METABOLISM OF GLYCOL-DERIVED LIPIDS IN NOVIKOFF HEPATOMAS*

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SUMMARY

[1,1,2,2-^{2}H]Ethanediol administered to Novikoff rat hepatomas was rapidly incorporated into glycol-derived neutral lipids and glycol phospholipids. The metabolic turnover rates of these diol-derived lipids and in particular of the diol phospholipids were at least an order of magnitude higher than those known for the corresponding glycerolipids. The data suggest that glycol phospholipids serve as precursors of the neutral glycol-derived lipids. Diol incorporation and diol lipid interconversions do not involve intermediary oxidation and reduction steps on the diol moiety. This was conclusively proven by mass spectrometry which showed that perdeuteration of the glycol moieties was retained in all instances.

Lipids derived from short-chain diols usually occur as minor constituents in animal and plant tissues, as well as in microorganisms (2-8). They have been found as neutral lipids (2-7) and phospholipids (8), in which the short-chain diols are linked to long-chain acyl (2-6), alkyl (7), or alk-1-enyl (4,8) moieties. At the present time, neither the biosynthesis nor the catabolism of diol lipids is understood, and their function has remained unknown.

The present study was undertaken to determine whether free glycol can serve as precursor in diol lipid biosynthesis, and to establish general patterns of diol lipid turnover and interconversion. Novikoff rat hepatomas (9) were chosen because we

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*"Naturally occurring diol lipids. X." For the preceding paper in this series, see reference 1.
Abbreviations: TLC, thin-layer chromatography; GLC, gas liquid chromatography; MS, mass spectrometry; ATP, adenosinetriphosphate.

have previously found diol lipids in these poorly differentiated, fast growing tumors (10), and because evidence exists that diol lipids may accumulate in tissues (5) during phases of rapid proliferation.

MATERIALS AND METHODS

Male rats (70 g) of the Sprague-Dawley strain (D. Rolfsmeyer, Madison, Wis.) were inoculated in each hind leg with $2x10^6$ Novikoff hepatoma cells (NlSl-67) grown in Swims 67 G medium (11). Ten days after inoculation, 0.1 ml of [1,1,2,2-^{2}H]ethanediol (Merck, Rahway, N. J.) was injected into each tumor. Rats were killed in groups of three, 3, 6, 12, 24 and 48 hours after glycol injection. The tumors (average weight, 7 g) were pooled within each group, homogenized, and extracted with 20 parts (v/w) of chloroform-methanol, 2:1 (v/v). The lipid extracts were not partitioned with water. The total lipids, 1.0 g, from each group were fractionated (6) on 3 mm layers of Silica Gel H (Merck, A. G., Darmstadt, Germany); developing solvent, hexane-diethyl ether, 80:20 (v/v). Three fractions were taken: The nonpolar glycol lipid fraction (R_F 0.6-1.0), 0.2 g, including triglycerides and less polar constituents; the glycol hydroxylipid fraction (R_F 0.05-0.6), 0.07 g, migrating off the origin, but being more polar than triglycerides; and the phospholipid fraction which was eluted from the origin with chloroform-methanol-water, 50:40:10 (v/v/v). The crude phospholipids were rechromatographed on 0.3 mm layers of Silica Gel H; developing solvent, chloroform-methanol-water, 65:35:8 (v/v/v) (6). Four lipid fractions were collected: The glycol phosphorylcholine fraction (R_F 0.05-0.3), 0.2 g, containing lipid constituents migrating off the origin but being more polar than

phosphatidyl choline; the glycol phosphorylethanolamine fraction (R_F 0.3-0.6), 0.2 g, comprising mostly phosphatidyl choline; the glycerol phosphorylethanolamine fraction (R_F 0.6-0.8), 0.1 g, consisting largely of phosphatidyl ethanolamine; and a fraction of least polar phospholipids (R_F 0.8-1.0), 0.03, g, containing phospholipids less polar than phosphatidylethanolamine.

The phospholipid fractions were hydrolyzed (6,8,12) with phospholipase C (EC 3.1.4.3) from Bacillus cereus (General Biochemicals, Chagrin Falls, Ohio), and the hydrolysis products were purified by TLC; developing solvent, hexane-diethyl ether, 50:50(v/v). These hydroxylipids derived from phospholipids as well as the native hydroxylipids and the nonpolar lipid fractions were taken up in diethyl ether, and the solutions were repeatedly extracted with water to assure complete removal of glycol precursor**. 1,3-Butanediol dihexadecanoate was added as internal standard (0.5% of fraction weight), and the lipid fractions were degraded chemically (6) essentially as we have described previously (1). Transesterification was achieved with $Ba(OH)_2$ in boiling methanol followed by brief treatment with methanolic BF_3. The hydrolysis products were reacted with hexadecanal (13,14) to form cyclic acetals of the polyhydric alcohols, and the acetalation mixture was subjected to hydrogenolysis with $LiAlH_4$. The fraction of C_{16}-acetals of diols was isolated by TLC, and further resolved by GLC (1). C_{16}-acetals of glycol were analyzed relative to 1,3-butanediol cyclic acetal derived

**Effective removal of free glycol from lipid-bound glycol was verified in a separate experiment in which [^{14}C]glycol was added to the tissue homogenate. After extraction, fractionation, and phospholipase C hydrolysis, the residual activities associated with the respective lipid fractions were found to be negligible.

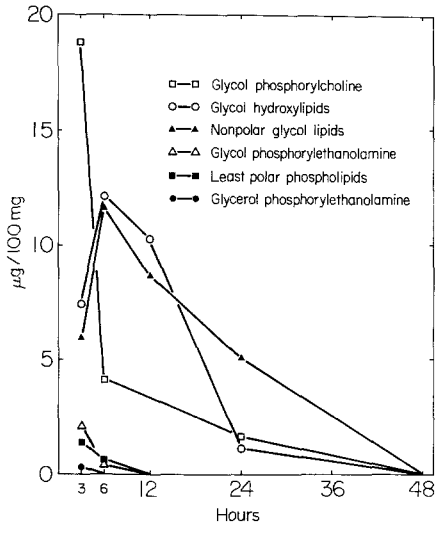

Figure 1. Distribution of [1,1,2,2-^{2}H]ethanediol-derived lipid constituents in neutral lipid fractions and phospholipid fractions at various time periods after administering perdeuterated ethanediol to Novikoff rat hepatomas.

from the internal standard, and the ratio of deuterated to nondeuterated glycol cyclic acetals was determined by GLC/MS using a Hitachi-Perkin Elmer single-focusing mass spectrometer, RMU-6D, at 70 ev ionization potential. Quantitations were based on the intensities of the prominent 1,3-dioxolane ions (65% of total ionization) at m/e 73 and m/e 77 for the nondeuterated and deuterated species, respectively (1,15). Dioxolane ions due to partially deuterated glycol acetals between m/e 73 and 77 were not observed.

RESULTS AND DISCUSSION

In the present *in vivo* study [1,1,2,2-^{2}H]ethanediol was administered to Novikoff rat hepatomas (9,11), and tumors were removed after 3, 6, 12, 24 and 48 hrs. The lipids were extracted under conditions precluding loss of water-soluble diol phospholipids (6) and were fractionated (*vide ante*). The neutral lipid

fractions, as well as the hydroxylipids produced through phospholipase C (EC 3.1.4.3) hydrolysis (6,8,12) of individual phospholipid fractions were transesterified (1), the polyhydric alcohols were acetalated with hexadecanal, and the long-chain cyclic acetals of diols were analyzed by gas chromatography/mass spectrometry (1,6). These analyses revealed that perdeuterated glycol had been incorporated readily into neutral lipids and phospholipids containing the glycol backbone.

Figure 1 shows the distribution of neutral lipids and phospholipids derived from perdeuterated glycol after different elapsed time periods. While the diol-bound lipophilic moieties may consist of acyl and alk-1-enyl ether chains, the amounts of glycol-derived constituents are uniformly expressed in μg of the [1,1,2,2-^{2}H]ethanediol hexadecanoate of the respective diol lipid class per 100 mg of total lipids. Surprisingly, the levels of deuterated diol lipids three to six hours after injection of substantial amounts of deuterated glycol were in the same order of magnitude as those found for natural diol-derived lipids in this tissue (10). In order to insure reliable identification of the metabolites at low levels, deuterium-labeling of the substrate and use of mass spectrometry appeared advantageous, realizing that the larger amounts of precursor required may, to some extent, affect the metabolic balance of the system (16).

The data indicate that glycol incorporation into neutral lipids and particularly into phospholipids proceeds at a rapid rate (Fig. 1). The apparent metabolic half-life of the glycol-derived lipid constituents associated with all four phospholipid fractions is at least an order of magnitude shorter (approx. 2 hrs.) than is known for rat liver glycerophospholipids (17,18), while the half-lives of glycol hydroxylipids and nonpolar glycol

lipids are approximately 3 and 12 hrs., respectively. Early in the experiment (3-6 hrs.), the levels of glycol phospholipids, and particularly the high levels of diol-derived lipid associated with the glycol phosphorylcholine fraction, are declining rapidly at a time neutral glycol lipid synthesis proceeds at an accelerated pace. This strongly suggests that phospholipid intermediates are precursors of neutral glycol-derived lipids. The data also show that dephosphorylation initially produced glycol hydroxylipids which were subsequently transformed into nonpolar glycol lipids, probably involving an acylation mechanism. At this point, assignments of diol lipid fractions are based on their chromatographic behavior, as compared to synthetic diol lipid standards (19,20), and on the fact that they are cleaved by phospholipase C and/or methanolysis. Definitive structural assignments must await further experiments involving radioactively labeled precursors and isolation of individual diol lipids by Sephadex chromatography (21,22).

Use of perdeuterated glycol as precursor and analysis of lipid-bound glycol as 2-pentadecyl-1,3-dioxolane by GLC/MS showed convincingly that glycol incorporation and glycol lipid interconversions do not involve intermediary oxidation and reduction on the glycol backbone. Direct incorporation and interconversion can be deduced from the complete absence of partially deuterated glycol-derived lipid constituents. Hence, routes of biosynthesis via oxidized glycol intermediates, such as glycolaldehyde (23), are not operative in glycol lipid metabolism with free glycol as substrate. Direct phosphorylation of the diol, similar to the glycerokinase catalyzed phosphate transfer from ATP to glycerol (24,25), appears more likely, although ATP:glycerol phosphotransferase (EC 2.7.1.30) itself was found unable to utilize

glycol as substrate in an *in vitro* system (25). Our present data do not necessarily preclude conversion of glycol phosphate to a glycol analogue of phosphatidic acid (26), which if attacked by phospholipase C could be partially responsible for the high glycol lipid level associated with the glycol phosphorylcholine fraction early in the experiment. Glycol phospholipids in turn would produce the monosubstituted glycol species present in the glycol hydroxylipid fraction acting as diglyceride analogues (27,28) in the formation of nonpolar lipids as well as phospholipids.

Our data suggest that the low levels of diol lipids commonly found in tissues are the result of the high turnover rate of these unusual constituents, rather than the consequence of impaired biosynthesis. It appears that a delicate equilibrium is maintained at a constant low level through carefully regulated metabolic processes, a phenomenon that can be rationalized on the basis of the strongly lytic activities found for diol-derived lecithin analogues (29-31).

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