Lipid composition of *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (Tn) insect cells used for baculovirus infection

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Abstract The lipid composition of two different insect cell lines from *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (Tn) which are established cell lines for infection with recombinant baculovirus was analyzed by high-performance liquid chromatography and gas-liquid chromatography. The major phospholipids found were phosphatidylcholine and phosphatidylethanolamine, the major mono-unsaturated fatty acids were oleic acid and palmitoleic acid, the major saturated fatty acid was stearic acid. The cholesterol to phospholipid ratio was demonstrated to be lower than in mammalian cell lines. Infection with a recombinant baculovirus *Autographa californica* resulted in increased levels of phosphatidylcholine in the insect cells. The baculovirus/insect cell system has become a popular system for heterologous protein production. Functional changes of membrane proteins produced in these two cell lines might be correlated to a different lipid profile of their cellular membranes.

Key words: Insect cell; Sf9 cell; Tn cell; Phospholipid; Fatty acid; Baculovirus

1. Introduction

The cultured *Spodoptera frugiperda* (Sf9), army fallworm, and the cabbage looper *Trichoplusia ni* have been established as cell lines for the production of foreign proteins after infection with respective recombinant baculoviruses AcMNPV (*Autographa californica* nuclear polyhedrosis virus). This system is a promising tool for the overproduction and large-scale purification of membrane proteins [1]. In numerous studies the function of membrane-bound enzymes, receptors and transport carriers was correlated with membrane fluidity [2–4]. This biophysical property is largely determined by the membrane composition at a certain temperature [5]. Although it has always been supposed that the lipid composition of insect cells significantly differs from that of mammalian cells [6–8], the lipid compositions of cultured Sf9 and Tn cells have never been extensively characterized. Recently, it has been shown that plasma membranes of Sf9 cells contain 10 times less cholesterol than plasma membranes isolated from mammalian cells [9]. In this study, we examined the phospholipid composition of two commonly used insect cell lines (Sf9 cells and Tn cells) by high-performance liquid chromatography (HPLC). HPLC has greatly simplified the separation of lipid classes as evaporative light-scattering detectors (ELSDs) were introduced. Separation of simple and complex lipids from animal tissues on a bonded silica gel column was shown to be successful in a single chromatographic run [10]. In addition, the fatty acid composition of whole insect cells was analyzed by gas-liquid chromatography (GLC). Both the phospholipid and the fatty acid compositions of the insect cell lines significantly differed from those of cultured mammalian cell lines. Thus, a different profile in membrane lipids might also result in different fluidity characteristics of a biological membrane and, therefore, could explain differences in the activity of membrane proteins produced in these insect cells after infection with a respective baculovirus.

2. Materials and methods

2.1. Materials

All solvents were Analar or HPLC grade and were supplied by Fisons (Loughborough, UK). Phospholipid standards and cell culture chemicals were purchased from Sigma.

2.2. Cell culture

S. frugiperda Sf9 cells were obtained from ATCC, *T. ni* cells were kindly provided by Dr. Klier, Max-Planck-Institut in Dortmund. The cells were grown at 27°C in TNM-FH medium supplemented with 5% fetal calf serum (Gibco) in monolayer culture as described [11]. Cells for large-scale experiments were grown in suspension in spinner flasks. For infection the insect cells were incubated with a recombinant baculovirus containing the cDNA of the $Na^+/K^+/Ca^{2+}$ exchanger [12] at a MOI (multiplicity of infection) of 10.

2.3. Extraction and analysis of lipids

The lipids were extracted from whole cells as described elsewhere [13]. Chromatography was performed on a 100×3 mm i.d. column packed with 5 mm Spherisorb S5CN (Chrompack, The Netherlands) as described previously [10]. Quantification of lipids by an ELSD was carried out with calibration lines of standards by electronic integration [10]. Separation of neutral and acidic phospholipids was carried out on Isolute Spe-NH_2 columns (IST) according to the manufacturer's description. Neutral lipids were eluted with 8 ml methanol, acidic lipids were eluted with 8 ml of chloroform/methanol/NH_4Ac (4:1:0.1). For calculations of phospholipid molarity an average molecular weight of M_r 750 was assumed.

Fatty acid methyl esters of total lipids and fractionated phospholipid classes were obtained according to Christie [14]. They were dissolved in hexane and the supernatant was used for GLC analysis after centrifugation (1500×*g*). To check for the presence of sphingolipids, ester bonds were hydrolyzed by transesterification with sodium methoxide, and the remaining lipids were analyzed by HPLC as described before.

The fatty acid profile determination of the different samples was done in a Hewlett Packard 5890 series II Plus capillary gas chromatograph fitted with a split/splitless injector and a flame ionization detector. The separation was carried out in a capillary column (0.25 mm i.d.×25.55 m in length) of fused silica coated with CP-wax 52CB. After holding the temperature at 170°C for 3 min the temperature was increased at 4°C/min to 210°C. Here, hydrogen was the carrier gas. Methyl esters were identified by the use of standards and quantified by electronic integration.

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Table 1
Optimum elution conditions for resolution of insect lipids

Time (min)	Solvent[a]			Flow rate (ml/min)
	A	B	C	
0	100			0.4
1	100			0.4
5	80	20		0.5
12	46	52	2	0.5
27	38	52	10	0.5
34	38	52	10	0.5
34.1	30	70		1.0
37	100			1.0
40	100			1.5

Lipids were extracted with chloroform/methanol and loaded on a Spherisorb S5CN column. A linear gradient was generated between each of the timed steps.
[a]A, iso-hexane-methyl-*tert*-butyl ether (98:2, v/v); B, propan-2-ol-chloroform-acetic acid (82:20:0.01, v/v); C, propan-2-ol-water-triethylamine (47:47:6, v/v).

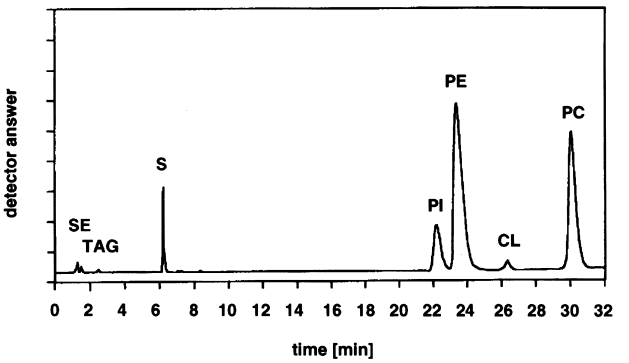

Fig. 1. Gradient elution of lipids isolated from non-infected Sf9 cells. Lipids were extracted from cells and separated on a Spherisorb S5CN column with ELSD under gradient conditions specified in Table 1 (SE, sterolesters; TAG, triacylglycerides; S, sterols; PI, phosphatidylinostol; PE, phosphatidylethanolamine; Cl, cardiolipin; PC, phosphatidylcholine).

3. Results

3.1. Phospholipid composition

The lipid classes in lipid extracts from either uninfected or infected Sf9 and Tn insect cells could be very well separated with a propionitrile-bonded column using a gradient system as presented in Table 1. As shown in the chromatographic profile for lipids isolated from uninfected Sf9 cells (Fig. 1) sterol esters eluted first, followed by triacylglycerols, sterols and the phospholipids phosphatidylinositol, phosphatidylethanolamine, cardiolipin and phosphatidylcholine. Glycolipids could not be detected. After separation of the neutral phospholipids from the acidic phospholipids, phosphatidylserine, which is frequently found in mammalian cell membranes [15], could not be detected. In addition, HPLC performed after hydrolysis of any lipids revealed the absence of any major sphingolipid. Comparable profiles were obtained from lipid extracts prepared from either infected or uninfected Sf9 and Tn cells. A high reproducibility was achieved in four independent experiments. The percentages of phospholipids in the insect cells are given in Table 2. The amount of these two major phospholipids, phosphatidylcholine and phosphatidylethanolamine, was equally high in Sf9 cells (35.1% and 36.1% respectively) whereas Tn cells contained more phosphatidylcholine (43.3%) than phosphatidylethanolamine (35.9%). The percentage of phosphatidylinositol was slightly increased in Sf9 cells (23%) compared to Tn368 cells (16.9%) and both insect cells showed an about threefold higher level of phosphatidylinositol than has been described for BHK cells [7]. The molar cholesterol/phospholipid ratio of 0.04 was in the same range as the value of 0.03 which was shown for Sf9 cells before [9] but was significantly lower than the value of 0.28 found in mammalian cells [7].

After infection of both types of insect cells with a recombinant baculovirus bearing the bovine $Na^+/Ca^{2+}/K^+$ exchanger cDNA under the control of the late polyhedrin promoter, an increase in phosphatidylcholine content of about 8–9% as well as a slight increase of the cholesterol to phospholipid ratio was observed in both cell lines.

3.2. Fatty acid composition

The fatty acid composition of Sf9 and Tn cell membrane lipid extracts was analyzed by gas chromatography (Table 3). The major unsaturated fatty acid in Sf9 and Tn cells was oleic acid (18:1ω9) with 48.0% and 53.9%, respectively, followed by palmitoleic acid (16:1ω7) with 21.5% and 16.8%. The main saturated acids were stearic acid (18:0) with 17.9% and 14.0% and palmitic acid (16:0) with 4.5% and 8.5. Only small amounts of dienoic and polyenoic fatty acids could be detected. These results are in agreement with the findings on whole Noctuidae [6] as well as on cultured Diptera cells of *A. albopictus* [8] with the difference of stearic acid and not palmitic acid being the major saturated fatty acid in Sf9 and Tn cells. The mean saturation in the fatty acid chains of the insect cell lipids was about 26% which is 5% less than in BHK-21 cells [7]. Additionally, we analyzed the fatty acid composition of each single phospholipid class. The profiles obtained were specific for each single lipid class and very similar in both insect cell lines (Table 3). Phosphatidylethanolamine and phosphatidylcholine contained the most unsaturated fatty acids. Infection with a recombinant baculovirus resulted in only minor changes in the levels of unsaturated

Table 2
Phospholipid composition of Sf9 and Tn cells

Phospholipid (w/w %)	Sf9	Tn	Sf9 (i)	Tn (i)
Phosphatidylinositol	23.0 ± 1.6[a]	16.9 ± 1.0	23.0 ± 0.3	14.1 ± 0.5
Phosphatidylethanolamine	36.1 ± 1.0	35.9 ± 1.3	32.4 ± 2.5	30.2 ± 1.2
Cardiolipin	4.6 ± 0.2	4.7 ± 1.1	3.7 ± 0.3	3.3 ± 0.5
Phosphatidylcholine	35.1 ± 1.0	43.3 ± 0.5	42.7 ± 0.6	52.3 ± 0.8
Cholesterol/phospholipid ratio	0.04 ± 0.003	0.03 ± 0.004	0.05 ± 0.001	0.04 ± 0.004

Lipids of non-infected cells and infected by recombinant baculovirus (i) were separated by gradient HPLC and peak areas quantified by electronic integration (normalised to 100%, w/w).
[a]Mean ± S.D. ($n = 4$).

Table 3
Fatty acid composition of total lipids and single phospholipid classes

Fatty acid (w/w%)	Sf9 cells					Tn cells				
	Total lipids[a]	Phospholipid class				Total lipids	Phospholipid class			
		PI	PE	Cl	PC		PI	PE	Cl	PC
14:0	0.2	0.3	0.2	1.3	0.9	1.0	0.1	0.8	1.8	1.4
16:0	4.5	3.4	4.0	13.4	9.1	8.5	5.2	10.8	8.0	11.1
16:1	21.5	12.7	19.8	29.5	17.3	16.8	8.4	16.3	44.0	17.3
18:0	17.9	27.3	15.9	6.2	13.1	14.0	25.4	12.0	8.6	9.8
18:1ω9	48.0	44.6	52.9	25.7	46.0	53.9	52.6	54.7	23.0	51.5
18:1ω7	2.6	2.6	2.8	3.7	3.0	1.2	1.1	1.2	1.2	1.9
18:2ω6	0.7	0.4	0.3	1.8	1.0	0.4	0.4	0.5	3.2	0.8
20:0–20:3	3.4	5.6	2.5	6.5	2.9	1.9	2.8	2.0	6.8	1.7
20:4ω6	0.6	1.2	0.6	0.5	0.3	0.7	1.7	0.5	0.3	0.3
20:5ω3	0.2	0.3	0.2	0.7	0.1	0.1	0.3	0.1	0.5	0.2
22:5ω3	0.1	0.3	0.1	1.5	0.5	0.1	0.3	0.2	0.0	0.6
22:6ω3	0.2	0.4	0.3	7.2	3.5	0.5	0.8	0.6	0.0	2.7
24:1	0.1	1.0	0.4	2.0	2.1	0.9	0.8	0.2	2.3	0.7

Lipids of uninfected Sf9 and Tn cells were transesterified, methylester of fatty acids isolated and separated by high-performance liquid-gas chromatography (PI, phosphatidylinositol; PE, phosphatidylethanolamine; Cl, cardiolipin; PC, phosphatidylcholine).
[a]All fatty acid compositions are normalized to 100%, after elimination of minor unidentified components.

lipid fatty acids in both cell lines and overall, the saturation of the fatty acid chains was not altered (data not shown).

4. Discussion

This study compares the lipid composition of two different insect cell lines which are commonly used as hosts for the baculovirus AcMNPV. The baculovirus/insect cell expression system is widely used for heterologous production of recombinant membrane proteins. However, membrane proteins produced in insect cells were often less active or inactive [16–18]. In analogy to the dependence of soluble enzymes on the composition and characteristics of their aqueous milieus the activity of functional membrane proteins is influenced by the properties of the lipid bilayer. Such a behavior has been observed with numerous membrane proteins such as the human erythrocyte sugar transporter [19], the nicotinic acetylcholine receptor [20,21] and the oxytocin receptor [9]. The molecular mechanisms of this modulation are not yet understood but could involve either specific interactions between boundary lipids, e.g. cholesterol, and membrane proteins or a modulation caused by the lipid bulk order or membrane fluidity [22,23]. Important factors that affect the fluidity of a membrane are the phosphatidylcholine to sphingomyelin ratio, the cholesterol to phospholipid ratio, the saturation and chain length of fatty acids and the temperature. It has been demonstrated recently that the addition of cholesterol into the medium of infected insect cells (Sf9) increased the low cholesterol to phospholipid ratio normally observed in these cells resulting in an increase in high-affinity agonist binding sites for the recombinant oxytocin receptor [9].

In our study, we found a similar low cholesterol content in Sf9 and Tn cells using HPLC gradient analysis. We show that both insect cell lines, Sf9 cells as well as Tn cells, contained phosphatidylcholine and most interestingly also phosphatidylethanolamine as a major component of total phospholipids. It has been reported that phosphatidylethanolamine is also the major lipid component in some fly species [6,8]. Furthermore, we found that the content of phosphatidylinositol appeared to be very high in whole cell extracts as well as in isolated insect cell membranes (data not shown). Comparable high levels of phosphatidylinositol have only been observed in mitochondrial outer membranes [15]. Analysis of isolated membranes from different membrane compartments might show if this high amount of phosphatidylinositol is due to its distribution and its role as a messenger molecule inside the cell. Finally it would be interesting to investigate if the observed differences in the phosphatidylcholine and phosphatidylinositol content between Sf9 and Tn cells could explain differences in the functional activity of expressed proteins as seen in these cell lines for the expression of the human GnRH receptor [16]. Novel theories implicating a role of non-bilayer lipids, such as phosphatidylethanolamine, maintaining the functional structure of integral membrane proteins [23,24] need to be considered.

After infection of insect cells with a recombinant baculovirus an increase of the phosphatidylcholine content in both cell lines was observed. This finding is in agreement with other studies which also revealed a higher phosphatidylcholine content in cells infected with the related chilo-iridescent virus type 6 [25]. The virus envelope of nuclear polyhedrosis viruses is synthesized de novo [26] and contains phosphatidylcholine as a major phospholipid component [27]. Thus, one possible explanation might be that after virus infection synthesis of phosphatidylcholine is induced which leads to an elevated phosphatidylcholine content in the insect cells after virus infection.

In summary, the phospholipid composition in the Sf9 and Tn insect cell lines differs significantly from that of mammalian cells [7]. The high content of phosphatidylethanolamine and phosphatidylinositol, the lack of detectable phosphatidylserine, glyco- and sphingolipids, and the lower cholesterol to phospholipid ratio is notable. The distribution of fatty acids in lipid classes also differed. Both phosphatidylcholine and phosphatidylethanolamine contained less saturated fatty acids than was reported for mammalian cells (around 50%) [15] which might be partially compensated in these cells by a higher saturation of other phospholipid classes or by longer fatty acid chains in average.

We conclude that although the lipid composition of the Sf9 and Tn insect cell lines clearly differed from that of mammalian cells, a similar degree of fluidity in the cellular membranes of these insect cell lines could be maintained due to the lower cultivation temperature (27°C) and a lower saturation of fatty

acids chains to antagonize the lower cholesterol content. Further studies are needed in order to understand the role of distinct membrane components, both phospholipid headgroups and fatty acids, for the activity of membrane proteins.

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