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ACTIVITIES OF ENZYMES INVOLVED IN THE METABOLISM OF ETHER-LINKED LIPIDS IN NORMAL AND NEOPLASTIC TISSUES OF RAT*

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Summary

We have compared the activities of three enzymes (acyl-CoA reductase, fatty alcohol:NAD$^+$ oxidoreductase, and alkyl synthase) involved in the metabolism of ether lipids in rat liver and two tumors that contained various levels of ether lipids. The activity of fatty alcohol:NAD$^+$ oxidoreductase was high and the activities of acyl-CoA reductase and alkyldihydroxyacetone-*P* synthase were low in liver, which has low amounts of ether glycerolipids. On the other hand in Fischer R-3259 sarcoma, where high concentrations of ether-linked lipids were present, the reverse pattern was observed. In Morris hepatomas 5123C, with an intermediate value of ether lipids, the activities of all three enzymes ranged between that found in liver and Fischer sarcomas.

Ether-linked lipids are found both in normal and cancerous mammalian cells [1, 2]. The biosynthetic and degradative pathways for these lipids and their precursors have been established and reviewed [3]. The level of ether lipids is increased in less differentiated Morris hepatomas when compared with that in normal livers [4,5] and the elevated levels of ether lipids have been correlated with low glycerol-*P* dehydrogenase [4] and alkylglycerol monooxygenase activities [6] in Morris hepatomas and other transplantable tumors. Optimal assay conditions for several other key enzymes involved in

the metabolism of ether lipids, acyl-CoA reductase [7, 8], alkyldihydroxyacetone-*P* synthase [9], and fatty alcohol:NAD^+ oxidoreductase [10] have only recently been established. Therefore, it became possible to also assess the activities of these enzymes in several tissues that differ in the amounts of ether lipids present.

The sources of the chemicals used were: NAD^+, $NADP^+$, glucose-6-*P*, and glucose-6-*P* dehydrogenase from P-L Chemicals; albumin (essentially fatty acid-free) from Sigma Chemical Co.; and [1-^{14}C]palmitic acid (58.9 Ci/mol), and [1-^{14}C]palmitoyl-CoA (56.9 Ci/mol) from New England Nuclear. [1-^{14}C]Hexadecanol was synthesized from [1-^{14}C]palmitic acid by reduction with Vitride [11]. Hexadecanoyldihydroxyacetone-*P* was synthesized as described previously [12]. Both [1-^{14}C]hexadecanol and hexadecanoyldihydroxyacetone-*P* were greater than 99% pure as determined by thin-layer chromatography (TLC) on silica gel G or H layers.

Normal livers from male Charles River (CD strain) rats, Fischer R-3259 sarcomas obtained 4—6 weeks after transplant in the CD rats, and Morris hepatomas 5123C obtained 2 months after transplant in Buffalo rats were used as enzyme sources. Necrotic tissue was carefully dissected from the tumors and discarded. The livers or tumors were then homogenized in a Potter-Elvehjem homogenizer in 4 vols. of buffered sucrose (0.1 M Tris-HCl (pH 7.4), 0.1 M KCl, 0.25 M sucrose). All homogenates were centrifuged at 150 × *g* for 10 min to remove the unbroken cells; the supernatants were used for enzyme assays.

Acyl-CoA reductase activity was assayed according to the optimal conditions established by Moore and Snyder [7]. The incubation mixture in a final volume of 1.0 ml, consisted of 6 mM ATP, 2 mM $NADP^+$, 10 mM glucose-6-*P*, glucose-6-*P* dehydrogenase (2—3 units; 1 unit equals 1 nmol NAD^+ or $NADP^+$ reduced/min at 30° C, pH 7.8), 0.4 mg albumin, 0.1 M phosphate (pH 6.8), [1-^{14}C]palmitoyl-CoA (15 nmol, 50 000 dpm) and homogenates. Fatty alcohol:NAD^+ oxidoreductase activity was determined as reported earlier [10]. Alkyldihydroxyacetone-*P* synthase was measured using the optimal conditions established by Rock et al. [9]. All enzyme assays were carried out at two or more concentrations of protein or at two different time intervals of incubation.

The ether lipid content of Morris hepatoma 5123C and Fischer R-3259 sarcoma was determined by first extracting the total lipids using the method of Bligh and Dyer [13], except that the methanol contained 2% acetic acid. Neutral lipids and phospholipids were separated on microcolumns of silicic acid [14]. The amounts of ether-linked lipids in each fraction were based on the alkylglycerols and alk-1-enylglycerols [14] produced after Vitride reduction [11]. The values were multiplied by 3 to express them in terms of percentages of intact lipid classes. Protein was determined by the method of Lowry et al. [15] using bovine serum albumin as the standard.

The level of total ether-linked lipids (both alkyl and alk-1-enyl types) is significantly higher in Morris hepatoma 5123C and Fischer R-3259 sarcoma than in normal liver (Table I). The activities of acyl-CoA reductase and alkyldihydroxyacetone-*P* synthase are also considerably higher in Morris hepatoma 5123C and Fischer R-3259 sarcoma than in normal liver, whereas

TABLE I

ALKYL AND ALK-1-ENYL GLYCEROLIPID CONTENT OF NORMAL LIVER, MORRIS HEPATOMA 5123C, AND FISCHER R-3259 SARCOMA

Values are averages of duplicate samples and varied less than 10% from the average. n.d. = not detectable.

	% of total lipids		
	Normal liver**	Morris hepatoma 5123C	Fischer R-3259 sarcoma
Neutral Lipids			
Alkyldiacyl	n.d.	0.5	0.5
Alk-1-enyldiacyl	n.d.	Trace	0.1
Phospholipids			
Alkylacyl	0.4	0.7	2.7
Alk-1-enylacyl	0.4	1.1	3.8
Total ether lipids	0.8	2.2	7.1

**Data calculated from Ref. 5.

TABLE II

ACTIVITIES OF ENZYMES INVOLVED IN THE METABOLISM OF ETHER-LINKED LIPIDS

n.d. = not detectable. The means ± S.E. are from three separate experiments. Values are in pmol/min per mg protein.

Enzyme	Liver	Morris heapatoma sarcoma 5123C	Fisher R-3259 sarcoma
Acyl-CoA reductase	2.7; 2.8	16; 17	13; 14
Alkyldihydroxyacetone-*P* synthase	n.d.	5.5; 5.1	7; 12
Fatty alcohol:NAD^+ oxidoreductase	1360; 1250	570 ± 4	66 ± 7

the activity of fatty alcohol:NAD^+ oxidoreductase is much lower in the tumors (Table II). Alkyldihydroxyacetone-*P* synthase catalyzes the first committed reaction step for the formation of the alkyl ether-linked glycerolipids. The level of one of its substrates, fatty alcohols, could be affected by the enzyme activities of both acyl-CoA reductase and fatty alcohol:NAD^+ oxidoreductase. Previously, a negative correlation between fatty alcohol:NAD^+ oxidoreductase activity and the content of ether-linked lipids [2, 10] was found in normal tissues. Tissues, such as liver, that are low in ether lipids have a high fatty alcohol:NAD^+ oxidoreductase activity and tissues, like brain and heart that are high in ether lipids, have low fatty alcohol:NAD^+ oxidoreductase activities. In addition, Ehrlich ascites carcinomas (5.5% of total lipids) and preputial gland tumors (5.8% of total lipids), which are similar to Fischer R-3259 sarcomas [16, 17] in their ether-lipid content, have specific activities of fatty alcohol:NAD^+ oxidoreductase of 33 and 55 pmol/min per mg protein, respectively. These values are similar in magnitude to those found in the Fischer R-3259 sarcoma (66 pmol/min per mg protein, Table II). Furthermore, the activities of alkyldihydroxyacetone-*P* synthase and acyl-CoA reductase seem to be high in several other normal and neo-

plastic tissues, such as developing rat brain [8], preputial glands [7], harderian glands, and Ehrlich ascites carcinoma [9] that are rich in ether-linked lipids. Therefore, it appears that the level of ether-linked lipids in tissues is regulated in a coordinated manner by the increased biosynthetic and decreased catabolic activities of the enzymes involved in their metabolism. This is further supported by the evidence that Δ 1-alkyl desaturase, which forms plasmalogens, is also low in liver and high in brain and tumors (Wykle, R.L., personal communication).

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