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Inadequacy of Myelin Phospholipids for Restoration of Succinoxidase Activity in Lipid-depleted Mitochondria

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Abstract

Succinoxidase activity in lipid-depleted mitochondria was not restored efficiently by mixed myelin phospholipids at difference with the natural mitochondrial phospholipids, yeast phospholipids, and Asolectin. Since similar differences in activity were present between pure phosphatidyl-ethanolamine fractions separated from myelin phospholipids and Asolectin, they should be due to the different fatty acid composition of the phospholipids. In contrast with the different ability in restoration of succinoxidase, all the phospholipids studied were bound to the lipid-depleted membranes to similar extents.

Introduction

Previous investigations in our laboratory have indicated that the bonds between phospholipids and proteins in the inner membrane of beef heart mitochondria are largely hydrophobic in nature;[1–4] phospholipid and protein must be therefore strictly interdigitated, in other words the membrane proteins must be deeply buried in the lipid bilayer. Theoretical considerations allow to postulate that the types of phospholipids present might affect the possibility and the extent of hydrophobic interactions with the proteins in the membrane: in particular the fatty acid nonpolar residues of the phospholipids should be more exposed to the protein than their polar part and thus affect the interactions more specifically, whereas the hydrophilic moiety of the lipid molecules should be less specific in this respect. O'Brien suggests that saturated lipids, like those prevailing in the myelin sheath[5] form thick stable bilayers, whereas double bonds confer an increased looseness to the bilayer;[6] by this way the possibility advanced by Benson[7] and Green and Tzagoloff[8] that proteins are included hydrophobically into the bilayer, appears highly plausible for membrane containing unsaturated fatty acids in large amounts.[9]

If these assumptions are correct, significant differences could be attained in the association to lipid-depleted mitochondria of phospholipids having different fatty acid composition. Besides unsaturation, also the chain length of the fatty acids may be meaningful in this respect. De Pury and Collins have found that the binding of lecithins from EFA-deficient rats (less unsaturated) and from normal rats to lipid-depleted mitochondria

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follow different kinetics;[10] the rate constants of the binding were much higher for the "deficient" lecithins containing large amounts of 5,8,11-eicosatrienoic acid than for the "normal" lecithins containing large amounts of arachidonic acid. This difference might reflect different stability of the membranous lipoproteins.

Certain enzymes require phospholipids for activity (cf. ref. 11): the electron transfer chain in mitochondria has a phospholipid requirement;[8, 12, 13] the role of phospholipids has been considered essentially structural;[8] several phospholipids were found effective in restoring succinoxidase activity in acetone-treated mitochondria;[12] in the experiments cited above, De Pury and Collins[14] have found that lecithins from normal and EFA-deficient rats have the same efficacy in restoring succinate-cytochrome *c* reductase activity.

In this communication we report that myelin phospholipids, which have a fatty acid composition far different from that of the mitochondrial phospholipids,[5] have little effect in restoring succinoxidase activity in lipid-depleted mitochondria (LDM), although they are bound to an almost normal extent.

Methods

Beef heart mitochondria were prepared according to Smith.[15] The yeast *Saccharomyces cerevisiae* was disrupted mechanically according to Schatz[16] and the membranous faction of the homogenate was collected by centrifugation at 105,000 × *g*. Bovine myelin was prepared according to Autilio *et al.*[17] Lipids were extracted by these different fractions with the method of Folch *et al.*;[18] phospholipids were separated according to Marks *et al.*[19] and micelles were obtained by sonication from these phospholipids and from Asolectin (Commercial soybean phospholipids) with the method of Fleischer and Fleischer.[20] Aliquots of the phospholipid dispersions were dried *in vacuo* and methyl esters were obtained by heating in methanolic HCl; gas-liquid chromatography was performed as previously described.[21]

DEAE-cellulose chromatography of the phospholipids was accomplished according to Rouser *et al.*[22] and the fractions were checked for purity by chromatography on silicic acid-impregnated paper.[23]

Lipid-depleted mitochondria were prepared as described previously.[3] Succinoxidase activity was measured by conventional manometry as described by Lester and Fleischer.[24] Phosphorus was assayed according to Marinetti[25] and protein with a biuret method.[26]

Results and Discussion

In Fig. 1 are reported the most significant parameters of the fatty acid composition of the phospholipid micelles used in this study: in comparison with mitochondrial phospholipids and Asolectin (commercial soybean phospholipids) and even of yeast phospholipids, myelin phospholipids have a greater amount of saturated fatty acids, lower total unsaturation, and a very large quantity of fatty acids having more than 20 carbon atoms.

Figure 2 shows that Asolectin, yeast phospholipids, and mitochondrial phospholipids appear almost equivalent in restoring succinoxidase activity, but myelin phospholipids have scarce efficacy even at high concentrations. Is this difference in relation with the quantity of phospholipids bound to the membrane?

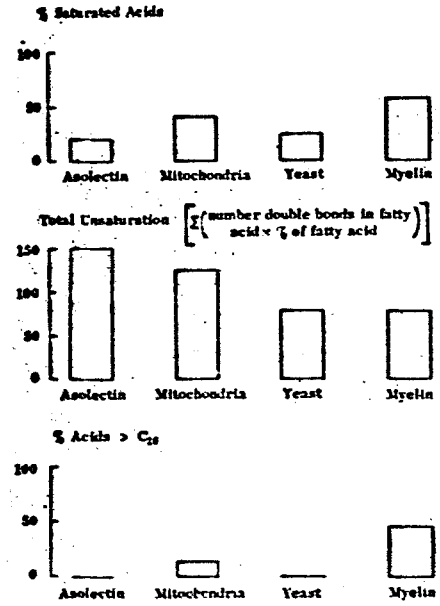

Figure 1. Fatty acid composition of phospholipid micelles of different origin.

Figure 3 shows the unexpected finding that the differences in the amount of P bound to the membrane are small, and even myelin phospholipids are reassociated to the delipidated mitochondria in normal amounts. The difference might be due to binding to "non-physiological" sites in the membrane; a possibility deserving to be explored is that myelin phospholipids are not capable to induce a correct conformation required for activity of the proteins of the electron transfer chain.

The differences we have observed have been obtained by comparing the effect of mixed phospholipids having a greatly different composition also in phospholipid classes, so that also differences in the polar moieties are present; for this reason we have separated pure fractions by DEAE-cellulose chromatography according to Rouser *et al.*;[22]

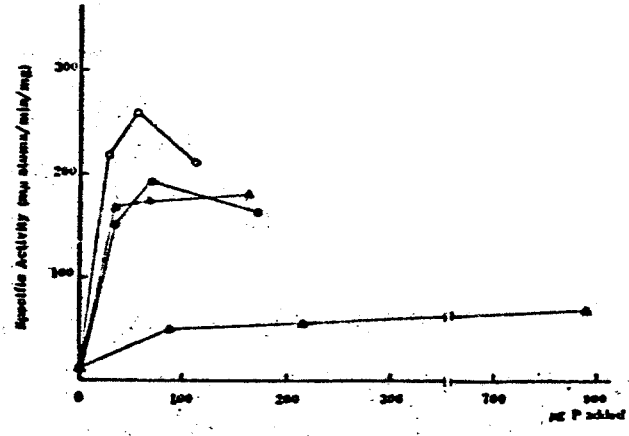

Figure 2. Effect of different phospholipids on succinoxidase activity of LDM. ●—●, Asolectin; ○—○, yeast phospholipids; Δ—Δ, mitochondrial phospholipids; ▲—▲ myelin phospholipids.

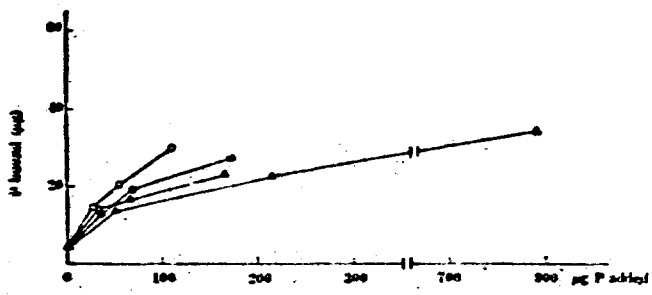

Figure 3. Binding of different phospholipids to LDM. The content of the Warburg flasks in the manometric experiments (Fig. 2) were centrifuged at 20,000 rpm for 10 min, the residue resuspended in sucrose-Tris, and phosphorus was assayed according to Marinetti.[25] ●—●, Asolectin; ○—○, yeast phospholipids; △—△, mitochondrial phospholipids; ▲—▲, myelin phospholipids.

phosphatidyl ethanolamine (PE) fractions separated from Asolectin and from myelin phospholipids keep, although attenuated, the differences in fatty acid composition of the mixed fractions (Fig. 4). Only PE from Asolectin is highly effective in restoring succinoxidase activity whereas the same fraction from myelin has little effect (Table I): deoxycholate (DOC) has been added to clarify the micelles, since PE does not easily form micelles in water.[20] Succinoxidase is greatly enhanced by PE from Asolectin but not by PE from myelin in presence of DOC, in spite of a general effect of the detergent of depressing succinoxidase at the concentration used.

In conclusion we have shown that the mitochondrial electron transfer chain requires phospholipids having a roughly specific type of fatty acid composition; it is known that there is large tolerance for phospholipids of different type;[12] but, when the fatty acid

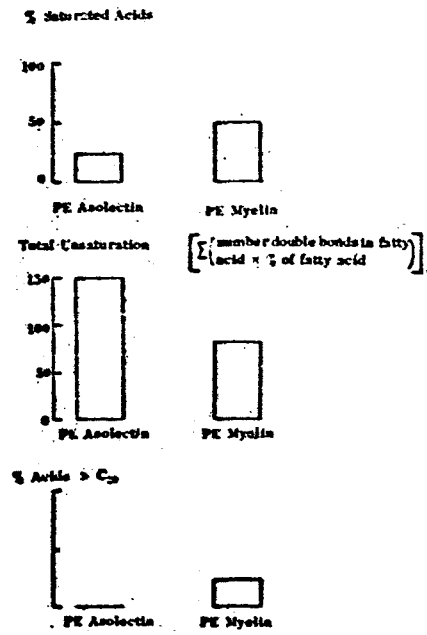

Figure 4. Fatty acid composition of PE micelles from Asolectin and myelin lipids.

TABLE I. Effect of two phosphatidyl ethanolamine fractions on succinoxidase activity in LDM

Phospholipid (17 μg P)	DOC (2 mg)	Specific activity* (mμ atoms O_2/min/mg)	
—	−	25	18
—	+	17	16
Asolectin	−	159	154
Asolectin	+	85	141
PE-Asolectin	−	57	36
PE-Asolectin	+	118	117
PE-Myelin	−	68	25
PE-Myelin	+	60	29

* The two columns represent two different experiments.

composition is far from physiological for mitochondria, like in the case of myelin phospholipids, in our experimental conditions there is not significant activity. Either unsaturation or a particular chain length of the fatty acids or both factors together may be the specific requirements for succinoxidase activity: the total unsaturation is the same in yeast phospholipids and in myelin phospholipids, but the amount of unsaturated fatty acids is higher in yeast and the types of fatty acids and positions of the double bonds are different.

The fact that normal binding of myelin phospholipids is not accompanied by significant activity raises interesting questions on the role of phospholipids in the membrane, and in particular whether specific phospholipids are required to induce specific conformational changes in the enzyme proteins of the respiratory chain.

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