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Characteristics of ether-linked glycerophospholipids in Friend erythroleukaemia cells differentiated by dimethyl sulphoxide or hexamethylenebisacetamide and in non-inducible clones treated with the inducers

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The present study deals with changes in ether-linked glycerophospholids which accompany differentiation of Friend erythroleukaemia (FEL) cells by dimethyl sulphoxide (DMSO) and hexamethylenebisacetamide (HMBA). We also tested clones of FEL cells non-inducible by DMSO or HMBA for ether-linked lipid changes not related to the differentiation process. FEL cells contained appreciable proportions of alkenylacyl and alkylacyl subfractions in phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Compared with FEL cells, clones non-inducible by DMSO or HMBA had a greater amount of alkenylacyl PE associated with a lack of alkenylacyl PC. The differentiation of FEL cells by DMSO or HMBA was accompanied by a reduction of alkylacyl PE and PC. DMSO- and HMBA-differentiated FEL cells showed changes in alkenyl- and alkyl-chain profiles, some of which were also observed in non-inducible FEL cells treated with DMSO or HMBA.

INTRODUCTION

Several lines of evidence lead us to believe that cell-surface lipids are involved in cellular differentiation. For instance, since chemico-physical properties have been found to modulate cellular differentiation (Arcangeli *et al.*, 1987), it may well be that lipids are implicated through their effect on membrane fluidity (van Blitterwijk, 1985). Moreover, specific lipid components influence some of the cell-surface properties involved in cellular differentiation such as receptorial (Ginsberg *et al.*, 1982; Spector & Yoreck, 1985) or enzymic activities (Sanderman, 1978), transport of nutrients (Stubbs & Smith, 1984), antigenicity (Hakomori, 1981; Shinitzky, 1984) and intercellular recognition (Hakomori, 1981). Indeed, in the course of cellular differentiation, both phospholipid structure and metabolism undergo modifications. These include: alteration of phospholid class composition (Honma *et al.*, 1980; Saito *et al.*, 1980; Zwingelstein *et al.*, 1981; Rittmann *et al.*, 1982) and of phospholipid transmembrane distribution (Van der Schaft *et al.*, 1987); increased synthesis of sphingomyelin, phosphatidylinositol and phosphatidylserine (O'Doherty, 1978); better metabolic control of acyl chain make-up in phopholipids (O'Doherty, 1978); and increased level of long-chain polyunsaturated fatty acids (Robert *et al.*, 1979; Perkins & Scott, 1978; Zwingelstein *et al.*, 1981, 1982). No information exists, however, as to whether cellular differentiation is accompanied by changes in ether-linked lipids, a family of lipid components whose biological significance is still obscure (Horrocks & Sharma, 1982). The behaviour of ether-linked lipids in artificial models is quite distinct from that of diacyl analogues (Boggs, 1980; Horrocks & Sharma, 1982; Paltauf, 1983), which points to differences in the biological function of these lipid components. It has been recently reported that certain biological properties related to cell-surface characteristics, such as metastatic potential (Friedberg *et al.*, 1986; Calorini *et al.*, 1987), tumorigenicity (Roos & Choppin, 1984) or susceptibility to cell fusion (Roos & Choppin, 1985), are associated with the occurrence of high levels of ether-linked lipids in alkyl form.

In the present study we explored whether the differentiation of Friend erythroleukaemia (FEL) cells induced by dimethyl sulphoxide (DMSO) or hexamethylenebisacetamide (HMBA) (Friend *et al.*, 1971) affects the composition and molecular structure of ether-linked glycerophospholipids. We also studied two clones of FEL cells non-inducible to differentiation by DMSO or HMBA in order to see whether changes of ether-linked phospholipids in differentiated FEL cells are related to the differentiation process or are produced by direct effects of the inducers on membrane lipids.

MATERIALS AND METHODS

Cells and culture conditions

FEL cells (clone 745 A), kindly donated by Dr. G. Rossi (Instituto Superiore di Sanità, Roma, Italy), were maintained in our laboratory under the growth conditions reported in a previous paper (Fallani *et al.*, 1988). Clones of FEL cells non-inducible by DMSO or HMBA were isolated as previously reported (Fallani *et al.*, 1988).

Abbreviations used: FEL, Friend erythroleukaemia; DMSO, dimethyl sulphoxide; HMBA, hexamethylenebisacetamide; PE, phosphatidylethanolamine; PC, phosphatidylcholine.

Lipid extraction and analysis of ether-linked lipids

FEL cells were washed twice by centrifugation (250 *g* for 10 min) and resuspension in Dulbecco & Vogt's (1954) phosphate-buffered saline, pH 7.2. Cell suspensions were sonicated and extracted with chloroform/methanol (2:1, v/v) by the method of Folch *et al.* (1957). Phospholipids were separated from neutral lipids by silicic acid column chromatography and then fractionated by two-dimensional t.l.c. under the conditions described by Ruggieri *et al.* (1979). Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were recovered from the absorbent by elution with a chloroform/methanol mixture (9:1, v/v) and analysed by Su & Schmidt's (1974) ether-linked lipid procedure. By this procedure, alkenyl-linked lipids are converted into cyclic acetals by reaction with 1,3-propane-1,3-diol and *p*-toluenesulphonic acid, and alkyl-linked lipids are converted into alkylglycerols by hydrogenolysis with sodium bis-(2-methoxyethoxy)-aluminium hydride in toluene (Eastman Kodak Co.). Cyclic acetals and isopropylidene derivatives (Wood, 1967) of alkylglycerols are then measured by quantitative g.l.c.

G.l.c. analyses

Cyclic acetals and isopropylidene derivatives of alkylglycerols were determined by quantitative g.l.c. with dimethoxytetradecane and pentadecylglycerol respectively as internal standards. Analyses were performed at 195 °C on glass columns packed with 10% EGSS-X on Gas Chrom P 100–120 mesh (Supelco, Bellefonte, PA, U.S.A.) and mounted in a Perkin–Elmer gas chromatograph (model 3920) equipped with hydrogen flame detectors. Peak areas were calculated by using a Perkin–Elmer Sigma 10 integrator.

RESULTS

FEL cells contained a high proportion of alkenylacyl molecules (ethanolamine plasmalogens) and an appreciable amount of alkylacyl molecules in PE (Table 1). Alkylacyl molecules were also present in PC, whereas alkenylacyl molecules (choline plasmalogens) were barely detected. This pattern is consistent with that found in ether-linked lipids of different types of cell grown *in vitro* (Anderson *et al.*, 1969; Fallani *et al.*, 1982; Yoshioka *et al.*, 1985; Calorini *et al.*, 1987). Clones non-inducible by DMSO and HMBA had a higher percentage of ethananolamine plasmalogens than FEL cells, but they lacked choline plasmalogens. Differentiation of FEL cells by either DMSO or HMBA was accompanied by a significant decrease of alkylacyl PE and PC.

The alkenyl-group composition of ethanolamine plasmalogens in FEL cells was characterized by a prevalence of $C_{16:0}$ over $C_{18:0}$ and $C_{18:1}$ chains (Table 2). A comparable pattern was also found in alkyl groups of alkylacyl PE, although this latter PE had a higher proportion of $C_{16:1}$ chains. Compared with FEL cells, clones non-inducible by DMSO or HMBA had a higher proportion of $C_{18:0}$ and a lower level of $C_{18:1}$ alkenyl chains in ethanolamine plasmalogens. Moreover, the clone non-inducible by DMSO showed a higher percentage of $C_{18:0}$ and only a trace amount of $C_{18:1}$ alkyl chains in alkylacyl PE. In FEL cells differentiated with either DMSO or HMBA, there was a marked increase of $C_{18:1}$ associated with a decrease of $C_{18:0}$ chains in both alkenylacyl and alkylacyl PE. There was a similar change in the alkenyl- and alkyl-group composition in the clone non-inducible by HMBA treated with HMBA, whereas in the clone non-inducible by DMSO the exposure to the inducer raised the $C_{18:1}$ alkyl-chain level.

Unlike alkenylacyl PE, alkenylacyl PC had $C_{18:1}$ as the major alkenyl group. In alkylacyl PC, $C_{16:0}$ prevailed over $C_{18:1}$ and $C_{18:1}$ alkyl chains (Table 3). FEL cells differentiated by DMSO or HMBA showed an increase of $C_{16:0}$ and $C_{16:1}$ and a decrease in $C_{18:1}$ alkenyl chains in alkenylacyl PC, and a decrease of $C_{16:0}$ and an increase of $C_{18:1}$ alkyl chains in alkylacyl PC. The $C_{18:1}$ alkyl chain was also increased in the clone non-inducible by HMBA exposed to HMBA, whereas it was decreased in the clone non-inducible by DMSO treated with the inducer.

DISCUSSION

Our study revealed that FEL cells contain high proportions of alkenylacyl and alkylacyl molecules in PE and PC. In a previous study (Fallani *et al.*, 1988), FEL cells were shown to contain small quantities of alkyldiacylglycerols which were not modified during erythroid differentiation. The levels of alkenylacyl and alkylacyl

Table 1. Changes in ether-linked (alkenylacyl- and alkylacyl) subfractions of PE and PC from FEL cells upon differentiation with DMSO or HMBA, and clones non-inducible by DMSO and HMBA treated with the corresponding inducer

Values, expressed as percentages of PE and PC, are means ± S.E.M. for three or four separate experiments; the remaining percentage is accounted for by the diacyl fraction. Values were calculated as described by Fallani *et al.* (1982). Statistical significance was determined by Student's *t* test; significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.005$, compared with the appropriate control. Abbreviation: N.D., not detectable under the analytical conditions used.

Ether-linked subfraction	Proportion of PE and PC (%)						
	FEL cells			Non-inducible clone (DMSO)		Non-inducible clone (HMBA)	
	Control	DMSO	HMBA	Control	DMSO	Control	HMBA
Alkenylacyl PE	34.0±6.0	35.9±4.0	49.5±10.0	56.2±8.0**	60.9±11.8	59.6±7.5**	51.1±3.7
Alkylacyl PE	10.1±3.2	6.9±1.3*	4.7±2.0*	7.0±2.9	10.0±0.9	7.3±2.4	6.9±1.4
Alkenylacyl PC	2.5±0.7	2.1±0.7	4.1±1.0	N.D.	N.D.	N.D.	N.D.
Alkylacyl PC	21.9±3.8	11.4±1.7**	7.7±1.2**	19.2±1.2	14.4±0.3	15.5±2.1	11.6±2.8

Table 2. Changes in alkenyl- and alkyl-group compositions in PE ether-linked subfractions from FEL cells upon differentiation with DMSO and HMBA, and from clones non-inducible by DMSO or HMBA treated with the corresponding inducer

Values are means ± S.E.M. for three or four separate experiments. Statistical significance was determined by Student's *t* test: $*P < 0.05$, $**P < 0.01$, $***P < 0.005$, compared with the appropriate control. Abbreviation: tr, trace.

Type of chain	Chain length	Composition (mol/100 mol)						
		FEL cells			Non-inducible clone (DMSO)		Non-inducible clone (HMBA)	
		Control	DMSO	HMBA	Control	DMSO	Control	HMBA
Alkenyl	$C_{16:0}$	49.6±3.5	41.5±1.3	43.7±1.1	46.3±3.7	44.7±2.8	43.5±3.2	52.8±1.1
	$C_{16:1}$	1.7±0.7	2.1±0.9	3.4±1.1	1.2±0.7	0.5±0.1	1.5±0.3	1.3±0.2
	$C_{18:0}$	32.0±1.8	28.0±0.6*	21.6±0.8*	43.3±3.6*	43.4±2.6	41.5±2.1*	23.3±1.9*
	$C_{18:1}$	16.7±1.5	28.2±2.5***	31.4±1.3***	9.3±0.5*	11.4±0.5	13.5±0.5	22.9±2.3*
Alkyl	$C_{16:0}$	43.5±4.6	37.5±3.6	38.1±0.5	38.9±5.4	40.5±0.6	43.6±0.7	43.9±1.5
	$C_{16:1}$	7.7±0.2	5.5±1.6	5.5±2.7	9.1±4.7	4.4±1.5	2.7±1.4	1.0±0.8
	$C_{18:0}$	36.6±6.1	26.9±1.3*	23.6±2.7*	52.0±1.5*	49.0±1.6	42.3±1.6	28.0±2.8*
	$C_{18:1}$	13.7±3.8	30.1±2.3***	32.8±0.5***	tr	6.1±3.7***	10.0±0.5	17.1±2.1*

Table 3. Changes in alkenyl- and alkyl-group compositions in PC ether-linked subfractions from FEL cells upon differentiation with DMSO or HMBA, and from clones non-inducible by DMSO or HMBA treated with the corresponding inducer

Values are means ± S.E.M. for three or four separate experiments. Statistical significance was determined by Student's *t* test: $*P < 0.05$, $**P < 0.01$, $***P < 0.005$ compared with the appropriate control.

Type of chain	Chain length	Composition (mol/100 mol)						
		FEL cells			Non-inducible clone (DMSO)		Non-inducible clone (HMBA)	
		Control	DMSO	HMBA	Control	DMSO	Control	HMBA
Alkenyl	$C_{16:0}$	26.7±1.0	57.3±3.7***	47.5±8.2**	—	—	—	—
	$C_{16:1}$	1.7±0.1	11.1±3.2***	3.7±0.2*	—	—	—	—
	$C_{18:0}$	19.6±5.7	22.3±3.1	26.9±6.8	—	—	—	—
	$C_{18:1}$	51.8±4.0	9.0±1.1	19.6±3.1***	—	—	—	—
Alkyl	$C_{16:0}$	60.5±1.5	44.3±2.2***	47.4±0.5***	63.8±3.2	64.7±3.2	67.2±2.3	57.3±1.6
	$C_{16:1}$	1.9±0.3	1.9±0.3	1.1±0.1	1.9±0.3	1.6±0.3	1.4±0.2	3.7±0.4
	$C_{18:0}$	18.5±1.0	14.7±1.4	9.9±0.6**	20.1±4.9	25.5±1.5*	20.4±2.1	13.1±1.1*
	$C_{18:1}$	19.1±1.5	39.1±2.1***	41.7±1.3***	14.2±1.3	8.4±1.3*	11.0±1.3	25.9±2.1**

PE and PC in FEL cells were comparable with those reported for simian-virus-40-transformed Balb/c 3T3 (Fallani *et al.*, 1982) and murine mastocytoma cell lines (Yoshioka *et al.*, 1985), but higher than those of LM cells (Anderson *et al.*, 1969) and B16 melanoma cell lines (Calorini *et al.*, 1987).

Clones non-inducible by DMSO or HMBA differed from inducible FEL cells in terms of a much higher level of ethanolamine plasmalogens and a lack of choline plasmalogens. Although microviscosity could be modified by an alteration in ethanolamine/choline ratio (Shinitzky & Henkart, 1979), there is no way of specifying the net effect, if any, of changes in ethanolamine and choline plasmalogen proportions, since total PE and PC levels were unchanged in inducible and non-inducible FEL cells (Fallani *et al.*, 1988). Moreover, non-inducible clones had higher proportions of $C_{18:0}$ alkenyl and alkyl chains than FEL cells, which are compatible with a diminished fluidity in view of the rigidity conferred on lipid assemblies by saturated chains (Demel *et al.*, 1972). Fisher *et al.* (1981), found a decreased fluidity in variants of FEL cells resistant to phorbol myristate acetate effects.

The levels of alkylacyl PE and PC subfractions tended to decrease in FEL cells differentiated by DMSO or HMBA. Their values, however, still exceeded the very low levels found in DBA/2 mouse erythrocytes (results not shown), which may be related to the incomplete stage of maturation reached by the differentiated FEL cells. The tendency of FEL cells to decrease their levels of alkylacyl PE and PC subfractions during erythroid differentiation suggests that differentiation process is associated with a change in the metabolic reactions, such as acylation of dihydroxyacetone phosphate (Hajra, 1968), replacement of acyl group by a long-chain alcohol to form alkyldihydroxyacetonephosphate (Hajra, 1970), reduction of fatty acids to the corresponding alcohols (Snyder & Malone, 1970; Bishop & Hajra, 1978), transfer of phosphocholine and phosphoethanolamine to alkylacylglycerol (Snyder *et al.*, 1970) and cleavage of ether bonds (Soodsma *et al.*, 1970), which regulate the ether-linked lipid levels in normal and transformed cells (Hajra, 1973; Snyder & Snyder, 1975).

Differentiated FEL cells showed changes in the molecular structure of their ether-linked lipids consisting of the replacement of saturated with monounsaturated chains both in alkylacyl PE and PC and in alkenylacyl PE; alkenyl groups of alkenylacyl PC, instead, showed a change in the opposite direction. Since acyl chains of glycerophospholipids were not modified in differentiated FEL cells (Fallani *et al.*, 1988), changes in ether groups do not seem to be related to an alteration of fatty acid precursors of long-chain fatty alcohols. Thus the differentiation process may affect the complement of fatty acid-CoA reductases which supply the various long-chain alcohol precursors of ether-linked lipid biosynthesis (Snyder & Malone, 1970; Bishop & Hajra, 1978). The increased level of the $C_{18:1}$ alkenyl group in ethanolamine plasmalogens of differentiated FEL cells is reminiscent of the increase of $C_{18:1}$ molecular species of ethanolamine plasmalogens in normal human myelin during development (Boggs & Rangaraj, 1984). However, some of the alkenyl- and alkyl-group changes observed in the differentiated FEL cells were also present in the clones non-inducible by DMSO or HMBA exposed to the corresponding inducer. Thus at least some of the alterations of the ether-linked lipid molecular structure in DMSO- or HMBA-differentiated FEL cells may not be related to the differentiation process, but may reflect a direct effect of the inducers on lipid metabolism. Alternatively, it is possible that the changes in the molecular structure of ether-linked lipids of differentiated FEL cells may be related to differentiation programs independent of haemoglobin synthesis and that these programs are expressed in both inducible and non-inducible FEL cells after treatment with DMSO or HMBA.

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