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EFFECT OF AGING ON THE COMPOSITION OF MITOCHONDRIAL MEMBRANES FROM POTATO SLICES

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato tuber; mitochondria; phospholipids; fatty acids; polypeptides; aging.

Abstract—The phospholipid content of mitochondrial membranes from slices of potato tuber (*Solanum tuberosum*) remains stable during aging. The phospholipid compositions of whole mitochondria and inner membranes do not vary during aging whereas the concentrations of phosphatidylinositol and phosphatidylglycerol in outer membranes are slightly amplified. The saturation of outer membrane fatty acids is slightly increased during aging. Gel electrophoresis of mitochondrial membrane proteins show slight variations of one polypeptide in outer membranes and of three polypeptides in inner membranes. These results suggest parallel variations of lipids and proteins in membranes during aging, in marked contrast with the large modifications observed in mitochondrial activities.

INTRODUCTION

During the aging of potato tuber slices, the development of a wound-induced respiration has been long known to be linked to an increase in nitrogen content and respiratory activity of the mitochondrial fraction [1–5]. This phenomenon is dependent upon an active synthesis of proteins and phospholipids observed at the tissue [6–10] as well as at the mitochondrial [2, 11, 12] levels. Thus, the changes observed in mitochondrial properties during aging could be the result either of a multiplication of mitochondria having new properties [13–15] or of synthesis of new lipoprotein components within preexisting mitochondria [2, 9, 16].

To see if these newly synthesized components are easily detectable, a careful comparison of the phospholipid and polypeptide compositions of mitochondrial membranes from aged and fresh potato slices was carried out. However, only slight variations were observed which could not be directly correlated with the important changes occurring in mitochondrial activities.

RESULTS

Phospholipid composition of mitochondrial membranes

The phospholipid contents of whole mitochondria and inner membranes (+matrix) are essentially the same (Table 1). The phospholipid content of outer membranes is higher than that of inner membranes (+matrix), partly due to the presence of matrix proteins in this latter fraction. No significant changes are observed during aging. This indicates that parallel variations in phospholipids and proteins are taking place as potato tissue slices are aging.

The quantitative distribution of the individual phospholipids in whole mitochondria and mitochondrial membranes are shown in Table 2. As the matrix is poor in phospholipids [17], the phospholipid content of inner membranes (+matrix) can be assimilated to that of pure inner membranes. The prevalence of two major phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) is observed. Whereas these two phospholipids are in equal proportions in inner membranes, the PC content is higher than that of PE in outer membranes, in accordance with other data [18, 19]. Diphosphatidylglycerol (DPG) is present in inner membranes whereas higher proportions of phosphatidylinositol (PI) and, to a minor degree, of phosphatidylglycerol (PG) are found in outer membranes. Aging brings about no change in the phospholipid composition of whole mitochondria and inner

Table 1. Phospholipid content * of mitochondrial membranes isolated from fresh and aged potato tuber slices

Membrane fraction	Tissue	
	Fresh	Aged
Mitochondria	150	135
Inner membranes (+matrix)	160	150
Outer membranes	280	300

*Expressed in μg phospholipid/mg protein.

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Table 2. Phospholipid composition of mitochondrial membranes isolated from fresh and aged potato tuber slices

Tissue	Membrane fraction	Per cent of total phospholipids				
		PC	PE	DPG	PI	PG
Fresh	Mitochondria	37	35	11	11	6
	Inner membranes (+matrix)	40	39	11	7	3
	Outer membranes	36	27	5	19	13
Aged	Mitochondria	37	36	12	10	5
	Inner membranes (+matrix)	40	38	12	7	3
	Outer membranes	33	26	5	21	15

mitochondrial membranes (Table 2). Only a slight increase in PI and PG contents can be observed in outer membranes.

The distribution of fatty acids in phospholipids reveals that linoleic acid is the major fatty acid present in whole mitochondria and membrane fractions (Table 3). Outer membranes appear to have a higher content of saturated fatty acids than inner membranes, in good agreement with previous results [18, 20]. No variation in the fatty acid distribution in inner membranes occurs during aging while a tendency to a higher degree of saturation is observed in fatty acids of outer membranes (Table 3). This fact can be explained by the analysis of the fatty acid composition of the various phospholipids (not presented here) which shows that PI has the highest content in saturated fatty acids and that fatty acids from PG become more saturated as aging proceeds.

Therefore, during aging, it appears that all types of phospholipids are equally synthesized in inner membranes but more saturated phospholipids are preferentially found in outer membranes.

Polypeptide composition of mitochondrial membranes

To follow the effects of aging on the polypeptide composition, Table 4 shows the analysis of polypeptides obtained on electrophoregrams of whole mitochondria from fresh and aged slices and of submitochondrial fractions derived from them. Several groups of mitochondrial polypeptides can be distinguished according to their MWs (k = 1000): 105 k, 55 k, 48 k, 44 k, 39 k, 34 k, 28 k, 25 k, 22 k and below 22 k.

In whole mitochondria from fresh tissues, the major polypeptides are found at 55, 44, 34 and 28 k. The inner membranes (+matrix) fraction is similar to whole mitochondria but shows a slight decrease in the 22 k group. This polypeptide characterizes outer membranes. The polypeptide groups around 105, 55, 34 and 28 k appear to be consistently present in all fractions studied. But, whereas the 105, 34 and 28 k groups show some relative variations in their percentages, it is remarkable that the 55 k polypeptide group is always prominent. The groups 44 k and 25 k are mostly represented in the matrix fraction but are almost absent in inner membranes (devoid of matrix) and in outer membranes.

Aging does not modify the polypeptide compositions of whole mitochondria and inner membranes (+matrix). By contrast, a marked decrease in polypeptides of low MW (below 22 k) can be observed in pure inner membranes after aging. This group of polypeptides increases in the matrix fraction. Moreover, a lower content in the 34 and 25 k groups is also observed in the matrix of aged tissues. There is also a slight increase of the groups 22, 25 and 44 k in inner membranes after aging. In outer mitochondrial membranes, a great increase of the 25 k group, a slight increase in the 22 k peak and a decrease in the polypeptides below 22 k can be observed. Finally, the stability of the major group at 55 k in all fractions must be noted.

DISCUSSION

Neither the phospholipid composition nor the fatty acid distribution of inner mitochondrial membranes vary significantly during aging. However, a weak tendency to a higher degree of saturation of fatty acids in outer mitochondrial membranes is observed.

Table 3. Fatty acid composition of mitochondrial membranes isolated from fresh and aged potato tuber slices

Tissue	Membrane fraction	Per cent of total fatty acids				
		C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Fresh	Mitochondria	23	5	2	56	14
	Inner membranes (+matrix)	21	4	1	59	15
	Outer membranes	34	8	6	43	9
Aged	Mitochondria	24	5	3	55	13
	Inner membranes (+matrix)	22	4	2	58	14
	Outer membranes	37	9	5	40	9

Table 4. Polypeptide composition* of mitochondrial membranes isolated from fresh and aged potato tuber slices.

Tissue	Membrane fraction	Molecular weights (k)										
		<22	22	25	28	34	39	44	48	55	60–85	105
Fresh	Mitochondria	11	6	6	13	13	3	15	4	18	5	6
	Inner membranes (+matrix)	10	4	7	13	13	4	14	4	19	6	6
	Inner membranes	22	4	2	19	16	2	3	2	20	5	5
	Matrix	6	4	10	7	10	5	18	5	21	6	8
	Outer membranes	14	11	2	14	15	4	4	5	20	5	6
Aged	Mitochondria	8	7	7	15	12	3	17	4	17	5	5
	Inner membranes (+matrix)	12	7	7	15	11	3	14	3	18	5	5
	Inner membranes	7	11	10	17	12	2	11	2	19	4	5
	Matrix	17	3	6	9	4	4	18	5	22	6	6
	Outer membranes	7	14	11	17	11	3	4	4	19	5	5

*Expressed in per cent of total polypeptides.

As for the polypeptide composition, the distributions of the polypeptide groups shown by whole mitochondria and the inner membranes (+matrix) do not change significantly after aging, as already observed on sweet potato roots [21]. However, there are some slight increases during aging, concerning the 25 k polypeptide in both outer and pure inner mitochondrial membranes and the 22 and 44 k groups in inner mitochondrial membranes.

It is known that the development of a wound-induced respiration during aging of potato slices is characterized both by an increase in respiratory activity and by the appearance of cyanide resistance [22]. These phenomena are reflected at the mitochondrial level by the onset of a cyanide-resistant electron transport pathway and the increment in the oxidation rate of exogenous NADH [1, 4, 12]. Moreover, the expression of these new properties is dependent upon a synthesis of phospholipids and proteins [2, 5, 9, 10, 12, 16].

However, it appears rather difficult to establish a clear relationship between the dramatic changes in mitochondrial properties and the production of specific phospholipid or protein species, since only slight variations in membranes compositions are observed during aging. This can be due to the fact that comparisons are made on membranes possessing the same set of oxidases. In particular, the alternative electron transport pathway responsible for cyanide resistance is present, although in a latent form, in inner membranes from fresh slices [4]. All these results suggest a biogenesis of mitochondrial membranes similar to the original ones rather than a biogenesis of a new type of mitochondria.

Finally, even if cytoplasmically-made proteins and phospholipids do not induce striking changes at the level of membrane composition, they must be obviously implied in the development of new mitochondrial properties. The proteins could participate in changes in the configuration of the membranes perhaps in connection with boundary lipids, with the expression of cyanide resistance as a result. As for the nature of the particular cyanide-resistant alternative oxidase, it will remain unknown until its selective solubilization from pure inner membranes is achieved.

EXPERIMENTAL

Isolation of mitochondrial fractions. Potato tubers (*Solanum tuberosum* L., cv Bintje) were kindly supplied by Dr. Grison (Institut Technique de la Pomme de terre, 78690 Saint-Rémy l'Honoré, France). Aging of slices as well as the isolation of purified mitochondria was performed as previously described [4, 12]. Outer mitochondrial membranes and inner membranes (+matrix) were prepared according to ref. [23]. The matrix compartment was separated from inner membranes by sonication followed by centrifugation [17]. Protein determinations were made according to ref. [24], using BSA as standard.

Lipid analysis. Lipids were extracted according to ref. [25]. Fractionation of total phospholipids was carried out by TLC using a mixture of $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (5:2:1:1:0.5) according to ref. [26]. Individual phospholipids were identified by exposure to I_2 vapours, using pure phospholipid samples as reference standards. Fatty acids released from individual phospholipids by methanolysis [27]. Me esters mixed with known amounts of Meheptadecanoate as a standard were extracted by petrol and analysed by GLC. Phospholipids were quantitatively estimated assuming their fatty acid content accounts for 70% of the total phospholipid mass. Phospholipid composition and fatty acid distributions were determined from 5 independent expts.

Polyacrylamide gel electrophoresis. Membrane fractions were incubated for 3 min at 100° in a soln containing 125 mM Tris (pH 6.8), 20% glycerol, 10% 2-mercaptoethanol, 4% SDS and 0.01% bromophenol blue. The ratio SDS/protein was generally equal to 4. The electrophoresis of the solubilized material (50 to 100 μg of proteins) was performed with cylindrical polyacrylamide gels (7 cm length, 6 mm int. dia). The gels, containing 0.1% SDS were polymerized according to ref. [28]. The length of the concn gel (upper gel, 3% acrylamide) was 5% of the total length of the gel. The concn of acrylamide in the separating gel (lower gel) was 7.5 or 10%. The current intensity during electrophoresis was 4 m amp. Staining with Coomassie blue and destaining were performed as indicated in ref. [29]. The coloured gels were read at 580 nm with an ISCO gel scanner. A calibration curve was established from the electrophoresis of proteins of known MW: subunit of RNA polymerase (160 k), BSA (68 k), ovalbumin (45 k), chymotrypsinogen (25 k), myoglobin (17.8 k) and cytochrome *c* (12.4 k). The relationship between log MW and mobility was linear between 160

and 25 k with an acrylamide concn of 7.5% and between 100 and 17.8 k with a concn of 10%. The apparent MW of each polypeptide peak was calculated from the calibration curve. The % age of different polypeptide groups was estimated by triangulation of each individual peak on the electrophoretograms. Polypeptide compositions were obtained from 4 independent expts.

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