Res. Commun.* **27:** 486–492.
12. Snyder, F., B. Malone, and M. L. Blank. 1970. Enzymatic synthesis of O-alkyl bonds in glycerolipids. *J. Biol. Chem.* **245:** 1790–1799.
13. Snyder, F., M. L. Blank, B. Malone, and R. L. Wykle. 1970. Identification of *O*-alkyl dihydroxyacetone phosphate, *O*-alkyl dihydroxyacetone, and diacylglyceryl ethers after enzymatic synthesis. *J. Biol. Chem.* **245:** 1800–1805.
14. Wykle, R. L., and F. Snyder. 1970. Biosynthesis of an *O*-alkyl ketone intermediate by microsomal enzymes in Ehrlich ascites tumors. *J. Biol. Chem.* **245:** 3047–3048.
15. Wykle, R. L., C. Piantadosi, and F. Snyder. 1972. The role of acyl dihydroxyacetone phosphate, reduced nicotinamide adenine dinucleotide, and reduced nicotinamide adenine dinucleotide phosphate in the biosynthesis of *O*-alkyl glycerolipids by microsomal enzymes of Ehrlich ascites tumor. *J. Biol. Chem.* **247:** 2944–2948.
16. Friedberg, S. J., A. Heifetz, and R. C. Greene. 1971. Loss of hydrogen from dihydroxyacetone phosphate during glyceryl ether synthesis. *J. Biol. Chem.* **246:** 5822–5827.
17. Friedberg, S. J., A. Heifetz, and R. C. Green. 1972. Studies on the mechanism of *O*-alkyl lipid synthesis. *Biochemistry.* **11:** 297–301.
18. Friedberg, S. J., and A. Heifetz. 1973. Hydrogen exchange in the synthesis of glyceryl ether and in the formation of dihydroxyacetone in *Tetrahymena pyriformis*. *Biochemistry.* **12:** 1100–1106.
19. Friedberg, S. J., and A. Heifetz. 1975. The formation of tritiated *O*-alkyl lipid from acyl dihydroxyacetone phosphate in the presence of tritiated water. *Biochemistry.* **14:** 570–574.
20. Friedberg, S. J., and R. D. Alkek. 1977. Absolute configuration of tritiated *O*-alkyl glycerol synthesized enzymatically from 1, 3-$^{3}H_2$, 1,3-$^{14}C_2$ dihydroxyacetone phosphate. *Biochemistry.* **16:** 5291–5294.
21. Davis, P. A., and A. K. Hajra. 1979. Stereochemical specificity of the biosynthesis of the alkyl ether bond in alkyl ether lipids. *J. Biol. Chem.* **254:** 4760–4763.
22. Friedberg, S. J., D. M. Gomillion, and P. L. Stotter. 1980. The mechanism of ether bond formation in *O*-alkyl lipid synthesis. *J. Biol. Chem.* **242:** 5709–5714.
23. Friedberg, S. J., and M. Gomillion. 1981. Hydrogen exchange in the formation of dihydroxyacetone phosphate from acyl dihydroxyacetone phosphate in *O*-alkyl lipid synthesis in Ehrlich ascites tumor cell microsomes. *J. Biol. Chem.* **256:** 291–295.
24. Brown, A. J., and F. Snyder. 1983. Alkyldihydroxyacetone-P synthase solubilization, partial purification, new assay method, and evidence for a ping-pong mechanism. *J. Biol. Chem.* **257:** 4184–4189.

25. Brown, J., and F. Snyder. 1983. The mechanism of alkyl dihydroxyacetone-P synthase. Formation of [^3H_2O] from acyl [1-R-^{3}H]dihydroxyacetone-P by purified alkyldihydroxyacetone-P synthase in the absence of acylhydrolase activity. *J. Biol. Chem.* **258:** 4184–4189.
26. Snyder, F., W. T. Rainey, Jr., M. L. Blank, and W. H. Christie. 1970. The source of oxygen in the ether bond of glycerolipids. *J. Biol. Chem.* **245:** 5853–5856.
27. Friedberg, S. J., S. T. Weintraub, M. R. Singer, and R. C. Greene. 1983. The mechanism of ether bond formation in *O*-alkyl lipid synthesis in Ehrlich ascites tumor: unusual cleavage of the fatty acid moiety of acyl dihydroxyacetonephosphate. *J. Biol. Chem.* **258:** 136–142.
28. Brown, A. J., G. L. Glish, E. H. McBay, and F. Snyder. 1985. Alkyl dihydroxyacetone phosphate mechanism: ^{18}O studies of fatty acid release from acyl dihydroxyacetone phosphate. *Biochemistry.* **24:** 8012–8016.
29. Davis, P. A., and A. Hajra. 1977. The enzymatic exchange of the acyl group of acyl dihydroxyacetone phosphate with fatty acid. *Biochem. Biophys. Res. Commun.* **74:** 100–105.
30. Friedberg, S. J., D. M. Peterson, S. T. Weintraub, and N. Satsangi. 1987. O-Alkyl lipid synthesis: the mechanism of the acyl dihydroxyacetone-fatty acid exchange. *Biochem. Biophys. Res. Commun.* **145:** 1177–1184.
31. Wells, M. A. 1971. Evidence for *O*-acyl cleavage during hydrolysis of 1,2-diacyl-*sn*-glycero-3-phosphorylcholine by the phospholipase A_2 of *Crotalus adamanteus* venom. *Biochim. Biophys. Acta.* **238:** 80–86.
32. Bloom, B., and Y. J. Topper. 1956. Mechanism of action of aldolase and phosphotriose isomerase. *Science.* **124:** 982–983.
33. Rose, I. A., and S. V. Rieder. 1958. Studies on the mechanism of the aldolase reaction. Isotope exchange reactions of muscle and yeast aldolase. *J. Biol. Chem.* **231:** 315–329.
34. Rieder, S. V., and I. A. Rose. 1959. The mechanism of the triosephosphate isomerase reaction. *J. Biol. Chem.* **234:** 1007–1010.
35. Rose. I. A. 1958. The absolute configuration of dihydroxyacetone phosphate tritiated by aldolase reaction. *J. Am. Chem. Soc.* **80:** 5835–5836.
36. Gupta, C. M., R. Radhakrishnan, and H. G. Khorana. 1977. Glycerophospholipid synthesis: improved general method and new analogs containing photoactivable groups. *Proc. Natl. Acad. Sci. USA.* **74:** 4315–4319.
37. Bligh, E. G., and W. J. Dyer. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–919.
38. Lowe, G., and R. F. Pratt. 1976. Proton exchange of the pro-S hydrogen at C-1 in dihydroxyacetone phosphate and D-fructose-1-phosphate catalyzed by rabbit-muscle aldolase. *Eur. J. Biochem.* **66:** 95–104.
39. Schlenk, H., B. G. Lamp, and B. W. De Haas. 1952. The synthesis of derivatives of dihydroxyacetone and of glycerides. *J. Am. Chem. Soc.* **74:** 2550–2552.
40. Hajra, A. K., T. V. Saraswathi, and A. K. Das. 1983. Chemical synthesis of 1-O-alkyl and 1-O-acyl dihydroxyacetone-3-phosphate. *Chem. Phys. Lipids.* **33:** 179–193.
41. Peterson, D. M., R. A. Martinez, N. Satsangi, S. T. Weintraub, P. L. Stotter, and S. J. Friedberg. 1988. The synthesis of regiospecifically labeled [^{18}O]glycolic acid and [^{18}O]acyl dihydroxyacetone phosphate. *J. Lipid Res.* **29:** 94–101.
42. Dittmer, J. C., and R. L. Lester. 1964. A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. *J. Lipid Res.* **5:** 127–128.
43. Davis, P. A., and A. K. Hajra. 1981. Assay and properties of the enzyme catalyzing the biosynthesis of 1-O-alkyl dihydroxyacetone-3-phosphate. *Arch. Biochem. Biophys.* **211:** 20–29.
44. Alworth, W. L. 1972. Stereochemistry and Its Application in Biochemistry. Wiley-Interscience, New York, NY. 75–81.