Are D- and L-*chiro*-Phosphoinositides Substrates of Phosphatidylinositol-Specific Phospholipase C?†

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ABSTRACT: Derivatives of *chiro*-inositol have been recently shown to mediate many important biological processes. This work addresses the question of whether phosphatidylinositol-specific phospholipase C (PI-PLC) could be involved in the generation of these *chiro*-inositol derivatives. Two diastereomers of the analog of phosphatidylinositol containing 1D- and 1L-*chiro*-inositol have been synthesized. 1D-2-*O*-(1,2-*O*-Dipalmitoyl-*sn*-glycero-3-phospho)-*chiro*-inositol (1D-*chiro*-PI) was synthesized in 12 steps starting from 1D-2,3,4,5-*O*-tetrakis(methoxymethylene)-*myo*-inositol by the inversion of the hydroxyl group at the 1-position of inositol followed by several protection/deprotection and phosphorylation steps. 1L-2-*O*-(1,2-*O*-Dipalmitoyl-*sn*-glycero-3-phospho)-*chiro*-inositol (1L-*chiro*-PI) was synthesized in eight steps starting from 1L-*chiro*-inositol using regioselective silylation of the hydroxyl group at the 2-position of *chiro*-inositol in a key synthetic stage. Both diastereomers were subjected to cleavage by PI-PLC from *Bacillus thuringiensis*. The reaction of 1L-*chiro*-PI produced *chiro*-inositol 1,2-cyclic phosphate, however, at the rate of 10^{-3} of that attained with the natural substrate, phosphatidylinositol. On the other hand, 1D-*chiro*-PI was found to be resistant to PI-PLC. These results suggest that the natural *chiro*-inositol derivatives should have the 1L-configuration if they are produced by PI-PLC, which is in contrast to the 1D-configuration reported by others. We therefore have isolated *chiro*-inositol from the total bovine liver lipid and determined its absolute configuration. The obtained *chiro*-inositol was found to be exclusively of the 1L-configuration, with the enantiomeric purity exceeding 99%.

Phosphatidylinositol-specific phospholipase C (PI-PLC)[1] is an important enzyme in cleaving anchors of some membrane proteins and in generation of various inositol derivatives (Deckmyn et al., 1990; Rhee et al., 1989; Rhee & Choi, 1992; Meldrum et al., 1991; Bruzik & Tsai, 1994). The important classes of these inositol derivatives include the various isomers of inositol phosphates, cyclic inositol phosphates, and phosphoinositolglycan insulin mediators. It was a general understanding that all naturally occurring inositol phosphatases have the *myo*-configuration. However, several recent publications reported the presence of *chiro*-inositol (Futerman et al., 1985; Low et al., 1987; Larner et al., 1988a,b; Mato et al., 1987; Pak & Larner, 1992; Ostlund et al., 1993) in animal tissues (Figure 1). The phosphoinositolglycan generated in rat liver in response to insulin stimulation (Larner et al., 1988a,b) and by treatment of hepatoma cells with *Staphylococcus aureus* PI-PLC (Mato et al., 1987) were reported to contain *chiro*-inositol exclusively or predominantly. The identity of the cyclitol in those *chiro*-inositol derivatives was determined as 1D-*chiro*-inositol (Larner et al., 1988a,b). Recently, Larner's group reported the presence of a family of at least eleven structurally distinct species of glycosylphosphatidylinositol (GPI) in bovine liver, five of which contained exclusively *chiro*-inositol (Pak & Larner, 1992). Both 1D-

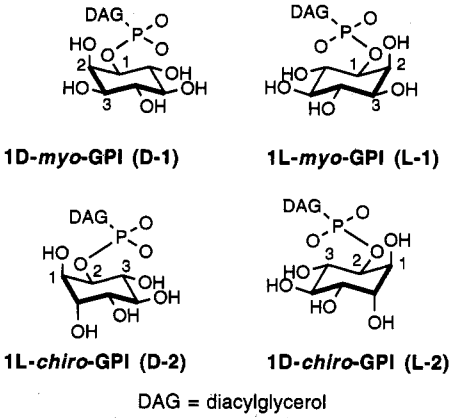

FIGURE 1: Structures of the *myo*- and *chiro*-phosphatidylinositols considered in this paper. Note that the phosphatidyl group is attached to the analogous hydroxyl group in *myo*- and *chiro*-PI despite different numbering of the inositol ring. The stereochemical designation and numbering follow IUPAC–IUB recommendations (IUPAC Commission on Nomenclature, 1976). In brief, numbering of *myo*-inositol starts from the carbon atom (C-1) adjacent to the carbon atom bearing the axial hydroxyl group (C-2) and increases passing the latter carbon first. The carbon atom C-1 is designated as having the 1D-configuration if numbering increases counterclockwise with the axial hydroxyl group oriented toward the viewer (clockwise in 1L). Numbering of *chiro*-inositol starts from the carbon atom bearing the axial hydroxyl group (C-1) and continues passing first the carbon with an equatorial hydroxyl group (C-2). In 1D-*chiro*-inositol numbering increases counterclockwise with the hydroxyl group at C-1 oriented toward the viewer (clockwise in 1L). Note that in *chiro*-inositol the following positions are equivalent: 1 and 6, 2 and 5, and 3 and 4, due to C_2 symmetry.

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[1] Abbreviations: GPI, glycosylphosphatidylinositol; IDDM, insulin-dependent diabetes mellitus; MOPS, 3-(morpholino)propanesulfonic acid; NIDDM, non-insulin-dependent diabetes mellitus; PI, phosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C; t_R, retention time; SDC, sodium deoxycholate; TBDPS, *tert*-butyldiphenylsilyl; TFA, trifluoroacetic acid.

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and 1L-*chiro*-inositols were found recently in urine and plasma of healthy human subjects (Ostlund et al., 1993), with the 1D-enantiomer predominating. There is, however, a disagreement over the levels of 1D-*chiro*-inositol in diabetes. According to the most recent work, the 1D-enantiomer was significantly elevated in NIDDM and IDDM patients (Ostlund et al., 1993). In contrast, an earlier study reported a deficiency of this enantiomer in NIDDM patients (Kennington et al. 1990) and in diabetic animals (Huang et al., 1993; Ortmeyer et al., 1993a,b). The concentration of 1D-*chiro*-inositol in urine and plasma has been proposed as a diagnostic parameter for NIDDM (Kennington & Larner, 1991), and dietary 1D-*chiro*-inositol has been used to lower blood sugar levels in human and animal subjects (Larner & Kennington, 1991; Larner et al., 1992). A synthetic method for 1D-*chiro*-inositol has been developed (Kennington et al., 1992). A hypothesis has been put forward that the insulin signal is transduced by the receptor-mediated hydrolysis of GPI by an insulin-dependent GPI-specific PLC (Romero & Larner, 1993; Pak et al., 1992; Romero, 1991; Low & Saltiel, 1987). GPI-specific PLCs have been found in several organisms (Fouchier et al., 1990; Fox et al., 1986, 1987; Stieger et al., 1991; Buelow & Overath, 1986), with GPI-PLC from *Trypanosoma brucei* being homologous (Hereld et al., 1988; Carrington et al., 1989) to PI-PLCs from bacteria (Kuppe et al. 1989).

Despite the proposed biological importance of the *chiro*-inositol derivatives and the implicit assumption that they are also generated by PI-PLC or GPI-PLC cleavage of the corresponding phospholipids (Romero & Larner, 1993; Low & Saltiel, 1987), it has never been demonstrated that any of the PI-PLC or GPI-PLC enzymes can accept *chiro*-phosphatidylinositides or *chiro*-glycosylphosphatidylinositides as substrates. Two main reasons for the paucity in such studies are the difficulty in synthesizing the *chiro*-inositol substrates and in obtaining some of the enzymes in pure forms. Furthermore, the natural *chiro*-inositol derivatives were reported to exist in the 1D-configuration (Larner et al., 1988a,b), but our stereochemical analysis predicts that PI-PLC is more likely to accept 1L-*chiro*-PI for the following reasons: Recent studies on the mechanism of bacterial and mammalian PI-PLC (Volwerk et al., 1990; Leigh et al., 1992; Bruzik et al., 1992; Bruzik & Tsai, 1994) indicated that both types of enzymes have an absolute requirement for the 1D-configuration of *myo*-PI (**D-1**, Figure 1) and for the presence of an axially oriented 2-hydroxyl group adjacent to the equatorially oriented phosphatidyl moiety. Such an arrangements enables the nucleophilic attack of the 2-hydroxyl group at the phosphorus atom in **D-1**, the first step in PI cleavage by both mammalian and bacterial PI-PLC (Bruzik et al., 1992; Volwerk et al., 1990). Analogs of PI with the 1L-configuration of *myo*-inositol such as **L-1** are not substrates for bacterial PI-PLC (Volwerk et al., 1990; Leigh et al., 1992). These results predict that, of the two diastereomers of *chiro*-PI (**L-2** and **D-2** in Figure 1), the 1L-isomer is more likely to be a substrate for PI-PLC since the orientation of the phosphatidyl moiety and adjacent hydroxyl groups in **L-2** are the same as those of **D-1**. Notice that the numbering system and D/L designation differ between *myo*- and *chiro*-inositols as explained in the legend of Figure 1.

This report describes the first synthesis of both 1D- and 1L-*chiro*-phosphatidylinositols in diastereomerically pure forms and their substrate properties toward bacterial PI-PLC as studied by ^{31}P NMR. In the absence of any information regarding the structure of natural *chiro*-inositol-containing phospholipids, the structure of analogs to be synthesized was selected based on the premise of the highest stereochemical similarity of these analogs to *myo*-inositol phospholipids. With these considerations the structure of 2-phosphatidyl-*chiro*-inositol was chosen (**L-2** and **D-2**, Figure 1). The only difference between 2-phosphatidyl-*chiro*-inositol and 1-phosphatidyl-*myo*-inositol is the inversion of the hydroxyl group at the 3-position (in **D-1**). For comparison, in the alternative 3-phosphatidyl-*chiro*-inositol the phosphatidate and the adjacent hydroxyl groups would be in the unfavorable diequatorial arrangement, while in 1-phosphatidyl-*chiro*-inositol the required equatorial/axial arrangement of phosphatidate and 2-hydroxyl group, respectively, would require a flip of the inositol ring, placing four hydroxyl groups in an unfavorable axial orientation.

The results indicate that 1L-*chiro*-PI was accepted by PI-PLC at a reduced rate whereas the 1D-diastereomer was not a substrate. Such a result implied that the naturally occurring *chiro*-inositol derivatives should have the 1L-configuration if they are derived from phosphatidylinositides by PI-PLC. This prompted us to isolate and characterize *chiro*-inositol from bovine liver lipid. The results indicated that the isolated natural *chiro*-inositol is indeed the 1L-enantiomer.

MATERIALS AND METHODS

Materials. 1D-2,3,4,5-*O*-Tetrakis(methoxymethylene)-*myo*-inositol (**3a**, $[\alpha]_D$ +3.6°) was obtained as described recently (Bruzik et al., 1992). 1L-*chiro*-Inositol was obtained from 1L-(−)-quebrachitol (Aldrich) by its demethylation with 50% hydroiodic acid at 70 °C during 48 h (Tegge & Ballou, 1989). The product was essentially free of other inositol isomers, but contained ca. 10% of unhydrolyzed quebrachitol. It was used for further syntheses without additional purification. 1D-*chiro*-Inositol [$[\alpha]_D^{20}$ +62.4° (*c* 1.2, H_2O)] was obtained by acidic hydrolysis of kasugamycin (Fluka) as reported (Kennington et al., 1992). Inositol 1-phosphate was synthesized as described earlier (Pietrusiewicz et al., 1992). PI-PLC from *Bacillus thuringiensis* was a generous gift from Dr. T. L. Rosenberry (Case Western University, Cleveland, OH).

Methods. NMR spectra were obtained with Bruker 250-, 300-, and 500-MHz AM spectrometers. ^1H and ^{13}C NMR chemical shifts were indirectly referenced to tetramethylsilane and those of ^{31}P to 85% H_3PO_4. The purity of products was assayed by spectroscopic methods (^1H, ^{13}C, and ^{31}P NMR) and TLC technique using aluminum-foil-based silica gel plates visualized with phosphomolybdic acid solution. Optical rotations were measured using Perkin-Elmer 241 MC spectropolarimeter. HPLC separations were performed using a Hitachi L-6200A system employing D-4500 photo-diode-array detector and Dionex PAD-2 electrochemical detector.

Synthesis of 1D- and 1L-2-O-(1,2-O-Dipalmitoyl-sn-glycero-3-phospho)-chiro-inositols (D-2 and L-2, Respectively)

1D-1-O-Benzoyl-2,3,4,5-O-tetrakis(methoxymethylene)-myo-inositol (4a). 1,6-Diol **3a** (520 mg, 1.46 mmol) in pyridine (4 mL) was treated with benzoyl chloride (186 μL, 10% excess) at −30 °C, and the progress of the reaction was monitored by TLC (hexane–acetone, 3:1). The mixture was kept at 4 °C for 12 h, and then water (10 mL) and ethyl acetate (20 mL) were added. The organic phase was separated, washed twice with water (10 mL) and concentrated. Crude product was chromatographed on silica gel (hexane–acetone, 10:1) to give pure **4a** (540 mg, 80%). A small amount of the 1,6-dibenzoyl derivative (4%) was also recovered. **4a**: TLC R_f 0.18 (hexane–

acetone, 3:1); ^{1}H NMR ($CDCl_3$) δ 8.1 (2H), 7.55 (1H), 7.40 (2H), (each m, Ph), 4.97 (dd, H-1, J 2.5, 10.2 Hz, 1H), 4.87–4.66 (m, OCH_2O, 8H), 4.27 (tr, H-2, J 2.4 Hz, 1H), 4.15 (brs, OH, 1H), 4.09 (brtr, H-6, J 9.9 Hz, 1H), 4.00 (tr, H-4, J 9.5 Hz, 1H), 3.66 (dd, H-3, J 2.3, 10 Hz, 1H), 3.44, 3.41, 3.38, 3.26 (each s, Me, 3H), 3.32 (tr, H-5, J 9.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 165.9 (C=O), 133.0, 129.8, 128.3 (Ph), 98.8, 98.0, 97.2, 95.8 (OCH_2O), 86.4, 76.6, 76.0, 73.7, 73.5, 70.2 (CHO), 56.0, 55.9, 55.7, 55.6 (OMe).

*1D-6-O-Benzoyl-2,3,4,5-O-tetrakis(methoxymethylene)-myo-inositol (**5a**).* The foregoing 1-benzoate **4a** was isomerized to 6-benzoate **5a** as follows: **4a** (540 mg) was dissolved in THF (3 mL) and 0.1 mL of 1.0 M tetra-*n*-butylammonium fluoride was added. The mixture was left at room temperature for 12 h. TLC (hexane–acetone, 3:1) indicated the presence of another product (R_f 0.20) in addition to the substrate (R_f 0.22). The ratio of the isomers was ca. 1:1 as determined by ^{1}H NMR. The mixture was concentrated and chromatographed on silica gel (Sigma, 10–40 μm), giving **4a** (240 mg, eluted off earlier) and **5a** (248 mg). The same 1:1 mixture of **4a** and **5a** was also formed when pure **5a** underwent isomerization under analogous conditions. The recovered isomer **4a** was recycled to give a total yield of 66% after two cycles. **5a**: ^{1}H NMR ($CDCl_3$) δ 8.1 (2H), 7.5 (1H), 7.4 (2H), (each m, Ph), 5.41 (tr, H-6, J 9.9 Hz, 1H), 4.82–4.63 (m, OCH_2O, 8H), 4.02 (tr, H-2, J 2.3 Hz, 1H), 4.00 (tr, H-4, J 9.7 Hz, 1H), 3.87 (d, 1H), 3.63 (tr, J 9.5 Hz, 1H), 3.52 (m, 2H), 3.52, 3.38, 3.35, 3.05 (each s, Me, 3H); ^{13}C NMR ($CDCl_3$) δ 166.7 (C=O), 132.8, 130.0, 129.6, 128.1 (Ph), 98.5, 98.3, 98.0, 96.6 (OCH_2O), 81.4, 78.5, 78.0, 76.9, 74.3, 70.2 (CHO), 56.3, 56.2, 55.9, 55.5 (OMe).

*1D-6-O-Benzoyl-2,3,4,5-O-tetrakis(methoxymethylene)-1-O-[(trifluoromethyl)sulfonyl]-myo-inositol (**6a**).* The solution of the 1:1 mixture of the foregoing alcohols **4a** and **5a** (483 mg, 0.52 mmol of **5a**) and pyridine (250 μL) in chloroform (5 mL) was treated with trifluoromethanesulfonic anhydride (311 mg, 5% excess) at –40 °C and the mixture was slowly warmed up to room temperature. The progress of the reaction was monitored by TLC (hexane–acetone, 3:1; substrate R_f 0.22, product R_f 0.27). Aqueous workup and chromatography using the above solvent afforded triflate **6a** (228 mg, 74% from **5a**) and another unidentified product (183 mg). **6a**: ^{1}H NMR ($CDCl_3$) δ 8.05 (2H), 7.55 (1H), 7.45 (2H) (each m, Ph), 5.87 (tr, H-6, J 9.9 Hz, 1H), 4.9–4.6 (m, OCH_2O and H-1, 9H), 4.35 (tr, H-2, J 2.5 Hz, 1H), 4.10 (tr, H-4, J 9.4 Hz, 1H), 3.66 (tr, H-5, J 9.6 Hz, 1H), 3.63 (dd, H-3, J 2.3, 9.8 Hz, 1H), 3.42, 3.41, 3.407, 3.00 (each s, Me, 3H); ^{13}C NMR ($CDCl_3$) δ 165.2 (C=O), 133.3, 129.8, 129.3, 128.3 (Ph), 118.3 (q, J 681 Hz, CF_3), 98.6, 98.5, 97.5, 96.4 (OCH_2O), 84.0, 78.7, 77.2, 75.5, 74.1, 70.3 (CHO), 56.4, 56.2, 56.1, 55.9 (OMe).

*1D-2-O-Benzoyl-3,4,5,6-O-tetrakis(methoxymethylene)-1-O-nitro-chiro-inositol (**7a**).* The triflate **6a** (228 mg, 0.385 mmol) in acetonitrile (1 mL) was treated with tetra-*n*-butylammonium nitrate (234 mg, 2-fold excess) at 75 °C during 24 h. Silica gel chromatography (hexane–acetone, 5:1, product R_f 0.3) afforded the pure nitro derivative **7a** (87 mg, 45%). **7a**: ^{1}H NMR ($CDCl_3$) δ 8.05 (2H), 7.56 (1H), 7.43 (2H) (each m, Ph), 5.70 (dd, H-1, J 3.5, 5.6 Hz, 1H), 5.59 (m, H-2, 1H), 4.85–4.70 (m, OCH_2O, 8H), 4.14 (dd, H-6, J 3.1, 4.6 Hz, 1H), 3.97 (m, 2H), 3.77 (m, 1H), 3.51, 3.42, 3.40, 3.14 (each s, Me, 3H); ^{13}C NMR ($CDCl_3$) δ 165.3 (C=O), 133.5, 129.7, 129.2, 128.5 (Ph), 98.4, 98.2, 98.0, 97.4 (OCH_2O), 77.6, 77.4, 77.1, 76.7, 76.6, 73.9 (CHO), 56.39, 56.35, 56.2, 55.8 (OMe).

*1D-2-O-Benzoyl-1,3,4,5,6-O-pentakis(methoxymethylene)-chiro-inositol (**9a**).* The foregoing nitrate **7a** was subjected to hydrogenolysis in THF over 10% Pd/C at ambient hydrogen pressure and room temperature during 12 h. The progress of the reaction was monitored by TLC (hexane–acetone, 3:1, product R_f 0.2). The catalyst was filtered off and the filtrate was concentrated to give crude alcohol **8a**. This product was treated with MOM-Cl (0.1 mL) and diisopropylethylamine (0.3 mL) in DMF (1 mL) at room temperature during 12 h followed by heating at 70 °C during 5 h (TLC, hexane–acetone, 3:1, product R_f 0.31). Silica gel chromatography (hexane–acetone, 10:1) afforded pure **9a** (78 mg, 90% in two steps). **9a**: ^{1}H NMR (C_6D_6) δ 8.27 (m, Ph), 7.03 (m, Ph), 5.87 (dd, H-2, J 3.2, 9.6 Hz, 1H), 4.91 (m, 3H), 4.78 (d, J 6.6 Hz, 1H), 4.71 (d, J 6.6 Hz, 2H), 4.58 (d, J 6.7, 1H), 4.51 (d, J 6.7 Hz, 1H), 4.39 (m, 5H), 4.21 (m, 2H), 3.31 (s, Me, 6H), 3.17, 3.10, 3.06 (each s, Me, 3H).

*1D-1,3,4,5,6-O-Pentakis(methoxymethylene)-chiro-inositol (**10a**).* The foregoing benzoate **9a** was cleaved by sodium hydroxide in MeOH (3%, 1 mL). Silica gel chromatography (hexane–acetone, 3:1, R_f 0.22) afforded pure alcohol **10a** (47 mg, 75%). **10a**: ^{1}H NMR ($CDCl_3$) δ 4.78–4.60 (m, OCH_2O, 10H), 3.97 (dd, H-6, J 3.0, 4.1 Hz, 1H), 3.90 (dd, H-1, J 3.4, 4.2, 1H), 3.82 (dd, H-5, J 2.8, 10.0 Hz, 1H), 3.74 (tr, H-4, J 10.5 Hz, 1H), 3.77 (ddd, H-2, 1H), 3.49 (tr, H-3, J 9.5 Hz, 1H), 3.38, 3.35, 3.33, 3.32, 3.30 (each s, Me, 3H), 2.93 (d, OH, J 4.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 98.56, 98.23, 98.20, 97.30, 96.7 (OCH_2O), 83.6, 78.0, 77.3, 76.2, 75.8, 69.9 (CHO), 56.0, 55.9, 55.8, 55.7, 55.5 (OMe).

*1D-2-O-(1,2-O-Dipalmitoyl-sn-glycero-3-phospho)-chiro-inositol (**D-2**).* The alcohol **10a** (45 mg, 112 μmol) and diisopropylethylamine (39 μL, 2-fold excess) in chloroform (0.5 mL) were treated with methyl *N,N*-diisopropylphosphoramidochloridite (26.5 mg, 20% excess) at room temperature. The progress of the reaction was followed by TLC (hexane–acetone, 3:1, substrate R_f 0.22, product R_f 0.41). After the phosphitylation had been completed, the mixture was evaporated to dryness and added with tetrazole (32 mg, 4-fold excess) and 1,2-dipalmitoyl-*sn*-glycerol (92 mg, 20% excess). The whole mixture was dissolved in THF–acetonitrile (1:1, 0.5 mL). The new product with an R_f of 0.5 (same solvent system as above) was formed within 0.5 h. The reaction mixture was treated with *m*-chloroperbenzoic acid (42 mg, 60%, 10% excess) at –30 °C and warmed up to room temperature within 0.5 h. The crude product (R_f 0.15) was purified by chromatography (hexane–acetone, 10:1) to give the pure phosphate triester **11a** (55 mg, 46%, $\delta_{^{31}P}$ –1.4 ppm, $CDCl_3$). The above product **11a** was dissolved in neat trimethylamine at –10 °C in a heavy-walled tightly closed screw-cap vial and heated at 50 °C during 21 h. TLC after this time showed complete demethylation. Trimethylamine was evaporated and the residue was treated with neat ethanethiol (1 mL) and boron trifluoride–ether (30 μL) during 30 min at room temperature. After this time TLC (chloroform–methanol–ammonia, 17:3:0.1, product R_f 0.26) showed a complete deprotection. The mixture was concentrated and the product was purified by chromatography (chloroform–methanol–ammonia, 70:30:0.6) to give pure **D-2** (30 mg, 71%). **D-2**: $[\alpha]_D$ +0.6 ± 0.2° (*c* 0.7, methanol–chloroform, 1:1); ^{1}H NMR (CD_3OD–$CDCl_3$, 5:1) δ 5.23 (m, H-2′, 1H), 4.44 (dd, H-1′A, J 3.1, 12.0 Hz, 1H), 4.24 (dtr, H-2, J 3.1, 9.1, 9.1 Hz, 1H), 4.18 (dd, H-1′B, J 6.8, 12.0 Hz, 1H), 4.13 (tr, H-1 (6), J 3.5 Hz, 1H), 4.04 (m, H-3′AB, 2H), 3.93 (tr, H-6 (1), J 3.3 Hz, 1H), 3.72 (tr, H-3, J 9.4 Hz, 1H), 3.68 (dd, H-5, J 3.1, 9.4 Hz, 1H), 3.59 (tr, H-4, J 9.6 Hz, 1H), 2.32, 2.29

(each tr, $CH_2C{=}O$, *J* 7.4 Hz, each 2H), 1.59 (m, 4H), 1.26 (brs, 48H), 0.87 (tr, 6H); ^{31}P NMR (CD_3OD) δ 1.08 ppm.

*1L-2-O-(tert-Butyldiphenylsilyl)-chiro-inositol (**12b**).* 1L-*chiro*-Inositol (50 mg) containing ca. 10% (–)-quebrachitol and imidazole (30 mg) in DMF (1 mL) was treated with *tert*-butyldiphenylsilyl chloride at –10 °C and kept at room temperature during 2 h. DMF was evaporated under vacuum and the residue was chromatographed (chloroform–methanol, 4:1). The fraction containing silylated quebrachitol (R_f 0.58) was eluted off first and was followed by the main fraction of 2-TBDPS-*chiro*-inositol (**12b**, R_f 0.5, 30 mg, 26%). The extension of the reaction time to 24 h resulted in the formation of a significant amount of another product with a slightly lower mobility (most likely 3-TBDPS-*chiro*-inositol). **12b**: ^1H NMR (DMSO-d_6) δ 7.75 (4H, Ph), 7.35 (6H, Ph), 4.5 (brs, OH), 3.87 (dd, H-2 (5), *J* 2.1, 9.6 Hz, 1H), 3.58 (m, 3H), 3.49 (dd, H-5(2), *J* 2.1, 9.6 Hz, 1H), 3.2 (tr, H-3 (4), *J* 9.2 Hz, 1H), 1.00 (s, Me, 9H); ^{13}C NMR (DMSO-d_6) δ 136.0, 135.7, 135.0, 134.2, 129.47, 129.43, 127.5, 127.4 (Ph), 73.6, 73.1, 72.34, 72.26, 70.6 (CHO), 27.1 (C-*Me*), 19.4 (C_q).

*1L-1,3,4,5,6-O-Pentakis(methoxymethylene)-chiro-inositol (**10b**).* 2-TBDPS-*chiro*-inositol (**12b**, 140 mg, 0.33 mmol) and ethyldiisopropylamine (0.5 mL) in DMF (1 mL) were treated with methoxymethylene chloride (0.25 mL) at room temperature for 12 h and for 3 h at 60 °C (product R_f 0.4, hexane–acetone, 3:1). The mixture was diluted with ethyl acetate and extracted three times with water and the organic phase was concentrated. The residue (204 mg) was rendered anhydrous by evaporation with dry dioxane. The above product was solubilized in a 1.0 M solution of tetra-*n*-butylammonium fluoride in THF (0.5 mL) and the mixture was heated at 55 °C during 3 h. The progress of desilylation was checked by TLC (product R_f 0.15). Chromatography on silica gel (hexane–acetone, 3:1) afforded pure **10b** (78 mg, 59% in two steps). This product gave essentially the same proton spectrum as that of **10a**, except that it contained 15% of the unidentified impurity. This product was used for further synthetic steps without additional purification.

*1L-2-O-(1,2-O-Dipalmitoyl-sn-glycero-3-phospho)-chiro-inositol (**L-2**).* Phosphitylation of alcohol **10b** was carried out essentially identically as described above for **10a**. Starting from 75 mg of **10b** 57 mg of 1L-*chiro*-PI (**L-2**) was obtained (38% in five steps). **L-2**: ^{31}P NMR (CD_3OD-$CDCl_3$, 5:1) δ 1.11 ppm; $[\alpha]_D^{23}$ –12.7° (*c* 0.6, methanol–chloroform, 1:1). Despite the fact that **L-2** and **D-2** are not enantiomers (they both contain L-α-glycerol residue) the ^1H NMR spectrum of **L-2** was essentially identical to that of **D-2**.

Reactions of chiro-Phosphatidylinositols with PI-PLC from B. thuringiensis

(A). 1D-*chiro*-PI (**D-2**, 2.4 mg, 3 μmol) was dispersed in MOPS–Na buffer (0.4 mL, 50 mM, pH 7.0) containing 10 mM SDC and 25% D_2O. This sample was treated with 3.5 μg of PI-PLC and the reaction was monitored by ^{31}P NMR. Only a single signal at –0.2 ppm due to the substrate was observed after 3 weeks at room temperature. To verify that PI-PLC was still active after such a long incubation time, synthetic dipalmitoylphosphatidyl-*myo*-inositol (1 mg) was added. A rapid conversion of *myo*-PI to IcP ($\delta_{^{31}P}$ 16 ppm) was observed within the time necessary to acquire the first ^{31}P NMR spectrum (5 min.).

(B). 1L-*chiro*-PI (**L-2**, 2.7 mg, 3.3 μmol) was treated with PI-PLC as above. The conversion of the substrate ($\delta_{^{31}P}$ –0.2 ppm) into the product ($\delta_{^{31}P}$ 15.6 ppm) was observed and was complete within 10 days.

*1L-chiro-Inositol 1,2-Cyclic Phosphate (**L-14**).* 1L-*chiro*-PI (**L-2**, 4.2 mg, 5 μmol) and SDC (5 mg) were dispersed in water (0.5 mL containing 20% D_2O). The dispersion was treated with PI-PLC (10 μg) and the reaction was monitored by ^{31}P NMR. After most PI had been used up (60 h) the mixture was passed through C-18 cartridge and further chromatographed on Dowex 1X8-200 (ammonium form) using a step gradient of ammonium carbonate. The fraction eluted at 50 mM buffer was evaporated under vacuum to give pure **L-14**: ^1H NMR (D_2O) δ 4.67 (dd, H-1, *J* 3.5, 5.2 Hz, 1H), 4.51 (ddd, H-2, *J* 5.3, 8.3, 13.6 Hz, 1H), 4.37 (brtr, H-6, *J* 3.2 Hz, 1H), 3.89 (dd, H-3, *J* 8.4, 9.7 Hz, 1H), 3.86 (dd, H-5, *J* 3.1, 9.2 Hz, 1H), 3.74 (tr, H-4, *J* 9.5 Hz, 1H); ^{31}P NMR (D_2O) δ 15.7 ppm.

Determination of Configuration of Naturally Occurring chiro-Inositol

Isolation and Determination of the Content of chiro-Inositol in the Total Lipid of Bovine Liver. Bovine liver purchased from a local store (330 g) was homogenized in iced 5% TFA (1 L) using a Waring blender. The homogenate was centrifuged at 7000*g*, and the pellet was rehomogenized in chloroform–methanol (1:2, 600 mL). Supernatants were pooled and extracted three times with chloroform–0.5 M KCl (1:1, 200 mL). Organic phases were collected, washed twice with 0.5 M KCl (100 mL), and concentrated to give dark brown thick oil (9 g). The foregoing lipid extract (1 g) was solubilized in dioxane and 3% methanolic KOH (10 mL) and stored for 0.5 h. TLC (chloroform–methanol–water, 65:35:4) indicated that all phospholipids have been saponified. The mixture was adjusted to pH 1.5 with HCl and extracted five times with chloroform–methanol (2:1). The organic phase was discarded and the aqueous phase was concentrated to dryness. ^{31}P NMR at this point indicated the presence of seven major signals in the range –1.2 to 1.0 ppm. The residue was redissolved in 6 N HCl (5 mL) and refluxed during 24 h. ^{31}P NMR showed only one signal of inorganic phosphate, indicating the completion of hydrolysis (at 12 h 33% of monoesters was still present). The mixture was concentrated to dryness and the residue redissolved in water, and passed through the cation-exchange column (H^+ form, Aldrich, Dowex 50X8, 1 cm × 12 cm) and the anion-exchange column (OH^- form, Bio-Rad, AG 1X8, 100–200 mesh, 1 cm × 12 cm). The final eluate was concentrated by evaporation. The oily residue contained >95% glycerol and the remainder was inositol. Glycerol was removed by distillation at 100 °C under high vacuum to give the product which contained chiefly *myo*- and *chiro*-inositols. The content of *chiro*-inositol was determined by integration of its H-1/H-6 proton signals at 4.10 ppm and H-2 proton signals of *myo*-inositol at 4.13 ppm. These measurements gave the value of 8%, consistent with determination of the perbenzoylated derivatives (see below).

Hexa-O-benzoylinositols. General Procedure. The suspension of inositol in dry pyridine was treated with excess of benzoyl chloride at 75 °C during 3 h. The product was purified by chromatography on silica gel (hexane–acetone, 3:1) and subsequent crystallization from acetone–hexane.

Hexa-O-benzoyl-myo-inositol: mp 265 °C (hexane–acetone, 3:1); ^1H NMR ($CDCl_3$) δ 8.17 (m, 2H), 7.9 (m, 10 H), 7.7 (m, 1H), 7.6 (m, 2H), 7.45 (m, 5H), 7.3 (m, 10H), 6.38 (tr, H-4, H-6, *J* 10.2 Hz, 2H), 6.34 (tr, H-2, *J* 2.9, 1H), 6.06 (tr, H-5, *J* 10.2 Hz, 1H), 5.85 (dd, H-1, H-3, *J* 10.5 Hz, 3.0 Hz, 2H).

Hexa-O-benzoyl-1L-chiro-inositol was obtained starting from 1L-quebrachitol: mp 243–245 °C; $[\alpha]_D$ –68°, $[\alpha]_{436}$

−157° (*c* 0.5, chloroform); ^{1}H NMR ($CDCl_3$) δ 8.15 (m, 4H), 7.88 (m, 8H), 7.67 (m, 2H), 7.58 (m, 4H), 7.45 (m, 4H), 7.28 (m, 8H), 6.31 (m, 2H), 6.13 (m, 2H), 6.05 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 165.6, 165.4, 164.8 (C=O), 133.8, 133.3, 133.0, 130.1, 129.8, 128.84, 128.79, 128.4, 128.3 (Ph), 70.27, 70.09, 68.47 (CHO).

*Hexa-O-benzoyl-1*D*-chiro-inositol* was obtained as above starting from 1D-*chiro*-inositol obtained from kasugamycin: mp 246–248 °C; $[\alpha]_D$ +64.8°, $[\alpha]_{436}$ +148° (*c* 0.41, chloroform).

Hexabenzoyl Derivatives of Inositols from Bovine Liver. Benzoylation of the mixture of inositols obtained from the bovine lipid and purification of the mixture of hexabenzoyl derivatives was performed analogously as described above. The content of *chiro*-inositol derivative was determined in two ways: (i) by the integration of the two-proton multiplet at 6.13 (from *chiro*-inositol) and the two proton doublet of doublets at 5.85 (of *myo*-inositol) and (ii) by integration of HPLC peaks of hexabenzoyl derivatives (see below). These determinations gave the values of 9% and 8.3%, respectively. The sample had the specific rotation of $[\alpha]_{436}$ −9° (*c* 0.3, chloroform).

HPLC Separations of myo- and chiro-Inositols

Underivatized Inositols. The mixture of *chiro*- and *myo*-inositols was chromatographed on the Dionex CarboPac PA1 column (4.6 × 250 mm column equipped with a guard) using 10 mM NaOH for elution and the Dionex pulsed amperometric detector (PAD-2) equipped with a gold electrode for monitoring and quantitation. Potentials and time intervals of their cycling were E_1 = 0.05 (480 ms), E_2 = 0.6 (120 ms), E_3 = −0.6 (60 ms). The time constant was 3 s and sampling time was 800 ms. The column was eluted at the flow rate 0.2 mL/min. Retention times were 8.81 min for *myo*-inositol and 10.57 min for *chiro*-inositol. Both components were baseline separated.

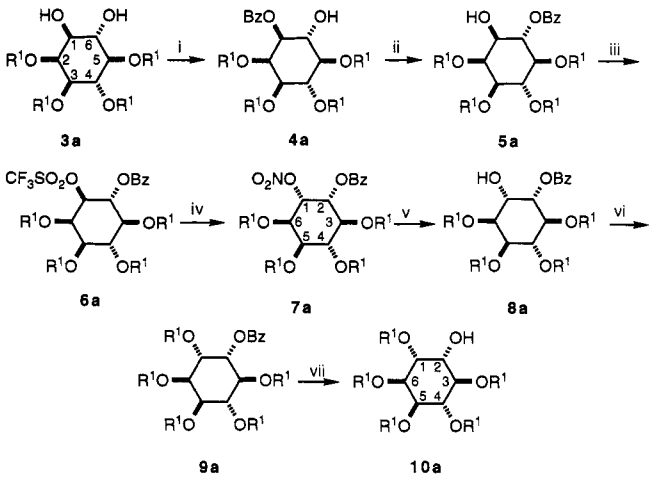
Hexabenzoyl-myo- and -chiro-Inositols. The mixture of *myo*- and *chiro*-derivatives was chromatographed on silica gel column (Rainin Microsorb-MV, 4.6 × 250 mm, 100-Å pore) using hexane–isopropyl alcohol (98:2, v/v) and a UV photo-diode-array detector. Chromatograms were quantitated using monitoring at 254 nm. Retention times were 11.4 min for the *chiro*-derivative and 12.7 min for the *myo*-derivative. This column was also used for preparative isolation of hexabenzoyl-*chiro*-inositol from natural lipid to be used for chiral chromatography (see below).

Chiral Separations of chiro-Inositol. Hexa-*O*-benzoyl-*chiro*-inositol was chromatographed on the CHIRALPAK OT (+) column (Chiral Technologies, Inc., 4.6 × 250 mm) with a helical acrylamide stationary phase. A normal C-18 guard was used. Near-baseline separations were obtained using methanol–hexane–isopropyl alcohol 50:25:25 as a mobile phase at 0.6 mL/min flow rate. Due to column instability at normal temperatures in the above solvent, separations were performed at 10 °C. The 1D-enantiomer was eluted at 25.3 min and the 1L-enantiomer at 32 min. The sample of hexabenzoyl-*chiro*-inositol obtained from the bovine liver lipid gave only the slow-eluting peak. The enantiomeric purity of natural *chiro*-inositol from bovine liver lipid is determined as >99%.

RESULTS

*Synthesis of 1*D*-chiro-Phosphatidylinositol (**D-2**).* Our recent synthetic study (Bruzik & Tsai, 1992) generated a number of intermediates useful toward synthesis of phosphoinositides including 1D-2,3,4,5-*O*-tetrakis(methoxymethylene)-*myo*-inositol (**3a**, Scheme 1). The conversion of this precursor into the starting material (**10a**) for the synthesis of *chiro*-PI necessitated only an inversion and a subsequent protection of the hydroxyl group at the 1-position of *myo*-inositol. Our synthetic procedure is shown in Scheme 1. The 1,6-diol **3a** was benzoylated regioselectively at the 1-OH group and the resulting 6-alcohol **4a** was subjected to base-catalyzed migration of the benzoyl group between 1- and 6-hydroxyl groups to give the derivative **5a**. The 1-alcohol **5a** was further treated with trifluoromethanesulfonic anhydride and the resulting sulfonyl ester **6a** was reacted with tetra-*n*-butylammonium nitrate to afford the corresponding 1-nitro derivative of 1D-*chiro*-inositol (**7a**) (Tagliaferri et al., 1990). The subsequent hydrogenolysis of the nitro group and methoxymethylation of the resulting 1-hydroxyl group followed by saponification of the 2-benzoyl group afforded the corresponding 2-alcohol (**10a**, note the numbering of the *chiro*-inositol). The alcohol **10a** was phosphitylated (Bruzik et al., 1992) to give a 1:1 mixture of the diastereomeric phosphotriesters (**11a**, Scheme 2). Demethylation of the phosphotriesters **11a** with trimethylamine and the subsequent exhaustive deprotection of the inositol moiety with ethanethiol/BF_3 completed the synthesis, affording 1D-*chiro*-PI (**D-2**). The 1L-isomer of *chiro*-PI (**L-2**) can be also obtained in an analogous way starting from 1D-1,2,5,6-tetrakis(methoxymethylene)-*myo*-inositol (enantiomer of **3a**). Since this derivative is less readily accessible than **3a**, an alternative synthesis was devised as described in the next section.

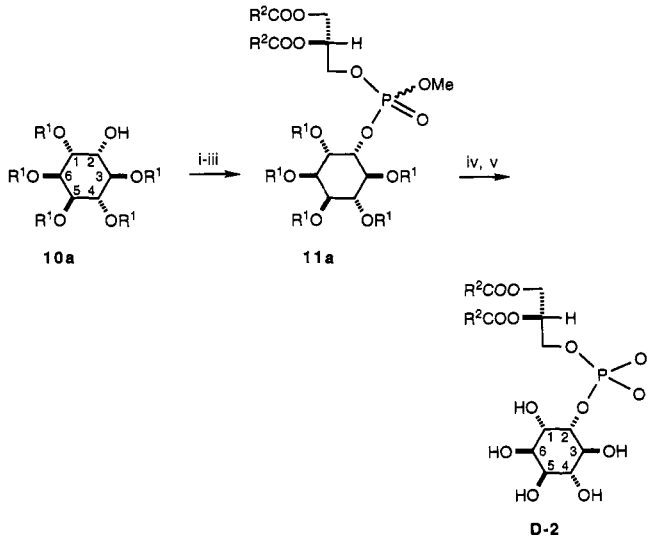
Scheme 1[a]



[a] R[1]: methoxymethyl. (i) BzCl/Py; (ii) $Bu_4N^+F^-$ (cat.); (iii) $(CF_3SO_2)_2O$/Py; (iv) $Bu_4N^+NO_3^-$; (v) H_2/Pd; (vi) MOM-Cl/EtiPr$_2$N; (vii) NaOH/MeOH.

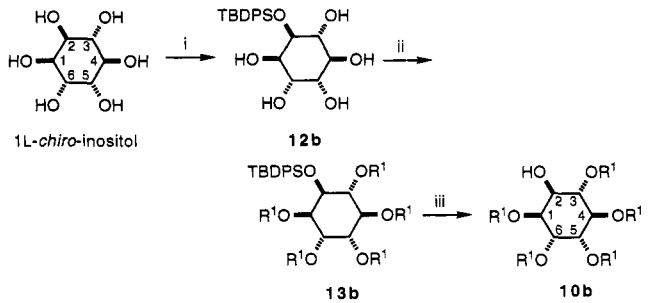
*Synthesis of 1*L*-chiro-Phosphatidylinositol (**L-2**).* As shown in Scheme 3, 1L-*chiro*-inositol obtained from 1L-(−)-quebrachitol (Tegge & Ballou, 1989) was treated with *tert*-butyldiphenylsilyl (TBDPS) chloride/imidazole in DMF to afford regioselectively 1-TBDPS-*chiro*-inositol (**12b**) in 26% yield. This derivative was treated with methoxymethylene chloride (MOM-Cl) to give the fully protected derivative **13b**. Further removal of the silyl group in **13b** with tetra-*n*-butylammonium fluoride provided 2-alcohol **10b**. Alcohol **10b** was then converted into 1L-*chiro*-PI (**L-2**) analogously as described for the conversion of **10a** into **D-2**. Despite the low yield obtained in the first silylation step, this scheme is superior to the synthesis used in the D-series due to the small number of steps needed to obtain PI-precursor **10b**. The alcohol **10a** could also be prepared in the analogous way to **10b** starting from 1D-(+)-pinitol.

Scheme 2[a]



[a] R^1: methoxymethyl. R^2: $C_{15}H_{31}$. (i) Cl-P(OMe)N(iPr)$_2$/Et(iPr)$_2$N; (ii) 1,2-dipalmitoyl-*sn*-glycerol/tetrazole; (iii) *m*-chloroperbenzoic acid; (iv) Me_3N/50 °C; (v) EtSH/BF_3–Et_2O.

Scheme 3[a]



[a] R^1: methoxymethyl. TBDPS: *tert*-butyldiphenylsilyl. (i) TDBPS-Cl/imidazole/DMF; (ii) MOM-Cl/Et(iPr)$_2$N; (iii) $Bu_4N^+{}_4F^-$.

Activity of PI-PLC toward 1D- *and* 1L-*chiro-Phosphatidylinositols.* Both diastereomers of *chiro*-PI (**D-2** and **L-2**) were subjected to the action of PI-PLC from *B. thuringiensis*. The 1L-isomer **L-2** was cleaved by PI-PLC at a low rate to give 1L-*chiro*-inositol 1,2-cyclic phosphate (Figure 2). The product of the cleavage has been identified as a cyclic phosphate by its ^{31}P NMR spectrum showing a signal at 15.6 ppm characteristic of five membered ring phosphates and ^1H NMR spectrum showing a large vicinal coupling constant of phosphorus to H-2 (19 Hz). The above values are very close to those of *myo*-inositol 1,2-cyclic phosphate (Cerdan et al. 1986). The rate of the cleavage of **L-2** is 3 orders of magnitude slower than that of 1D-*myo*-PI (**D-1**). The 1D-isomer **D-2** was found resistant to PI-PLC, even when treated with a large amount of enzyme and employing long incubation times. We estimate that the activity of PI-PLC toward 1D-*chiro*-PI under our assay conditions is <1 nmol/mg·min, or $<10^{-6}$ of that with 1D-*myo*-PI.

Although our result concerns only one type of bacterial PI-PLC and one type of *chiro*-phosphatidylinositol substrate, the stereochemical properties of enzymes are usually conserved among the same class of enzymes. For example, all known types of PI-PLC cleave substrate with the 1D-configuration of *myo*-inositol. Our results therefore implies that if the natural *chiro*-inositol-containing insulin mediators are derived from PI-PLC-catalyzed reactions, they should have the 1L-instead of the 1D-configuration. Such a possibility prompted us to isolate and characterize naturally occurring *chiro*-inositol as described in the following section.

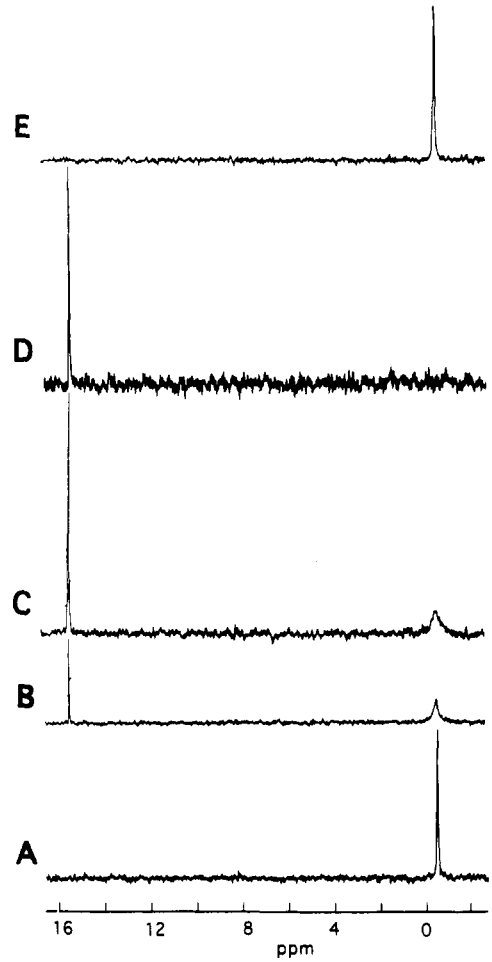


FIGURE 2: Time courses of PI-PLC-catalyzed reaction with 1L- and 1D-2-phosphatidyl-*chiro*-inositol (**L-2** and **D-2**, respectively) as monitored by ^{31}P NMR. Dispersion of **L-2** (7.5 mM) in SDC (10 mM, pH 7.0) was treated with PI-PLC (3.5 μg) and ^{31}P NMR spectra were acquired after 1 h (A), 18 h (B), 100 h (C), 10 days (D). Spectrum E was obtained with analogously treated sample of **D-2** after 3 weeks. Identities of peaks at –0.2 and 15.6 ppm are *chiro*-PI and *chiro*-IcP, respectively.

Isolation of chiro-Inositol from Bovine Liver Lipid. The total liver lipid was hydrolyzed with sodium hydroxide in methanol and the resulting water-soluble phosphodiesters were further hydrolyzed in refluxing 6 N HCl. The hydrolysate was deionized to remove inorganic phosphate and then was evaporated under high vacuum to remove glycerol. The resulting mixture of *myo*- and *chiro*-inositol was subjected to exhaustive benzoylation to give a mixture of hexabenzoyl-*myo*- and *chiro*-inositols. The composition of this mixture was determined by: (i) the ^1H NMR spectra of crude inositols and of the mixture of hexabenzoylated derivatives and (ii) HPLC of the mixture of hexabenzoyl derivatives using silica gel column with hexane–isopropyl alcohol (98:2) elution and UV detection at 254 nm. The elution profiles are shown in Figure 3, parts A–C. The composition of the inositol mixture was found to be 91.5 ± 0.5% *myo*-inositol and 8.5 ± 0.5% *chiro*-inositol by both NMR and HPLC methods.

Configuration of chiro-Inositol from Bovine Liver Lipid. The standard compounds, hexabenzoyl derivatives of 1L-*chiro*-inositol and 1D-*chiro*-inositol, were synthesized from 1L-quebrachitol and kasugamycin (Tegge & Ballou, 1989; Kennington et al., 1992), respectively. The $[\alpha]_{436}$ values were –157° and +148° for the 1L- and 1D-derivatives, respectively. The mixture of *chiro*- and *myo*-hexabenzoylinositols obtained from the bovine lipid exhibited $[\alpha]_{436}$ –9° (*c* 0.3, chloroform).

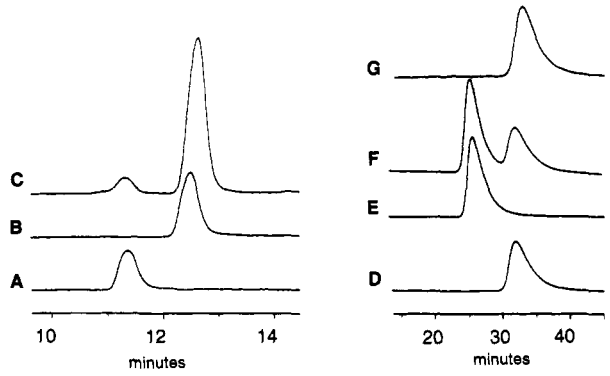


FIGURE 3: Determination of the content and configuration of *chiro*-inositol from bovine liver lipid by HPLC. Conditions: (A–C) HPLC on Microsorb-MV, 4.6 × 250 mm, detection at 254 nm, isocratic elution with hexane–isopropyl alcohol (98:2, v/v), flow rate 1 mL/min; (D–G) HPLC on the polymethacrylate CHIRALPAK OT (+) column, 4.6 × 250 mm, detection at 230 nm, flow rate 0.6 mL/min, isocratic elution with methanol–hexane–isopropyl alcohol (50:25:25, v/v) at 10 °C. Samples: (A) 1D-hexabenzoyl-*chiro*-inositol, retention time (t_R) 11.4 min.; (B) hexabenzoyl-*myo*-inositol, t_R 12.5 min.; (C) hexabenzoylinositols from bovine liver lipid, t_R 11.4 and 12.7 min.; (D) 1L-hexabenzoyl-*chiro*-inositol, t_R 31.8 min.; (E) 1D-hexabenzoyl-*chiro*-inositol, t_R 25.4 min.; (F) the mixture of 1D- (60%) and 1L- (40%) enantiomers, t_R 25.3 and 32.0 min.; (G) Hexabenzoyl-*chiro*-inositol from bovine liver, t_R 32.8 min.

Since hexabenzoyl-*myo*-inositol is optically inactive, $[\alpha]_{436}$ should be −106° after correcting for 91.5% *myo*-derivative. However, the quantitative polarimetric determination of the enantiomeric purity of *chiro*-inositol was difficult due to a large error of such measurement at a low concentration. We therefore developed an HPLC method for quantitative analysis of the *chiro*-inositol derivatives (for an alternative method see Ostlund et al., 1993). The benzoylated *chiro*- and *myo*-derivatives were preparatively separated by HPLC on silica gel (Figure 3C). The isolated hexabenzoyl-*chiro*-inositol along with the synthetic standards were subjected to analysis on the chiral HPLC column [CHIRALPAK OT (+), Chiral Technologies, Inc.] with a helical polymethacrylate phase. This column was found to distinguish between individual enantiomers of hexabenzoyl-*chiro*-inositol as shown in Figure 3 (traces D–F). The elution profile G shows very clearly that the *chiro*-inositol from bovine liver is entirely of 1L-configuration.

The possibility of epimerization of *myo*-inositol into *chiro*-inositol during the acidic hydrolysis of inositol phosphates was excluded by a control experiment in which samples of *myo*-inositol and synthetic *myo*-inositol 1-phosphate were separately refluxed in 6 N HCl and 50% TFA over 48 h. The final hydrolysates contained only *myo*-inositol (>99%) as indicated by their ^{1}H NMR spectra.

DISCUSSION

The results presented in this paper are significant in several aspects: (a) 1D- and 1L-*chiro*-PI have been synthesized in the diastereomerically pure forms (D and L represent the configurations of the inositol moiety; the diacylglycerol moiety is L-α in both cases). The configuration of **D-2** is assigned as 1D based on the reaction sequence starting with a known precursor (Bruzik & Tsai, 1992), and that of **L-2** is assigned as 1L based on the configuration of the starting material 1L-(−)-quebrachitol assigned by comparison with 1D-(+)-*chiro*-inositol (Posternak, 1936). These compounds will be useful for substrate-specificity studies of other PI-related enzymes. (b) The PI-PLC from *B. thuringiensis* has been shown to cleave 1L-*chiro*-PI at a substantially reduced rate, 10^{-3} relative to the natural substrate, 1D-*myo*-PI. 1L-*chiro*-PI and 1D-*myo*-PI differ only in the configuration at carbon 3 (in the numbering system of 1D-*myo*-PI, see Figure 1) of the inositol ring. This result indicates that the 3-OH group of 1D-*myo*-PI is important in PI-PLC–substrate interactions. (c) In spite of the low activity of 1L-*chiro*-PI, the result suggests that it is possible for PI-PLC to produce 1L-*chiro*-inositol derivatives. It further implies that the insulin signal may be mediated by GPI-PLC homologous to known PI-PLC. On the other hand, PI-PLC showed no activity at all toward the 1D-diastereomer (<10^{-6} relative to 1D-*myo*-PI). In terms of stereochemistry, this result is consistent with the earlier finding that PI-PLC is specific to 1D-*myo*-PI and does not hydrolyze 1L-*myo*-PI (Volwerk et al., 1990; Leigh et al., 1992). Although our results on *chiro*-PI are limited to one specific type of PI-PLC, they imply that, if PI-PLC or GPI-PLC is indeed responsible for the generation of *chiro*-inositol-containing insulin mediators, the *chiro*-inositol should have the 1L- instead of the 1D-configuration. (d) We then proceeded to isolate inositols from bovine liver according to published procedures and showed that the *chiro*-inositol, which constitutes 8.5% of total inositol, indeed has the 1L-configuration, with an enantiomeic purity of >99%. Again, this experiment was performed only for the lipid from a single source and is insufficient to conclude whether earlier reports are in error. However, our results suggest that the authors of earlier reports should re-evaluate their findings.

The finding of the 1L-configuration of naturally occurring *chiro*-inositol phospholipids is significant in the context of the latest finding of the occurrence of both enantiomers of *chiro*-inositol in human biological fluids (Ostlund et al., 1993). Our result suggests that only 1L-*chiro*-inositol is incorporated into a phospholipid pool of bovine liver. This result is also consistent with stereochemical similarity between 1D-*myo*- and 1L-*chiro*-phosphatidylinositols as explained in Figure 1. As shown in this work, PI-PLC, which requires the 1D-configuration of *myo*-phosphatidylinositols, can also cleave 1L-*chiro*-phosphatidylinositol. This result implies that 1L-*chiro*-inositol can be metabolized by enzymes known to accept *myo*-inositol. For example, phosphatidylinositol synthase from mouse brain, which selects the hydroxyl group at the 1D-position of *myo*-inositol to produce phosphatidylinositols, accepts small structural variations of *myo*-inositol at the 3-position (Johnson et al., 1993). It remains to be seen whether it would accept inositol analogs with an inverted 3-hydroxyl group such as in 1L-*chiro*-inositol. Incorporation of 1D-*chiro*-inositol into phospholipids and their further degradation would most likely require existence of an entirely different enzymatic system with a reversed stereospecificity.

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