Facile acylation of glycerophosphocholine catalyzed by trifluoroacetic anhydride

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Summary A simplified procedure for the synthesis of short acyl chain phosphatidylcholines is described. A mixed fatty acid-trifluoroacetic anhydride is used to acylate *sn*-glycero-3-phosphocholine (GPC) which has been dissolved in trifluoroacetic acid. Yields exceeding 70% are achieved in 30-min reaction time using 1.5 to 2.0 equivalents of mixed anhydride per GPC hydroxyl. This allows a more economical use of labeled short chain acids.—**Kanda, P., and M. A. Wells.** Facile acylation of glycerophosphocholine catalyzed by trifluoroacetic anhydride. *J. Lipid Res.* 1981. **22:** 877–879.

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Phosphatidylcholines with fatty acyl chains of eight carbon atoms or less have proved useful with regard to physical–chemical studies of lipid–lipid and protein–lipid interactions, e.g., when used as substrates for phospholipases (1–4). The most attractive methods for obtaining such compounds require *sn*-glycero-3-phosphocholine (GPC) or its cadmium chloride adduct as starting material in a partial synthetic approach. The most widely used procedure for acylating GPC is that of Cubero Robles and Van den Berg (5), which employs a fatty acid anhydride as acylating agent with the tetraethylammonium salt of the same acid as catalyst. Unfortunately, a large excess of anhydride over the stoichiometric amount must be used to effect the normally sluggish introduction of the second fatty acid into GPC. This is not only costly when using labeled acids, but also complicates the purification of the resulting phosphatidylcholine product. Furthermore, the insolubility of GPC in nonhydroxylic solvents that might otherwise be suitable requires the use of elevated temperatures to obtain a homogeneous "melt" of the GPC, anhydride, and fatty acid salt mixture. This condition has been shown to promote phosphoryl migration, allowing the formation of 1,3-diacyl lecithins, which are difficult to separate from the desired 1,2-diacyl products (6).

In recent years, a number of improvements have been made in this synthesis, most notably the use of more powerful catalysts and more reactive acylating agents (6–11). Such modifications have generally been directed toward the preparation of long acyl chain phosphatidylcholines, although many appear suitable for the synthesis of short acyl chain homologues. In this regard, the use of 4-pyrrolidinopyridine as a catalyst has permitted a substantial reduction in the amount of fatty acid anhydride needed to obtain high yields (11). We describe here a convenient synthesis of short acyl chain phosphatidylcholines using a mixed fatty acid-trifluoroacetic anhydride as acylating agent (7) with trifluoroacetic acid as solvent for GPC. High yields (>70%) have been obtained using 1.5 to 2.0 equivalents of the mixed anhydride per hydroxyl group. The reaction is complete within 30 min as judged by TLC. The requirement for a relatively small excess of the mixed anhydride makes the use of labeled fatty acids more feasible.

MATERIALS AND METHODS

Materials

GPC was prepared according to Brockerhoff and Yurkowski (12) and purified by repeated precipitation by ethyl ether from a methanol solution. Trifluoroacetic acid and trifluoroacetic anhydride were from Aldrich, Milwaukee, WI. Hexanoic and heptanoic acids were from Eastman Kodak, Rochester, NY. Octanoic acid was from Pierce, Rockford, IL. Chloroform, methanol, and Darco G-60 charcoal were from Matheson, Coleman and Bell, Norwood, OH. Bio-Rex 70 and AG 3X-4A were from Bio-Rad, Richmond, CA and were further purified as described elsewhere (13). Silicic acid (CC-7) was from Mallinckrodt, St. Louis, MO. Silica gel G for thin-layer plates was from EM Laboratories, Elmsford, NY. Total phosphorus was determined by the method of Bartlett (14).

Preparation of 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine

Two mmol of GPC was dried in a 50-ml round-bottom flask in vacuo over P_2O_5 for 24 hr. Under dry nitrogen atmosphere, 50 mmol (3.7 ml) of trifluoroacetic acid (freshly distilled under anhydrous conditions) was added, and the flask was stoppered and shaken until all GPC dissolved. The flask was chilled in an ice bath, and a stirring bar was introduced, followed by an addition funnel. Eight mmol (1.0 ml) of freshly distilled hexanoic acid and 8 mmol (1.13 ml) of trifluoroacetic anhydride (freshly distilled from P_2O_5) were mixed briefly in the funnel, then added dropwise to the chilled, stirred GPC solution under

Abbreviations: GPC, *sn*-glycero-3-phosphocholine; TLC, thin-layer chromatography.

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TABLE 1. Yields and optical rotations of synthesized phosphatidylcholines

Lecithin	% Yield[a]	$[\alpha]_{546}^{22}$
Dibutyroyl	74	+12.5 (3.8 in chloroform–methanol 1:1)
Dihexanoyl	71	+11.8 (c, 2.3 in chloroform–methanol 1:1)
Diheptanoyl	70	+11.5 (c, 10 in chloroform–methanol 4:1)
Dioctanoyl	79	+11.0 (c, 9 in chloroform–methanol 1:1)

[a] Based on glycerophosphocholine.

nitrogen. After an additional 20 min at room temperature, the flask was chilled in an ice bath. Methanol (1.0 ml) was added and the solution was reduced to approximately half its volume in vacuo at 40°C. The residual solution was diluted with two to three volumes of chloroform and applied to a column containing 100 g of silicic acid packed in chloroform. The column was eluted successively with chloroform (1 liter), chloroform–methanol 4:1 (1.5 liters), then chloroform–methanol 4:7, at which point fractions were collected. The fractions were checked by TLC, and those containing pure dihexanoyl-*sn*-glycero-3-phosphocholine were pooled and the solvent was evaporated in vacuo. The residue was taken up in 25 ml of methanol, treated with charcoal, filtered, and the solution was then evaporated in vacuo. The phosphatidylcholine was dissolved in a minimal amount of water and percolated over a mixed bed AG 3X-4A, Bio-Rex 70 (15 g each) deionizing column. Two-hundred ml of water was passed through the column, and the eluent was lyophilized. The resulting dihexanoyl-*sn*-glycero-3-phosphocholine was stored in methanol at −20°C. It showed as a single spot when visualized with Dittmer-Lester reagent (15) on silica gel G plates chromatographed in the solvent system chloroform–methanol–water 65:25:4. The optical rotation is given in **Table 1.** The diheptanoyl and dioctanoyl compounds were prepared in a similar manner with yields as shown in Table 1.

RESULTS AND DISCUSSION

The success of the synthesis described here is made possible by the discovered solubility of GPC in trifluoroacetic acid. This could be due to salt formation between the two species, since the pKa of the trifluoroacetyl group is lower than that of the phosphoryl oxygen. This provides a convenient reaction medium, allowing maximal yields of product. In cases where more GPC is to be acylated than described here, one should dry the GPC over some glass beads (3–4 mm diam) to allow more rapid solubilization. This minimizes any acid-catalyzed phosphoryl migration that might occur.

The reactivity of the mixed fatty acid-trifluoroacetic anhydride is demonstrated by the facile acylation of the poorly nucleophilic secondary hydroxyl of GPC. This mixed anhydride can also be prepared and purified by distillation in vacuo in greater than 70% yield (16). The unreacted fatty acid here can also be recovered. The use of the purified mixed anhydride lowers the excess of acylating agent needed to about 1.5 equivalents per hydroxyl of GPC to obtain comparable yields.

Presently, we find this procedure to be unsuitable for the synthesis of long chain saturated or unsaturated phosphatidylcholines (yields are less than 40%). This may be due to the lower reactivity of the resulting mixed anhydrides. This necessitates longer reaction times that permit acid-catalyzed phosphoryl migration, as evidenced by increasing amounts of 1,3-diacyl-*sn*-glycero-2-phosphocholines seen on TLC with time. No evidence for phosphoryl migration was observed with short chain fatty acids based on TLC and optical rotation.

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