SOLUBILITY OF THE INTRAMITOCHONDRIALLY SYNTHESIZED PROTEIN AND OTHER MEMBRANE PROTEINS OF RAT LIVER MITOCHONDRIA IN ACIDIC OR NEUTRAL CHLOROFORM-METHANOL

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SUMMARY: Almost all protein species of submitochondrial particles from rat liver identified by SDS-polyacrylamide gel electrophoresis were extracted into acidic /2 mM/HCl/ chloroform:methanol /2:1, v/v/, whereas a single protein /or lipoprotein/ with molecular weight of 9.000 was extracted into neutral chloroform-methanol mixture. Evidence for intramitochondrial synthesis of this hydrophobic protein in rat liver *in vivo* is presented.

INTRODUCTION

Importance of the product of the mitochondrial protein synthesis for normal mitochondrial structure and function is well documented /for rev.see 1/. However, numerous attempts to elucidate the nature of this product have been essentially unsuccesful until the recent two years, when a fruitful research on the partcipation of the product of mitochondrial protein synthesis in the formation of cytochrome oxidase and cytochrome *b* has been accomplished /for rev.see 2/. Moreover, a new approach to the characterization of the proteins synthesized in mitochondria raised from the discoveries of Kadenbach /3/ and Tzagoloff and coworkers /4,5/ that at least some of the products of the mitochondrial protein synthesis are soluble in organic solvents. Some controversial data have been obtained on the number and the molecular weight of proteins representing the product of the mitochondrial protein synthesis that could be

extracted into chloroform-methanol. Thus Kadenbach /3/ found that rat liver mitochondria synthesize in vivo a peptide soluble in neutral chloroform-methanol with molecular weight of 2.000. Burke and Beattie /6/ extracted from in vitro labelled rat liver mitochondria by neutral chloroform-methanol a fraction containing several labelled proteins among which those with molecular weight of 40.000 were maximally labelled. A protein with molecular weight of 7.800 was extracted into neutral chloroform-methanol from yeast mitochondria labelled in vivo in the presence of cycloheximide by Tzagoloff et al./5/. All types of intramitochondrially synthesized proteins were extracted from the same source by acidic chloroform-methanol /5/.

This study has been devoted to the further characterization of the product of the mitochondrial protein synthesis. It has been found that almost all protein species of submitochondrial particles could be extracted into acidic chloroform-methanol, whereas a single, intramitochondrially synthesized low molecular weight protein /or lipoprotein/ could be extracted from these particles by neutral chloroform-methanol.

MATERIALS AND METHODS

Wistar rats /150-200 g body weight/ fasted overnight were i.p. injected with cycloheximide /62 mg/kg b.w./, 15 min later with ^{14}C-leucine /7 mC/kg b.w., spec.act. 105 mC/mmole/, after two hours with ^{12}C-leucine /30 mg/kg b.w./ and then sacrificed 40 min after ^{12}C-leucine injection.

Liver mitochondria were isolated /7/, suspended in the preparation medium /20 mg protein/ml /, then frozen at -30°, thawed in ice , 4 times diluted with the preparation medium and centrifuged 20 min at 20.000g. Sediment was suspended in 10 mM Tris-phosphate pH 7.4 to concentration 10 mg protein/ml and

TABLE I

In vivo incorporation of ^{14}C-leucine into mitochondrial fractions in the presence of cycloheximide

Fraction	Spec.activity /cpm.mg^{-1}protein/	Relative spec. activity /%/	Inhibition by chloramphenicol^{+} in %
Mitochondria	3.750	100	21.3
"Soluble"	923	24.6	-36.3
Particles	9.766	260.4	46.1
Acidic chloroform--methanol extract	10.037	267.7	53.5
Neutral chloroform--methanol extract	18.040	481	70.9

$^{+}$labelled in the presence of cycloheximide and chloramphenicol /500 mg chloramphenicol per kg b.w./.

Experimental details are given in Material and methods. For chloroform-methanol extraction 5 mg protein containing aliquots were used.

after standing 30 min at 0° centrifugated 30 min at 105.000g. The supernatant representing the "soluble" mitochondrial fraction was decanted and the sediment reextracted in the same manner. When the material labelled with ^{14}C-leucine was processed, 10 mM ^{12}C-leucine was included into the solutions used. The resulting sediment, in further text reffered to as the mitochondrial particles,was extracted with NaBr and NH_4OH according to Tzagoloff /8/. For chloroform-methanol extraction

untreated or NaBr, NH_4OH extracted mitochondrial particles were dissolved in 0.5 ml 0.01 M sodium phosphate buffer pH 7.2 containing 1% SDS /final concentration of protein specified in further text/ and extracted by 4 ml of methanol 20 min at room temperature. The supernatant was discarded and the sediment, extracted twice with 2 ml of neutral or acidic /2 mM HCl/ chloroform:methanol /2:1, v/v / for 20 min at 50°. The pooled extracts were centrifugated for 15 min at 200.000g at 20° and dried at 50° under the jet of argon. Specific radioactivity of mitochondrial subfractions examined was determined as described elsewhere /7/. SDS-polyacrylamide gel electrophoresis and the determination of molecular weights of proteins was performed according to Weber and Osborn /9/. The gels were either stained with Coomassie brilliant blue /ref. 9/ or sliced and processed for the determination of the distribution of radioactivity as described elsewhere /7/. Proteins were determined according to Lowry et al./10/.

RESULTS AND DISCUSSION

Rat liver mitochondria were labelled with ^{14}C-leucine in the presence of cycloheximide and specific activity of mitochondrial subfractions was determined /Table I/. As revealed by the increase in specific activity and chloramphenicol sensitivity of the incorporation of ^{14}C-leucine, in contrary to the neutral, the acidic chloroform-methanol extract of mitochondrial particles was not significantly enriched by the product/s/ of mitochondrial protein synthesis as compared to the unextracted particles.

It has been found that higher percentage of protein was extracted from the mitochondrial particles by acidic than by neutral chloroform-methanol. Probably due to the buffering

TABLE II

Recovery of protein in chloroform-methanol extracts

Fraction	Amount used for extraction /mg protein/	Extraction with chloroform--methanol	% of the original amount of protein extractable
Particles	14.0	neutral	0.83
		acidic	1.35
Particles	2.2	neutral	0.45
		acidic	12.50
NaBr, NH_4OH extracted particles	2.1	neutral	2.66
		acidic	22.0

Experimental details are given in Material and methods.

ability of proteins the effect of the acidity of chloroform--methanol was much less pronounced when the samples with higher protein concentration were extracted. Significantly higher amount of protein was extractable from NaBr, NH_4OH-treated than from untreated particles by both acidic and neutral chloroform-methanol indicating higher proportion of hydrophobic proteins in this fraction /Table II/.

To elucidate the implicated /see Table I/ presence of large amount of extramitochondrially synthesized proteins in acidic chloroform-methanol extracts and to characterize the hydrophobic product/s/ of mitochondrial protein synthesis electrophoretic patterns of fractions studied were compared. It was found that almost all the protein species identified by SDS-polyacrylamide gel electrophoresis in NaBr, NH_4OH-extracted and/or untreated particles were also present in the acidic chloroform-methanol extracts from these particles /Fig. 1/.

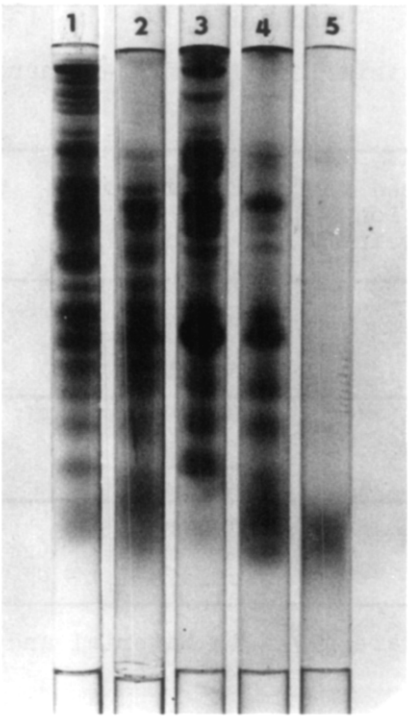

Fig. 1. Electrophoreograms of mitochondrial fractions studied: 1 – mitochondrial particles, 2 - acidic chloroform-methanol extract of mitochondrial particles, 3 - NaBr, NH_4OH treated particles, 4 - acidic chloroform-methanol extract of NaBr, NH_4OH treated particles, 5 - neutral chloroform-methanol extract of mitochondrial particles.

Accordingly, elsewhere described /7/ the complex pattern of the radioactivity distribution in gels after electrophoresis of particles labelled *in vivo* in the presence of cycloheximide was very similar to that obtained with the acidic chloroform--methanol extracts. Extraction of SDS-solubilized mitochondrial particles with acidic chloroform-methanol thus appears to be a useless step in attempts for isolation of the product/s/ of mitochondrial protein synthesis. After electrophoresis of neutral chloroform-methanol extracts from both types of particles single protein with molecular weight of 9.000 was identified by staining /see Fig. 1/ and determining the distribution of radioactivity in gels /see Fig. 2/.

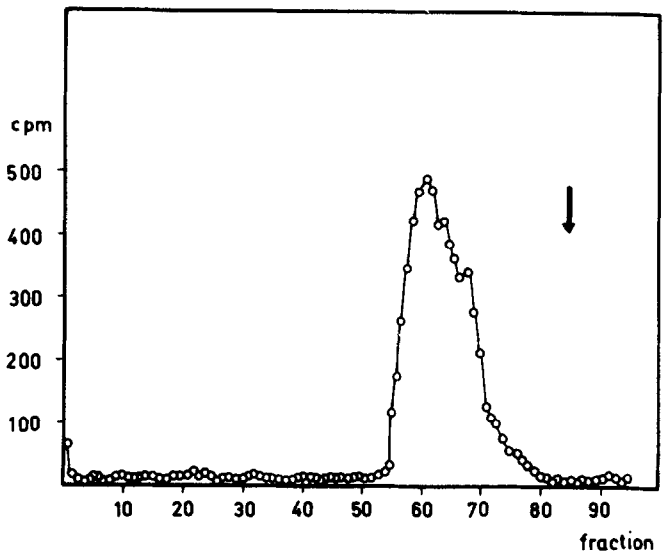

Fig. 2. Distribution of radioactivity in gel after electrophoresis of labelled neutral chloroform-methanol extract from mitochondrial particles.

The results presented above are in a good agreement with those obtained with yeast mitochondria by Tzagoloff and Akai /5/. It can be concluded that one of the products of the protein synthesis in rat liver mitochondria in vivo is a hydrophobic protein /or lipoprotein/ with molecular weight of 9.000. This value differs from that reported by Kadenbach /3/ who ascribed molecular weight about 2.000 to this product. However, his determination of molecular weight was based on gel filtration, whereas more accurate SDS-polyacrylamide gel electrophoresis was employed in the present study. The different findings of Burke and Beattie /6/ on the number and molecular weight of the chloroform-methanol soluble product/s/ of protein synthesis in rat liver mitochondria may reflect the in vitro labelling of the intramitochondrially synthesized proteins and the minor deviations in the chloroform-methanol extraction procedure used in their experiments.

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