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Lipid Composition of Mitochondrial Outer and Inner Membranes of *Neurospora crassa*

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Dedicated to Prof. Dr. Dr. h. c. Theodor Bücher on the occasion of his 60th birthday

Summary: The lipid composition of outer and inner mitochondrial membranes of *Neurospora crassa* was analyzed quantitatively. The outer membrane is made up of 59% lipid and 41% protein, the inner membrane of 23% lipid and 77% protein. The outer membrane is one of the most lipid-rich membranes described in the literature. The main lipid components of the outer membrane are phospholipid and ergosterol. They occur in a molar ratio of 3:1. No ergosterol is found in the inner membrane. Ergosterol is released from the outer membrane in the form of needle-like crystals, when outer membrane preparations are stored as concentrated suspensions. Simultaneously, outer membrane vesicles fuse to generate large membrane systems. The phospholipid compositions of the two membranes are different. This difference is small with respect to the major components phosphatidylcholine and phosphatidylethanolamine. On the other hand, cardiolipin occurs predominantly – if not exclusively – in the inner membrane. The carotenoid pigment neurosporaxanthin is localized selectively in the outer mitochondrial membrane.

Lipidzusammensetzung der mitochondrialen Außen- und Innenmembranen von Neurospora crassa

Zusammenfassung: Mitochondriale Außen- und Innenmembranen von *Neurospora crassa* wurden einer quantitativen Bestimmung ihrer Lipidzusammensetzung unterzogen. Die Außenmembran enthält 59% Lipid und 41% Protein, die Innenmembran 23% Lipid und 77% Protein. Die Außenmembran ist eine der lipidreichsten Membranen, die in der Literatur beschrieben sind. Die wesentlichen Lipidkomponenten der Außenmembran sind Phospholipide und Ergosterin. Ihr molares Verhältnis beträgt ca. 3:1. In der Innenmembran kommt kein Ergosterin vor. Ergosterin kristallisiert aus der Außenmembran in Form von Nadeln aus, wenn Außenmembranpräparationen als konzentrierte Suspensionen aufbewahrt werden. Gleichzeitig mit diesem Vorgang verschmelzen Außenmembranvesikel. Dies führt zum Auftreten ausgedehnter Membransysteme. Die Phospholipidzusammensetzungen der beiden Membranen sind unterschiedlich. Im Fall der Hauptkomponenten Phosphatidylcholin und Phosphatidyläthanolamin sind die relativen Konzentrationsunterschiede nur gering. Cardiolipin andererseits kommt überwiegend – wenn nicht ausschließlich – in der inneren Membran vor. Der Karotinoidfarbstoff Neurosporaxanthin stellt eine spezifische Komponente der mitochondrialen Außenmembran dar.

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Enzymes: Kynurenine 3-monooxygenase, L-kynurenine, NADPH:oxygen oxidoreductase (3-hydroxylating) (EC 1.14.13.9)

Succinate dehydrogenase (succinate cytochrome *c* reductase), succinate:(acceptor) oxidoreductase (EC 1.3.99.1).

The outer and inner membranes of mitochondria differ with respect to structure and function[1–3]. Whereas the inner membrane can be regarded as a multienzyme complex responsible for the major part of cellular energy production, the outer membrane contains a heterogeneous group of enzymes. Separation methods for the two membrane systems were first developed with the aid of mammalian liver mitochondria, and hitherto most of our information has come from this source.

Studies with *Neurospora crassa* have shown that mitochondrial membranes from organisms phylogenetically as distant as mammals and fungi display the same building principles.

With respect to the lipid part of outer and inner membranes, data are only available for liver mitochondria[1,2,4–8]. They show that each membrane has a characteristic lipid pattern. The present communication supplies data in answer to the following questions: Are the lipid patterns of the outer and inner mitochondrial membranes in *Neurospora* and liver constant parameters, like the specific distribution of enzymes? How are lipids which are specific for animals, such as cholesterol, substituted in fungi? Are common physical properties of the individual mitochondrial membranes from different organisms related to a common lipid structure?

Experimental

Preparation of mitochondrial membranes

Neurospora crassa wild type 74A was grown in Vogel's minimal medium containing 0.81mM phosphate[3]. The cells were harvested 16 h after inoculation. Mitochondria and other cell fractions were prepared by a procedure described previously[3]. Mitochondrial outer and inner membranes were separated by a combined technique of swelling, shrinking and sonication followed by centrifugation through a linear sucrose density gradient[3]. The gradient was divided into 11 fractions of 5 m*l* each. Fraction 1 (top of gradient) (outer membrane) and fraction 9 (inner membrane) were collected and centrifuged as described. The final pellets were resuspended in Tris-HCl, 10mM, pH 7.2.

Labeling of Neurospora cells with [^{32}P]*phosphate*

For the homogeneous labeling of lipid-phosphorus, 1 mCi [^{32}P]orthophosphate (1600–4480 Ci/mmol) (Radiochemical Centre, Amersham, England) was added to a 1 *l* culture immediately after inoculation.

Enzyme and cytochrome determinations

Kynurenine 3-monooxygenase, succinate, cytochrome *c* reductase and cytochromes aa_3 and *b* were measured as described elsewhere[3]. Protein was determined by the method of Lowry *et al.*[9].

Neurosporaxanthin and ergosterol

The acidic carotenoid neurosporaxanthin was extracted according to the method of Harding *et al.*[10]. The visible spectrum was recorded in hexane. The content of neurosporaxanthin was calculated from the extinction at 477.5nm ($\varepsilon = 85.4\,l \times \text{mmol}^{-1}\text{cm}^{-1}$)[11]. Ergosterol was extracted with methanol and acetone and transferred into hexane. UV-spectra were recorded. The content of ergosterol was estimated from the extinction at 282 nm. Authentic ergosterol (Fa. Fluka, Buchs, Switzerland) was employed as a standard for these analyses.

Phospholipids

Lipid extraction. The total lipids of cell fractions and mitochondrial membranes were extracted with chloroform/methanol (2:1, by vol.) at 22°C for 12 h. The extracts were washed with one-fifth vol. of 0.034% $MgCl_2$ according to the method of Folch *et al.*[12]. After removal of the upper aqueous phase, samples were either taken for phosphorus analysis, or portions (20–35 µg total phospholipid phosphorus) were vacuum dried for thin layer chromatography. Extracts were stored under nitrogen at −20°C.

Two-dimensional thin-layer chromatography. The dried extracts were taken up in chloroform/methanol (2:1, by vol.) and applied to silica gel thin-layer plates (20 × 20 cm, 0.25 mm thick; Merck AG., Darmstadt) under an atmosphere of nitrogen. The plates were developed two-dimensionally using chloroform/methanol/25% ammonia (65:35:5, by vol.) in the first direction, and chloroform/acetone/methanol/acetic acid/water (56:20:10:10:4, by vol.) in the second direction. The chromatography jars were lined with filter paper and saturated with the appropriate solvent. Two 4 cm lanes were separated along two intersecting edges of the thin-layer plates. These were used for the chromatography of appropriate standards in either direction.

Detection of spots. The plates were dried and exposed to iodine vapor. The spots were immediately encircled with a pencil. The individual spots were identified by comparison with the position of the standard compounds which were chromatographed on the two lanes mentioned above. Alternatively, single lipid standards were co-chromatographed with *Neurospora* lipid extract to obtain accentuated spots. In addition, lipid spots were identified with the following reagents: a) molybdenum reagent[13,14], for phospholipids; b) ninhydrin reagent (Merck AG., Darmstadt), for phospholipids containing free amino groups; c) orcinol-sulfuric acid

reagent[15,16], for glycolipids; d) diphenylamine reagent[16–19], for glycolipids. Phospholipid standards: phosphatidylcholine (lecithin), phosphatidylethanolamine (cephalin) and sphingomyelin were purchased from C. Roth OHG., Karlsruhe, lysophosphatidylcholine and lysophosphatidylethanolamine from Sigma Chemical Co., St. Louis, USA. Diphosphatidylglycerol (cardiolipin), phosphatidylserine, phosphatidylinositol and phosphatidic acid were obtained from Koch-Light-Laboratories, Colnbrook, England. Cerebrosides, sulfatides and gangliosides were kindly supplied by Dr. E. Mehl, Max-Planck-Institut für Psychiatrie, München.

Colorimetric phospholipid phosphorus assay. The procedures of Rouser *et al.*[20,21] and McClare[22] were used in a slightly modified way. The spots, detected with iodine vapor, were encircled with a fine dissecting needle. Subsequently most of the iodine was allowed to evaporate. The phospholipid-containing spots and blank areas corresponding in size to these spots were transferred into centrifuge tubes and digested with 0.25 m*l* 70% perchloric acid at 170–180°C for 2 h. After cooling 4.75 m*l* of a reagent containing 20 m*l* perchloric acid (70%), 2.5 g ammonium molybdate and 0.5 g ascorbic acid per 250 m*l*, were added. After 1 h at 50°C the tubes were centrifuged and the extinction at 660 nm was measured.

Analysis of ^{32}P-labeled phospholipids. Phospholipid spots were detected with iodine vapour and outlined. Most of the iodine was allowed to evaporate. In order to avoid quenching effects, the plates were treated with SO_2 to reduce the remaining iodine. The scrapings were transferred into scintillation counting flasks and suspended in 15 m*l* scintillation mixture (6 g butyl-PBD in 1 *l* toluene/ethoxyethanol 3:2, by vol.) containing 4% Cab-O-Sil (Thixotropic Gel Powder, Packard Instruments Co., Frankfurt). Radioactivity was measured in a Packard Tricarb scintillation counter (92% yield).

Results

Characteristics of outer and inner mitochondrial membranes

The purity of the mitochondrial membrane preparations, obtained by a combined technique of swelling-shrinking-sonication[3] was checked by determining marker enzyme activities and cytochrome contents. Kynurenine 3-monooxygenase was chosen as a marker for the outer membrane[23], succinate cytochrome *c* reductase and cytochromes *aa*$_3$ and b as markers for the inner membrane[3]. Table 1 gives the data of a typical experiment. In outer membrane preparations, inner membranes make up 9–20% of total protein, while in inner membrane preparations, outer membranes make up 2–5% of total protein. With these data, the lipid contents of "pure outer membranes" and "pure inner membranes" were calculated.

Table 1. Marker enzyme activities and cytochrome contents of outer and inner mitochondrial membrane preparations from *Neurospora crassa*.

Kynurenine 3-monooxygenase activity is expressed as (nmol hydroxykynurenine produced) × min^{-1} × (mg prot.)$^{-1}$, succinate cytochrome *c* reductase activity as (nmol cytochrome *c* reduced) × min^{-1} × (mg prot.)$^{-1}$; cytochrome contents are expressed as nmol × (mg prot.)$^{-1}$.

Enzyme activities and cytochrome contents are related to insoluble membrane protein.

	Outer membrane preparation (fraction 1)	Inner membrane preparation (fraction 9)
Kynurenine 3-monooxygenase	25.4	0.8
Succinate cytochrome *c* reductase	85	885
Cytochrome *aa*$_3$	0.05	0.31
Cytochrome *b*	0.23	1.15

Outer membrane preparations display an interesting behavior. Crystals are formed in pellets or concentrated suspensions of outer membrane if they are allowed to stand at 0° or 22°C. These crystals are ergosterol as the following properties show: 1) If the membrane is solubilized in sodium dodecylsulfate the crystals remain. Further washing yields a pure crystal fraction; 2) The crystals are soluble in chloroform and hexane; 3) The melting point of the crystals agrees with that of pure ergosterol (163°C); 4) UV spectra of solutions of the crystals in hexane are identical with spectra of authentic ergosterol; 5) Using the Liebermann-Burchard reaction[24], the crystals and ergosterol give a colour with identical spectral characteristics.

Electron micrographs were made of outer membrane preparations before and after ergosterol crystals were formed. Fig. 1a shows the original preparation. It consists of small vesicles of rather uniform size (average contour length ca. 0.4 μm). After standing for 5 h at 20°C large membrane systems are observed (Fig. 1b). The contour length of these membranes is up to 5–10 μm or even more. Simultaneously, in phase contrast pictures, long needles of ergosterol can be seen (Fig. 2).

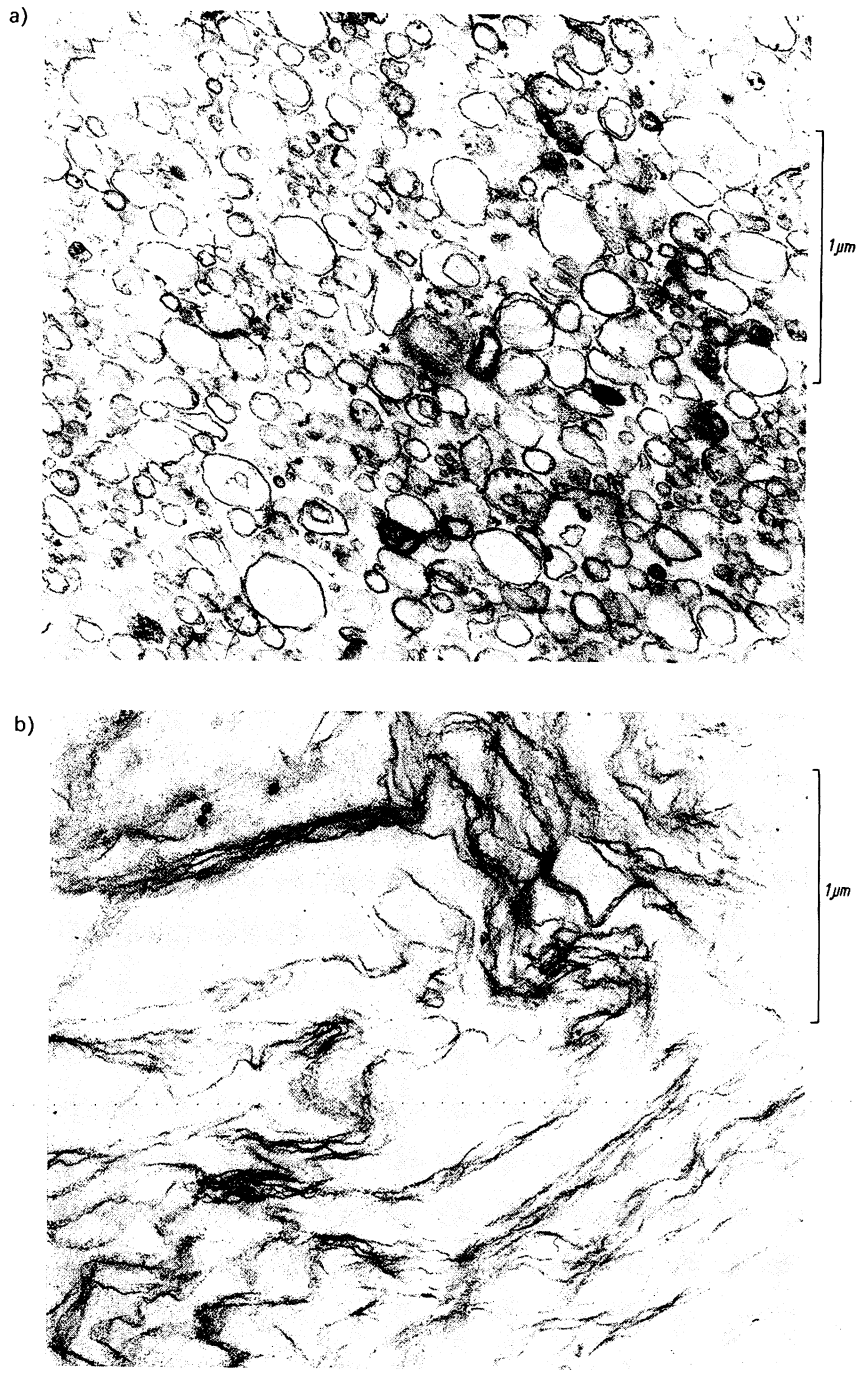

Fig. 1. Electron micrograph of an outer mitochondrial membrane preparation (× 33600).
a) Original preparation
b) after ageing for 5 h at 20°C in 10mM Tris-HCl, pH 7.2.

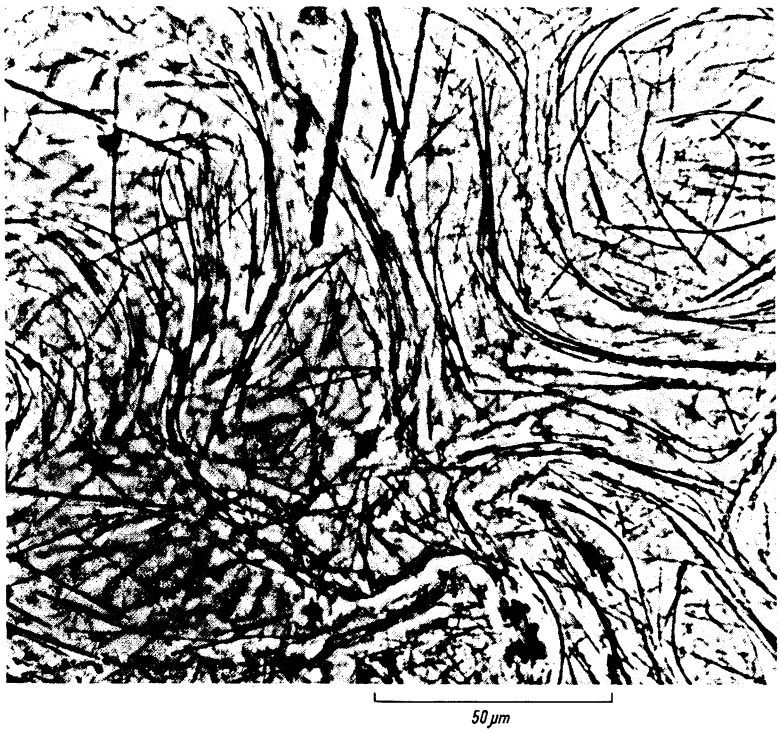

Fig. 2. Phase contrast picture of an outer mitochondrial membrane preparation after ageing for 5 h at 20°C (× 630).

Lipid composition of mitochondria and mitochondrial membranes

The main lipid components of *Neurospora* mitochondria appear to be ergosterol and phospholipids. Small amounts of an acidic red carotenoid pigment and of glycolipids are also found. Data from quantitative analyses are shown in Table 2. The lipid contents of outer and inner membrane preparations, as well as the values for "pure outer membrane" and "pure inner membrane" are also presented in Table 2. It is obvious from these data that ergosterol and the carotenoid pigment are located exclusively in the outer mitochondrial membrane.

The ergosterol content of the outer membrane is quite high. It ranges between 200 and 300 µg per mg protein. Ergosterol comprises some 15–20% of total outer membrane lipid.

The carotenoid pigment is neurosporaxanthin as judged from its acidic solubility characteristics (see Experimental) and its absorption spectrum (maximum at 477 nm and shoulders at 450 and 510 nm). Neurosporaxanthin has been found to be the main acidic carotenoid in *Neurospora*[10,11]. Spectral analysis showed that neurosporaxanthin is the only carotenoid present in mitochondria. On the other hand, some 60–80% of the total cellular neurosporaxanthin is recovered in the mitochondrial fraction. Since the outer membrane is partly lost during the isolation of mitochondria, it can be concluded that the vast majority of the cellular neurosporaxanthin is located in the outer mitochondrial membrane.

The phospholipid contents listed in Table 2 indicate another difference of outer and inner mitochondrial membranes. On an average, "pure outer membrane" contains 1.2 mg total phospholipid per mg insoluble membrane protein, whereas "pure inner membrane" contains 0.28 mg total phospholipid per mg insoluble membrane protein. Thus, the lipid to protein ratio appears to be five times higher in the outer membrane, if compared to the inner

Table 2. Lipid contents of mitochondria and outer and inner mitochondrial membranes of *Neurospora crassa*.
The lipid contents of "pure outer membrane" and "pure inner membrane" were calculated taking into account the mutual contamination of outer and inner membranes. All concentrations are related to insoluble membrane protein.
Molecular weights: ergosterol, 397; neurosporaxanthin, 498; phospholipids (average molecular weight) 775.

	Mitochondria	Outer membrane prepar. (fraction 1)	Inner membrane prepar. (fraction 9)	Pure outer membrane	Pure inner membrane
Ergosterol (μg/mg prot.)	15	192	5	214	0
Neurosporaxanthin (μg/mg prot.)	0.11	2.28	0.05	2.40	0
Total phospholipid phosphorus (μg P/mg prot.)	13.1	44.0	12.4	47.4	11.2
Total phospholipid (μg/mg prot.)	328	1100	310	1185	280
Molar ratio ergosterol/phospholipid	1:11.2	1:2.9	1:32	1:2.8	–
Molar ratio neurosporaxanthin/ergosterol/phospholipid	1:171:1916	1:106:310	1:125:4000	1:112:317	–

membrane. This difference is expressed in the densities of the two membranes which were estimated to be 1.07 – 1.10 (outer membrane) and 1.22 – 1.25 (inner membrane) by isopycnic centrifugation.

Phospholipid compositions of mitochondrial membranes

Phospholipids were separated by two-dimensional thin-layer chromatography. Fig. 3 shows a diagram of a thin-layer plate on which inner membrane lipids were separated. The relative proportions of the individual phospholipids were determined by phosphorus analysis. For this purpose, cells were homogeneously labeled with [^{32}P]orthophosphate. After chromatography, the radioactivity in the individual spots was measured.
The reliability of this rapid method was controlled by colorimetric phosphorus determination. In Table 3 the results of both methods are compared. Since the colorimetric assay has a relatively low sensitivity, only phospholipids which comprise more than 2% of total phospholipid are listed in this table. Obviously, the results obtained with the two methods agree very well.
Table 4 gives the complete phospholipid analysis of outer and inner mitochondrial membranes from a representative experiment. For comparison, the table includes the phospholipid compositions of whole cells, mitochondria and post-mitochondrial fraction ("78000 × *g* pellet").

Table 3. Phospholipid composition of *Neurospora* mitochondria. Comparison of colorimetric assay and radioactivity assay of phospholipid phosphorus.
Values are expressed as percentage of total phospholipid phosphorus. Since the colorimetric assay is less sensitive than the radioactivity assay only phospholipids are included which make up more than 2% of total.
Abbreviations refer to key given in Fig. 3.

	Colorimetric assay	Radioactivity assay
PC	37.5 ± 1.6	36.6 ± 0.7
PE	39.8 ± 1.2	42.6 ± 0.2
DPG	5.9 ± 0.7	5.7 ± 0.4
PI	7.8 ± 0.1	7.7 ± 0.3
PS	4.3 ± 0.4	3.5 ± 0.1
LPC	4.9 ± 0.3	4.1 ± 0.3

The main phospholipid components of mitochondrial membranes are phosphatidylcholine (lecithin), phosphatidylethanolamine (colamine cephalin), diphosphatidylglycerol (cardiolipin) and phosphatidylinositol. Phosphatidylcholine and phosphatidylethanolamine together make up ca. 70% of total phospholipid in the outer membrane, and ca. 54% in the inner membrane. In both membranes these components occur in nearly equal molar

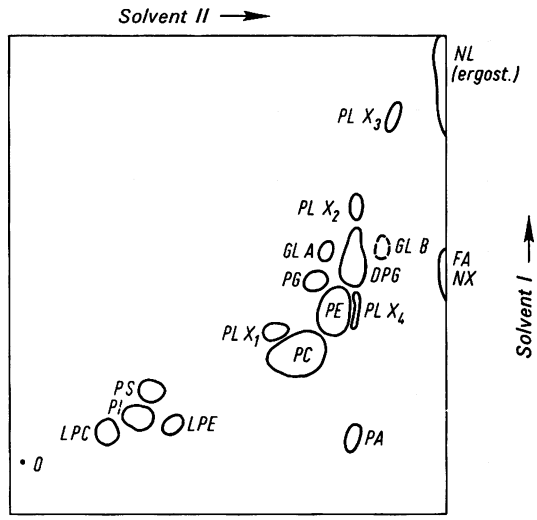

Fig. 3. Two-dimensional thin-layer chromatogram of the total lipid extract from a preparation of inner mitochondrial membrane of *Neurospora crassa*.

The spot encircled with a dotted line is not present in chromatograms of mitochondrial lipids. It is only seen in extracts from whole cells and from the "78000 × *g* pellet".

Abbreviations: O, origin; PA, phosphatidic acid; PC, phosphatidylcholine (lecithin); PE, phosphatidylethanolamine (colamine cephalin); PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine (serine cephalin); DPG, diphosphatidylglycerol (cardiolipin); LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PL X_{1-4}, unidentified phosphorus-containing lipids; GL A and GL B, glycolipids; NL, neutral lipids; FA, fatty acids; NX, neurosporaxanthin.

Solvent I: chloroform/acetone/methanol/acetic acid/water 56:20:10:10:4; solvent II: chloroform/methanol/25% ammonia 65:35:5.

proportions. On the other hand, diphosphatidylglycerol is almost exclusively found in the inner membrane. The relative concentration of phosphatidylinositol is somewhat higher in the outer than the inner membrane. Phosphatidylserine is a minor component which is present in both membranes to a similar degree.

Further minor components are the following phosphatides: phosphatidic acid, phosphatidylglycerol, lysophosphatidylcholine and lysophosphatidylethanolamine. In the outer membrane phosphatidic acid, lysophosphatidylcholine and lysophosphatidylethanolamine are present to about 4% each. In the inner membrane only lysophosphatidyl-

Table 4. Phospholipid composition of mitochondrial outer and inner membranes, mitochondria, post-mitochondrial fraction ("78000 × *g* pellet") and whole cells of *Neurospora crassa*.
Values are expressed as percentage of total phospholipid phosphorus.
Abbreviations refer to key given in Fig. 3.

	Whole cells	"78000 × *g* pellet"	Mitochondria	Outer membrane preparation (fraction 1)	Inner membrane preparation (fraction 9)	Pure outer membrane	Pure inner membrane
PC	36.8	24.8	33.9	35.4	28.3	37.2	28.1
PE	33.8	32.0	39.4	31.7	26.1	33.2	25.9
DPG	4.5	1.9	5.3	5.5	22.0	1.2	22.6
PI	6.2	17.1	7.1	7.4	6.0	7.8	5.9
PS	3.3	0.9	3.2	4.7	4.1	4.9	4.1
PA	3.7	2.4	0.7	3.4	1.1	4.0	1.0
PG	0.7	1.5	1.2	0.6	1.5	0.4	1.5
LPC	4.1	8.6	3.8	4.2	4.3	4.2	4.3
LPE	2.1	3.1	1.1	3.1	0.1	3.9	0.0
PL X_1	0.7	1.1	1.8	2.2	5.4	1.4	5.5
PL X_2	2.5	1.5	1.8	0.9	0.5	1.0	0.5
PL X_3	1.6	5.1	0.7	0.4	0.6	0.4	0.6

choline is found in this concentration. It may well be that these components are decomposition products of the major phospholipids. So it has been reported that during extraction diphosphatidylglycerol may undergo a rapid degradation which leads to the appearance of additional spots on thin-layer chromatograms[25].

Four phosphorus-containing spots were detected which have not yet been identified. They are designated PL X_1, PL X_2, PL X_3 and PL X_4 (see Fig. 3). Incomplete resolution was obtained between phosphatidylethanolamine and PL X_4. However, PL X_4 is a minor component compared to phosphatidylethanolamine. Therefore, the amount of phosphatidylethanolamine is not influenced in a noticeable way when determined together with PL X_4. Consequently, PL X_4 is not specified in Table 4.

Sphingomyelin was neither detected in mitochondrial membranes nor in whole cells of *Neurospora crassa*.

After incorporation of ^{3}H-labeled inositol into *Neurospora* cells, radioactivity was detected in phosphatidylinositol and in no other spot. Hence, the mitochondrial membranes of *Neurospora* contain no other inositol-containing lipids, and in particular no phosphorylated derivatives of phosphatidylinositol.

Among the polar lipids, two glycolipids were detected on chromatograms of extracts of *Neurospora* cells (GL A and GL B in Fig. 3). They yield a positive reaction with orcinol and diphenylamine.

Glycolipid A was found in extracts of mitochondria and inner mitochondrial membranes in very low amounts (less than 2% of total phospholipids, roughly estimated by the color developed with iodine). The outer membrane preparation clearly contained more. Quite remarkable quantities of this glycolipid were found in the post-mitochondrial fraction ("78000 × *g* pellet"). So it is highly probable that the presence of glycolipid A in the outer mitochondrial membrane preparation is caused by a contamination of mitochondria with the post-mitochondrial fraction.

Glycolipid B could not be detected in mitochondria, but it was found in small amounts in the "78000 × *g* pellet". This fraction also contains a remarkable amount of phosphatidylinositol (17%). Since glycolipids and phosphatidylinositol are described as characteristic components of the cell membrane[26], the postmitochondrial fraction may at least partly consist of fragments of the cell membrane.

Discussion

The data presented in this report clearly show that outer and inner membranes of mitochondria from *Neurospora* are different with respect to their lipid contents and their lipid composition.

The outer membrane is rich in lipid compared to the inner membrane. This is shown by the lipid-protein ratios of 1.4 − 1.5 in the outer membranes, and 0.28 in the inner membrane. With a lipid content of some 59%, the outer membrane of *Neurospora* mitochondria is one of the most lipid-rich membranes described in the literature (for review see[27]). The inner mitochondrial membrane in contrast has a relatively low lipid content of 23%.

It is interesting to note that also in the case of mammalian liver mitochondria the outer membrane has a higher lipid content than the inner membrane[1,2,4–8,28]. However, in liver the difference between the two membranes is not so large. The lipid contents of the inner mitochondrial membranes of *Neurospora* and liver are quite similar (24% according to Parsons and Yano[5] for guinea pig liver, and 25.4% for rat liver according to Levy *et al.*[28]. Outer membranes of mitochondria from guinea pig liver have been reported to contain 48% lipid[5], whereas for rat liver a value of 39% can be calculated from the data available in the literature[28].

The individual lipid components of mitochondrial membranes of *Neurospora* can be subdivided into two groups. Lipids of the first group occur in both outer and inner membranes to a similar extent. The other group consists of lipids which are found exclusively or predominantly in one of the two membranes. To the first group belong: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine. The second group includes ergosterol and neurosporaxanthin, which are only detected in the outer membrane, and diphosphatidylglycerol (cardiolipin) which is found highly concentrated in the inner membrane.

Similar aspects can be pointed out for the composition of liver mitochondrial membranes. In mitochondria of liver as well as of *Neurospora* the two main phospholipid components are phosphatidylcholine and phosphatidylethanolamine. Together they make up some 60 − 80% of the total phospholipid in outer as well as in inner membranes. However, the ratios of phosphatidylcholine to phosphatidylethanolamine in outer and inner membranes of liver are much higher than those in

Neurospora mitochondrial membranes. Furthermore, in liver the relative content of phosphatidylinositol is about 2–4 times higher in the outer membrane than in the inner membrane [4, 6, 8]. Phosphatidylserine is only detected in traces in liver mitochondrial membranes, whereas in *Neurospora* mitochondrial membranes it is present in an appreciable amount (ca. 5%).

One of the most interesting aspects concerning the phospholipid composition of mitochondrial membranes is the high concentration of cardiolipin in the inner mitochondrial membrane, as first shown by Parsons *et al.*[4] with guinea pig liver. Corresponding reports were made for rat liver by Stoffel and Schiefer[6], Levy and Sauner[7] and McMurray and Dawson[8]. In this paper it is shown, that also in *Neurospora*, cardiolipin appears to be a specific component of the inner mitochondrial membrane. Moreover, the relative proportions of cardiolipin are nearly equal in the different organisms.

These observations suggest that there are some invariable rules concerning the presence and concentrations of certain lipid components in mitochondrial membranes, even in phylogenetically distant organisms. On the other hand, some lipid components seem to be quite variable.

Similar considerations may be made regarding the presence of ergosterol in the mitochondrial membranes of *Neurospora*. This sterol is found in an unusually high concentration in the outer membrane. Parsons and Yano[5] have shown that cholesterol is concentrated in the outer membrane of guinea pig liver mitochondria, though the concentration (30.1 ± 12.8 μg/mg prot.) is far below that of ergosterol in *Neurospora* outer mitochondrial membrane. Correspondingly, the molar ratio of cholesterol to phospholipid in liver is much lower than the molar ratio of ergosterol to phospholipid in *Neurospora*. Parsons and Yano have reported values of 1:11–1:29. For rat liver outer mitochondrial membrane Levy *et al.*[28] have found molar ratios of 1:9–1:14. In *Neurospora* outer mitochondrial membrane the molar ratio of ergosterol to phospholipid is as high as 1:2.8.

This high concentration of ergosterol is probably the reason why it is released from the membrane under certain conditions to form needle-like crystals.

The function of these sterols in the outer mitochondrial membrane is not known. It may be speculated that they confer the rigid character to the outer membrane. In contrast to inner membranes outer membranes rupture upon swelling of mitochondria and open vesicles are frequently seen. It seems quite reasonable to assume that ergosterol plays the same role in *Neurospora* mitochondria as cholesterol in liver mitochondria.

A particular property of *Neurospora* outer mitochondrial membrane is the presence of the carotenoid pigment neurosporaxanthin. However, the concentration of this component is rather low. The molar ratio of neurosporaxanthin to phospholipid in the outer membrane is about 1:300. The function of this pigment is not known.

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