Specific Positional Distribution of Acyl Moieties in Phospholipids is not Generally Deleted in Neoplastic Cells

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The distribution of acyl moieties at *sn*-1 and *sn*-2 positions of cholinephosphoglycerides (CPG) and ethanolaminephosphoglycerides (EPG) has been determined for neurosarcoma, sarcoma 180 and leukemia L 1210. In all the three samples, the positional distribution of acyl moieties in the two major classes of phospholipids is found to be similar to that in cellular phospholipids of most mammalian tissues. The saturated acyl moieties are located predominantly at *sn*-1 and polyunsaturated acyl moieties at *sn*-2, whereas the monounsaturated acyl moieties are randomly distributed between these two positions. Apparently, a disruption of specific positioning of acyl moieties in phospholipids, which hitherto has been considered to be a general metabolic deletion in neoplasia, does not exist in all neoplastic cells.

Introduction

Most cellular phospholipids exhibit specific positioning of saturated acyl moieties at *sn*-1 and polyunsaturated acyl moieties at *sn*-2 of the glycerol backbone, whereas monounsaturated acyl moieties are randomly located at these two positions [1, 2]. This specificity in the positional distribution of acyl moieties in phospholipids has been shown to be disrupted in several types of neoplastic cells [3–7] and such a metabolic deletion is considered to be one of the characteristic features of neoplasia [6]. A recent study has shown, however, that the positional specificity of acyl moieties in phospholipids of normal lymphocytes is essentially retained in rapidly growing leukemia L 1210 ascites cells [8].

In order to examine, whether the abnormal positioning of acyl moieties in phospholipids is a general characteristic of neoplastic cells, we have studied the distribution of acyl moieties in the major phospholipids of neurosarcoma, a slowly growing tumor, and of sarcoma 180 ascites cells. Moreover, we have reinvestigated the positioning of acyl moieties in the phospholipids of leukemia L 1210 cells. We have found that in the solid tumor as well as in the two types of ascites cells the specific positional distribution of saturated and unsaturated acyl moieties in the major classes of phospholipids is not disrupted.

Experimental

Neurosarcoma was grown in the back of male Sprague-Dawley rats as described elsewhere [9]. The tumors were removed from the adhering muscle tissue on the 22nd day after transplantation of the tumor. Ascites cells of sarcoma 180 and leukemia L 1210 were grown in the peritoneal cavity of female NMRI mice and transplanted weekly. The ascites cells were collected on the fifth day after transplantation. The cells were rinsed repeatedly with Hank's medium and isolated by centrifugation.

Tumor tissue as well as ascites cells from five animals, each, were pooled and lipids were extracted with chloroform-methanol (1:2) according to an established procedure [10]. Total lipids were fractionated on layers of silica gel H using chloroform-methanol-water (65:25:4) and the fractions of cholinephosphoglycerides (CPG) and ethanolaminephosphoglycerides (EPG) were, each, isolated [11]. These phospholipid fractions were hydrolyzed by phospholipase A_2 from porcine pancreas [12] (Boehringer-Mannheim GmbH, Mannheim, Germany). The products of enzymatic hydrolysis, *i.e.* 1-acyl phosphoglycerides and the fatty acids liberated from *sn*-2 of the glycerol backbone, were isolated by chro-

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matography on layers of silica gel H using chloroform-methanol-water (65:25:4) [11]. 1-Acylphosphoglycerides and the fatty acids were then converted to the methyl esters [13] and analyzed by gas chromatography in a Perkin-Elmer F 22 instrument (Bodenseewerk Perkin-Elmer & Co. GmbH, Überlingen, Bundesrepublik Deutschland), equipped with flame ionization detectors. The methyl esters were separated on glass columns, 1.8 m by 3 mm, packed with EGSS-X on Gas Chrom P, 100–120 mesh (Applied Science Laboratories, Inc., State College, Pennsylvania, USA).

Results

Table I shows the positional distribution of acyl moieties in CPG and EPG of neurosarcoma, sarcoma 180 and leukemia L 1210. The data show distinctly that in both major classes of phospholipids in the three samples investigated the saturated and the polyunsaturated acyl moieties are located specifically at *sn*-1 and *sn*-2, respectively, whereas the monounsaturated acyl moieties are randomly distributed between positions *sn*-1 and *sn*-2.

Although the general pattern of distribution of acyl moieties in CPG and EPG of the solid neurosarcoma is similar to that in the sarcoma 180 and leukemia L 1210 ascites cells, several quantitative differences are observed in the distribution of individual acyl moieties in the tumor, as compared to the ascites cells (Table I). A striking feature of neurosarcoma is the occurrence of relatively large proportions of 15:0 and 17:0 acyl moieties, which occur almost exclusively at the *sn*-1 position of EPG. A low level of 16:0 acyl moieties at both *sn*-1 and *sn*-2 in EPG of neurosarcoma, as compared to the corresponding positions in CPG of neurosarcoma as well as both CPG and EPG of the two types of ascites cells, is noteworthy. As compared to neurosarcoma, the ascites cells are characterized by

Table I. Composition (%) of acyl moieties at *sn*-1 and *sn*-2 positions of phospholipids in neurosarcoma solid tumor and ascites cells of sarcoma 180 and leukemia L 1210.

Acyl moieties*	Neurosarcoma				Sarcoma 180				Leukemia L 1210			
	CPG		EPG		CPG		EPG		CPG		EPG	
	sn-1	*sn*-2	*sn*-1	*sn*-2	*sn*-1	*sn*-2	*sn*-1	*sn*-2	*sn*-1	*sn*-2	*sn*-1	*sn*-2
14 : 0	0.2	0.6	0.6	0.2	1.0	0.7	3.4	1.5	1.3	1.0	3.7	0.6
15 : 0	0.3	0.2	10.2	n. d.	n. d.	0.1	n. d.	0.3	n. d.	0.1	0.2	n. d.
16 : 0	27.2	11.4	7.8	2.1	23.7	10.0	22.3	13.7	16.2	13.7	19.5	10.3
16 : 1	n. d.	2.7	n. d.	0.6	1.5	2.2	2.4	2.4	1.7	3.2	2.5	2.1
17 : 0	n. d.	n. d.	8.3	n. d.	0.5	n. d.	0.9	0.7	1.0	n. d.	1.0	0.6
18 : 0	52.4	1.6	64.3	4.0	48.6	5.7	35.0	13.6	52.0	7.8	39.4	12.1
18 : 1	10.2	15.7	4.4	9.7	17.3	17.8	28.0	29.2	19.7	24.2	26.6	27.5
18 : 2 w 6	2.6	29.5	1.0	13.8	3.4	26.0	3.4	13.4	2.9	31.9	2.4	26.0
18 : 3 w 3	2.8	1.0	1.1	2.2	1.7	0.6	2.0	0.7	2.6	0.8	2.0	1.1
20 : 0	n. d.	n. d.	n. d.	n. d.	0.4	n. d.	0.5	n. d.	n. d.	n. d.	0.3	n. d.
20 : 2 w 6	2.5	1.8	0.8	1.4	1.5	0.6	n. d.	n. d.	2.5	0.8	1.3	0.6
20 : 3 w 6	n. d.	5.3	n. d.	6.5	n. d.	2.0	n. d.	1.0	n. d.	1.3	n. d.	0.6
20 : 4 w 6	n. d.	26.0	n. d.	38.8	n. d.	26.3	n. d.	15.4	n. d.	10.5	n. d.	7.2
22 : 1	1.8	n. d.	1.0	n. d.	n. d.	n. d.	n. d.	n.d.	n. d.	n. d.	n. d.	n. d.
22 : 3 w 6	n. d.	2.6	n. d.	11.3	n. d.	2.2	n. d.	2.3	n. d.	1.8	n. d.	n. d.
22 : 4 w 6	n. d.	n. d.	n. d.	2.7	n. d.	0.7	n. d.	0.7	n. d.	n. d.	n. d.	1.1
22 : 6 w 3	n. d.	n. d.	n. d.	4.4	n. d.	5.1	n. d.	5.0	n. d.	2.8	n. d.	6.4
Unidentified	0.0	1.6	0.5	2.3	0.4	0.0	2.1	0.1	0.1	0.1	1.1	3.8
Total saturated	80.1	13.8	91.2	6.3	74.2	16.5	62.1	29.8	70.5	22.6	64.1	23.6
Total monoenoic	12.0	18.4	5.4	10.3	18.8	20.0	30.4	31.6	21.4	27.4	29.1	29.6
Total polyenoic	7.9	66.2	2.9	81.1	6.6	63.5	5.4	38.5	8.0	49.9	5.7	43.0

* Number of carbon atoms: number of double bonds; w 6 and w 3 stand for methylene interrupted unsaturated acyl moieties with the first double bond located at the sixth and third carbon atom, respectively, from the terminal methyl group.
n. d. Not detected.

a low level of 18:0 at *sn*-1 position of EPG, elevated levels of 18:1 at both *sn*-1 and *sn*-2 of CPG as well as EPG, and a low level of 20:4 w 6 at *sn*-2 of both classes of phospholipids. The only exception is CPG of sarcoma 180, in which the level of 20:4 w 6 at *sn*-2 is as high as that at *sn*-2 in CPG of neurosarcoma. The levels of 18:2 w 6 at *sn*-2 of CPG and EPG of neurosarcoma are similar to those of the ascites cells, with the exception of leukemia L 1210, which contains a higher level of 18:2 w 6 at *sn*-2 of EPG than that at *sn*-2 in EPG of neurosarcoma.

Discussion

Our results (Table I) show that the "normal" positional distribution of acyl moieties in phospholipids [1, 2] is not disrupted in the three types of neoplasia investigated. Apparently, a disruption of specific positioning of acyl moieties in phospholipids, as reported for hepatomas [4–7], nephroma [6] and Ehrlich ascites cells [3], is not a general feature of neoplasia.

The "abnormal" positioning of acyl moieties in phospholipids of certain types of hepatoma has been attributed to an alteration in the mechanism which controls the normal asymmetric distribution of fatty acids in naturally-occurring phospholipids [14]. It is generally agreed that the asymmetric positioning of saturated and unsaturated acyl moieties in diacylglycerolipids occurs, partly, during the stepwise acylation of *sn*-glycerol 3-phosphate, which is catalyzed by acyl-CoA: *sn*-glycerol-3-phosphate acyltransferase and acyl-CoA: acyl-*sn*-glycerol-3-phosphate acyltransferase [15, 16]. Since all species of diacylglycerophospholipids should arise through the key intermediate, acyl-*sn*-glycerol-3-phosphate, the specific positioning of acyl moieties in such lipids is likely to be regulated by acyl-CoA: *sn*-glycerol-3-phosphate acyltransferase. This enzyme has been detected in both microsomes and mitochondria of several mammalian organs [17]. The mitochondrial acyl-CoA: *sn*-glycerol-3-phosphate acyltransferase, in contrast to its microsomal counterpart, specifically catalyzes the acylation of *sn*-1 with saturated acyl moieties, and this particular enzyme is believed to be primarily responsible for the specific positional distribution of acyl moieties in phospholipids [17]. Cultured cells and Ehrlich ascites tumor cells have been shown to be deficient in mitochondrial acyl-CoA: *sn*-glycerol-3-phosphate acyltransferase, which seems to have a bearing on the abnormal positioning of acyl moieties in the phospholipids of these cells [17, 18]. Our data on the distribution of acyl moieties in the major phospholipids of solid neurosarcoma, sarcoma 180 and leukemia L 1210 ascites cells (Table I) as well as previous data on leukemia L 1210 cells [8] suggest that a deficiency of the mitochondrial acyl-CoA: *sn*-glycerol-3-phosphate acyltransferase might not be a characteristic feature of all tumor cells.

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