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THE DISTRIBUTION OF MOLECULAR CLASSES OF PHOSPHATIDYLGLYCEROL IN THE MEMBRANE OF *ACHOLEPLASMA LAIDLAWII*

E.M. BEVERS, J.A.F. OP DEN KAMP and L.L.M. VAN DEENEN

Laboratory of Biochemistry, State University of Utrecht, Utrecht (The Netherlands)

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Summary

A double-label technique has been applied to study the distribution of different molecular classes of phosphatidylglycerol in the membrane of *Acholeplasma laidlawii*. After growth on oleic acid, 16% of the total phosphatidylglycerol contains two oleic acid residues and 84% contains one oleic acid and one saturated fatty acid. The dioleoyl phosphatidylglycerol is present in equal amounts in the outer and the inner layer of the membrane. Phosphatidylglycerol which is associated with membrane proteins consists exclusively of the class containing only one oleic acid.

Phospholipases have been used extensively to study the localization and physicochemical properties of phosphatidylglycerol in the membrane of *Acholeplasma laidlawii* [1,2]. It was found that three different pools of this phospholipid are present but the detection of these pools is possible only under the following conditions [1]. The temperature at which the incubations with phospholipases are carried out should be as low as possible in order to prevent rapid mixing of the different pools by lateral diffusion and transbilayer movements. On the other hand, the lipids have to be, also at the low incubation temperatures, in a liquid-crystalline condition to allow phosphatidylglycerol hydrolysis to proceed [2].

Both conditions can be met when cells are used which contain lipids enriched in oleic or linoleic acid. In oleate-enriched *A. laidlawii* membranes the following pools of phosphatidylglycerol can be detected: 40% of the phosphatidylglycerol is present in the outer leaflet of the membrane bilayer; 30% is present in the inner layer; the residual 30% is associated with intrinsic membrane protein [3] but the location of this pool is unknown.

Furthermore, using a double-label technique, we have been able to estimate the amounts and the physicochemical behaviour of the different species of

phosphatidylglycerol in the membrane [2]. The same double-label technique is applied now on the localization studies mentioned above in order to determine the molecular species of phosphatidylglycerol in the three different pools.

A. laidlawii strain B was grown on a lipid-poor tryptose medium, supplied with [1-^{14}C]oleic acid and ortho[^{32}P]phosphate, as described elsewhere [2]. Cells and membranes were isolated and incubated with an excess of phospholipase A_2 from pig pancreas for 15 min at 0°C. The reaction was stopped by the addition of EDTA and phosphatidylglycerol was isolated by extraction and thin-layer chromatography [2]. The extent of hydrolysis was measured by ^{32}P counting of the residual phosphatidylglycerol using a phosphorylated glycolipid as internal standard. The ^{14}C/^{32}P ratio was determined from the residual phosphatidylglycerol. Analysis of the species composition of the original phosphatidylglycerol was carried out by fatty acid analysis and by the determination of the ^{14}C/^{32}P ratio of total phosphatidylglycerol from untreated cells. These analyses were performed also on the 1-acyl lyso derivative obtained after incubation of isolated phosphatidylglycerol with phospholipase A_2 [2]. The data are presented in Table I. The data allow a calculation of the molecular species composition of the phosphatidylglycerol provided that we assume that all phosphatidylglycerol molecules contain at least one oleic acid residue. Furthermore, we have based the calculations on the accuracy of the radioactivity counting which results in an oleic acid content of 27% in the isolated lysoderivative instead of the 22% measured by gas-liquid chromatography. Calculations, details of which have been presented before [2], show that the phosphatidylglycerol consists of the two major species: 16% contains two oleic acid residues (dioleoyl phosphatidylglycerol) having a ^{14}C/^{32}P ratio of 7.0 and 84% contains one residue (monooleoyl phosphatidylglycerol) with a ^{14}C/^{32}P ratio of 3.5.

The results of phospholipase A_2 incubation on intact cells and isolated membranes are presented in Table II. As was found before, 30% of the phosphatidylglycerol cannot be hydrolyzed even when both sides of the membrane are exposed to the phospholipase [3]. This protein-protected pool of phosphatidylglycerol has a ^{14}C/^{32}P ratio of 3.5 and therefore contains exclusively mono-

TABLE I

FATTY ACID COMPOSITION AND ^{14}C/^{32}P RATIO OF PHOSPHATIDYLGLYCEROL AND 1-ACYL-LYSOPHOSPHATIDYLGLYCEROL IN OLEIC ACID-ENRICHED MEMBRANES FROM *A. LAIDLAWII*

Fatty acid composition *	Phosphatidylglycerol (%)	Lyso phosphatidylglycerol (%)
14 : 0	1	—
16 : 0	32	61
18 : 0	8	16
18 : 1*c*	58 **	22
^{14}C/^{32}P	4.1 **	0.95

* Only major fatty acids found in the membrane are presented.

** Assuming a uniform distribution of the ^{32}P label this means that 3.5 counts ^{14}C correspond to one oleic acid residue. From this figure one can calculate the ^{14}C/^{32}P ratio of dioleoylphosphatidylglycerol (7.0) and the species containing one oleate (3.5).

TABLE II

AMOUNT AND $^{14}C/^{32}P$ RATIO OF RESIDUAL PHOSPHATIDYLGLYCEROL FOUND AFTER PHOSPHOLIPASE INCUBATION OF INTACT CELLS AND ISOLATED MEMBRANES OF *A. LAIDLAWII*

	Cells	Membranes
Residual phosphatidylglycerol *	60	30
$^{14}C/^{32}P$	4.0	3.5

* Data are expressed as a percentage of the original amount of phosphatidylglycerol present.

oleoyl phosphatidylglycerol species (Table III). The second pool of phosphatidylglycerol which can be assayed directly is the one which is localized in the outer membrane layer. In intact cells 40% of the total phosphatidylglycerol is directly accessible to phospholipase A_2 and it is found that this pool contains both the dioleoyl species (8% of the total phosphatidylglycerol) as well as monooleoyl phosphatidylglycerol (32% of the total). It is obvious then that the rest of the dioleoyl phosphatidylglycerol (8% of the total) and the monooleoyl species (22%) is located in the inner layer (Table III).

The data described here have led to the following conclusions. First, the degree of unsaturation in the freely accessible inner and outer layer pools of phosphatidylglycerol differs only slightly. The value of this observation with respect to the difference in overall lipid fluidity between inner and outer layer as described by Rottem [4] is hard to assess because phosphatidylglycerol comprises only 30% of the total lipid content of this membrane. Data on the distribution of different molecular species of a given phospholipid over the two layers of a membrane are scarce. In human and rat erythrocyte membranes, phosphatidylcholine is distributed unequally over the two layers, the majority being outside. Both pools, however, are identical with respect to the molecular species composition [5,6]. A similar observation was made by Litman, studying the localization of egg yolk phosphatidylethanolamine in mixed liposomes [7]. A direct comparison, however, of this data with the results described here is premature because a substantial part of the phosphatidylglycerol has not been localized so far and furthermore because of the remarkable fatty acid composi-

TABLE III

TRANSBILAYER DISTRIBUTION OF PHOSPHATIDYLGLYCEROL SPECIES IN MEMBRANES FROM *A. LAIDLAWII* GROWN IN THE PRESENCE OF OLEIC ACID

Localization	% of total phosphatidyl-glycerol	Species composition (% of total amount of phosphatidyl-glycerol)	Species composition (% of total amount of phosphatidylglycerol in particular pool)
Outer monolayer	40%	32% Monooleoyl PG *	80% Monooleoyl PG
		8% Dioleoyl PG	20% Dioleoyl PG
Protein protected	30%	30% Monooleoyl PG	100% Monooleoyl PG
Inner monolayer	30%	22% Monooleoyl PG	73% Monooleoyl PG
		8% Dioleoyl PG	27% Dioleoyl PG

* PG, phosphatidylglycerol.

tion of this pool. No dioleoyl phosphatidylglycerol is associated with proteins under these conditions. The second conclusion which can be made, therefore, is that the intrinsic membrane proteins are surrounded in this membrane and under these experimental conditions preferentially with phospholipid molecules containing two different fatty acyl chains. Studying *Escherichia coli* membranes, Baldassare et al. [8] already demonstrated that such phospholipids have an important role in conserving membrane-bound enzymatic activities. In *A. laidlawii* the Mg^{2+}-dependent ATPase has an absolute requirement for phosphatidylglycerol [9]. Therefore, it can be speculated that this enzyme is surrounded preferentially with monooleoyl phosphatidylglycerol molecules. More direct experimental evidence is required, however, to prove such a specific enzyme-phospholipid interaction, especially because the technique we have employed can only be used at low temperatures and not at temperatures at which the intrinsic membrane proteins function.

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