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ATPase COMPLEX AND OXIDATIVE PHOSPHORYLATION IN CHLORAMPHENICOL-INDUCED MEGAMITOCHONDRIA FROM MOUSE LIVER

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SUMMARY

1. Functional properties of the ATPase complex are investigated in megamitochondria isolated from livers of weanling mice fed a diet containing 2 % chloramphenicol, as an inhibitor of mitochondrial protein synthesis.

2. Whereas the specific activity of ATPase remains unchanged in chloramphenicol-induced megamitochondria, about 40 % of the enzyme activity is resistant to inhibition by oligomycin, triethyltin or venturicidin. It is concluded that the ATPase complex lacks one or more components whose synthesis or accumulation is dependent on mitochondrial translation. The inhibitor-resistant ATPase portion appears tightly bound to the mitochondrial membrane.

3. Respiratory chain phosphorylation is tightly coupled in isolated megamitochondria. ATP synthesis and ATP-P_i exchange are diminished by 40 %, as compared to control mitochondria, but both processes are sensitive to oligomycin, triethyltin or venturicidin.

4. The decrease in ATP synthesis and ATP-P_i exchange in megamitochondria correlates quite well with the emergence of inhibitor-resistant ATPase.

5. The following electron transport activities in the megamitochondria are reduced: NADH-cytochrome *c* reductase, by 60 %, cytochrome oxidase, by 80 %; the amount of antimycin required to gain complete inhibition of the bc_1-segment is diminished by more than 50 %. On the other hand succinate dehydrogenase activity is increased by 50 %.

6. Chloramphenicol-induced megamitochondria appear to be a useful system for studying the role of mitochondrial translation in the assembly of mammalian mitochondria.

INTRODUCTION

The role of the mitochondrial protein synthesizing system in the biogenesis of mitochondria has been studied extensively through the last years (for reviews see

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoro methoxyphenylhydrazone; duroquinone, 2, 3, 5, 6-tetramethyl-1,4-benzoquinone; HEPES, *N*-2-hydroxyethylpiperazine-N^1-2-ethanesulphonic acid.

refs 1, 2). For a variety of microorganisms it is now well established that a small minority of mitochondrial proteins, necessary for the assembly of the inner membrane, is translated on mitochondrial ribosomes. Most of this information was gained from investigation on *Neurospora* or yeasts and their extrachromosomally inherited mutants. Our knowledge on the role of the mitochondrial protein synthesizing system in mammalian tissues is still limited, however. Recent investigations on liver mitochondria from weanling mice, fed a diet containing chloramphenicol, a specific inhibitor of mitochondrial translation, revealed the formation of extremely enlarged mitochondria (megamitochondria) in the liver parenchymal cell [3]. Morphological changes of these matrix-enriched mitochondria with reduced cristae are accompanied by alterations of enzyme contents [4]; these changes apparently result from a diminished activity of the mitochondrial protein synthesizing system. This communication describes studies on the influence of chloramphenicol on the various catalytic properties of the ATPase complex, i.e. ATPase, ATP synthetase and the ATP-P_i exchange in mouse liver mitochondria. The reduced capacity of the energy-conserving system and the decreased sensitivity of the ATPase to oligomycin, venturicidin and triethyltin in megamitochondria is discussed with regard to alterations in the membrane sector of the ATPase complex as a consequence of blocking mitochondrial translation by chloramphenicol.

METHODS AND MATERIALS

Experimental animals

Weanling male mice (NMRI strain, Ivanovas, Kisslegg, Germany) weighing 8–9 g were placed on a powdered diet (Altromin R), supplemented with chloramphenicol/stearylglycolate (actual drug concentration in the lab chow 2 %) for 9–11 days. This method was previously shown to induce matrix-enriched megamitochondria in the mouse liver cell; i.e. the number of mitochondria is reduced, whereas the diameter of the remaining organelles is significantly increased (2–10 μm [3]). Control animals were fed on the same diet but without added chloramphenicol.

Preparation of mitochondria

Since megamitochondria induced by chloramphenicol in mouse liver are extensively damaged if isolated according to the commonly used methods (unpublished results), a modified preparation procedure was developed. After perfusion of livers in situ with 1–2 ml of the isolation medium by the splenic route, the tissue was homogenized in 10 mM HEPES buffer (pH 7.3), 220 mM mannitol, 70 mM sucrose, 1 mM EDTA and 0.6 % bovine serum albumin, using a glass-Teflon homogenizer with a loose-fitting pestle.

To prepare mitochondria used for measuring oxidative phosphorylation and ATP-P_i exchange, the homogenate was centrifuged at $250 \times g$ for 10 min (Sorvall centrifuge RC-2B, rotor HB 4) to remove cell debris and nuclei; the megamitochondria were sedimented in two fractions: a "heavy fraction" was spun down at $1500 \times g$ (10 min), the rest of the mitochondria at $7000 \times g$ (10 min). Centrifugation of the heavy fraction at $7000 \times g$ causes severe damage of the mitochondria during the resuspension procedure; it is supposed that this fraction of most fragile organelles contains megamitochondria with extreme diameters. The pellets were carefully resus-

pended in isolation medium by gentle shaking in a centrifuge tube. To remove the major part of contaminating microsomes, centrifugations at $1500 \times g$ (10 min) and $7000 \times g$ (10 min) were repeated once. To avoid damage of the mitochondria, no further washing procedures were performed. The pellets were combined to a final suspension containig 8–12 mg mitochondrial protein/ml.

Modified preparation techniques were applied to isolate mitochondria for further separation of mitochondrial membranes and for enzyme assay. For this purpose the cell debris was sedimented at $150 \times g$ (5 min); the supernatant was layered on a linear density gradient (Ficoll 25–40 % in isolation medium) and centrifuged at $104\,000 \times g$ for 30 min (Beckman Spinco ultracentrifuge, rotor SW 25.2); the layer containing the mitochondria was carefully resuspended with isolation medium and the organelles were sedimented at $6500 \times g$ (15 min). The final suspension contained about 10 mg mitochondrial protein/ml.

As judged by electron microscopic examination and determination of the loss of the marker enzyme glutamate dehydrogenase during the preparation, this isolation procedure yields morphologically intact mitochondria including giant mitochondria up to a diameter of 10 μm (unpublished results). However, functional conditions of the mitochondria, especially with respect to oxidative phosphorylation, were found to be better with the first mode of preparation whereas the density gradient method leads to a mitochondrial fraction of higher purity. All operations were performed at 0–2 °C.

Liver mitochondria from control animals were prepared in the same manner.

Fractionation of Mitochondria

A crude mitochondrial membrane fraction (inner+outer membrane) was prepared according to the method of Winkler and Lehninger [5] with minor modifications. Mitochondria were suspended in 10 mM Tris · Cl (pH 7.3), 250 mM sucrose and 1 mM EDTA at a protein concentration of 20 mg/ml. To 1 ml of this suspension an aqueous solution (1.9 %) of the non-ionic detergent Lubrol WX was added, so that the final mixture contained 0.15 mg Lubrol/mg mitochondrial protein. The suspension was gently stirred for 30 min at 0 °C, diluted with buffer to a final volume of 5 ml and centrifuged for 30 min at $198\,000 \times g$ (Beckman Spinco ultracentrifuge, rotor SW 50.1). The pellet containing the membrane fraction was suspended in the medium described above.

Another fractionation procedure was carried out by sonicating the mitochondrial suspension twice for 10 s (Branson sonifier, step 3) followed by 30 min centrifugation at $198\,000 \times g$.

In case of testing functions on submitochondrial level 2 ml of the mitochondrial suspension was sonicated in approx. 5×10 s intervals until the turbid suspension became opalescent. During sonication the mixture was kept below 5 °C.

Enzyme assays

Succinate-cytochrome *c* reductase, NADH-cytochrome *c* reductase and cytochrome oxidase were assayed at 25 °C according to Sottocasa et al. [6]. NADH-duroquinone reductase was determined at 25 °C according to Ruzicka and Crane [7]. Succinate dehydrogenase was assayed at 38 °C as described by Veeger et al. [8]; concentrations of phenazine methosulfate applied in a test varied from 0.12–1.2 mM; *V*

was calculated from a double reciprocal plot. (Below 38 °C, preactivation of the enzyme with succinate is essential if full activity is to be measured [9].)

ATPase was measured spectrophotometrically at 366 nm and pH 7.0 according to the method of Pullman et al. [10]. This method is based on coupling the formation of ADP to the oxidation of NADH via pyruvate kinase and lactate dehydrogenase in the presence of KCN. Comparable results were obtained when ATPase activity was determined in the absence of an ATP-regenerating system and KCN by following the release of inorganic phosphate, estimated as described by Baginski et al. [11]. In all experiments, 4.3 mM $MgCl_2$ was present; 1 μM FCCP was added when indicated. When the action of inhibitors, i.e. oligomycin, venturicidin, Dio-9 and triethyltin, was tested, the mixture was preincubated for 6 min. Concentrations of the added inhibitors are indicated in the text. The reaction was started with ATP (3 mM).

Oxidative Phosphorylation and ATP-P_i exchange

Oxidative phosphorylation was determined at 25 °C by incubating 0.5 mg mitochondrial protein in 0.5 ml of 10 mM triethanolamine/HCl buffer (pH 7.2), 250 mM sucrose, 1 mM EDTA, 2 mM $MgCl_2$, 5 mM potassium phosphate containing 1 Ci $^{32}P_i$/mol P_i, 10 mM glucose and hexokinase (2 units/ml). The concentration of the respiratory substrates is given in the text. Oxygen consumption was measured with a Clark oxygen electrode and ATP synthesis by determining the amount of $^{32}P_i$ incorporated into organic phosphate [12].

ATP-P_i exchange was assayed as described by Pullman [13]. The medium contained 55 mM triethanolamine /HCl buffer (pH 7.2), 55 mM sucrose, 10 mM $MgCl_2$, 10 mM ATP and 20 mM potassium phosphate (0.5 Ci $^{32}P_i$/mol P_i).

Protein determination

Protein was determined with a modified biuret method [14].

Materials

Chloramphenicol (Paraxin®/stearyl glycolate) was a generous gift from Boehringer Mannheim GmbH, Mannheim, Germany. Oligomycin was obtained from Calbiochem, Los Angeles, U.S.A. and triethyltin from Merck-Schuchard, München, Germany. Dio-9 was purchased from Royal Netherlands Fermentation Industry, Delft, Holland, venturicidin from British Drug Houses Ltd, Poole, Dorset, U.K. Lubrol WX was from Sigma Chemical Co., St. Louis, U.S.A., Ficoll from Deutsche Pharmacia GmbH, Frankfurt, Germany. $^{32}P_i$ was from Amersham Buchler GmbH, Braunschweig, Germany. All other chemicals were of analytical grade.

RESULTS

The specific activity of mitochondrial ATPase was measured with intact organelles in the presence of FCCP and on sonically disrupted mitochondria with Mg^{2+} in the absence of FCCP. There is virtually no change in ATPase activity as a result of chloramphenicol-treatment of the animals. Inhibition by Dio-9, which reacts specifically with F_1-ATPase [15], is also unaffected by chloramphenicol (Table I). A considerably modified response is found with inhibitors which presumably react with one or more of the hydrophobic subunits of the ATPase complex [16, 17, 18]. Inhi-

TABLE I

SPECIFIC ACTIVITY OF ATPase AND INHIBITION BY Dio-9 IN LIVER MITOCHONDRIA FROM CONTROLS AND CHLORAMPHENICOL-TREATED MICE

ATPase activity in intact mitochondria was measured in the presence of 1 μM FCCP. Temperature was 25 °C. Experimental details are described in Methods. Results are expressed as mean values $\pm$S.E., the number of experiments is given in parenthesis.

	Addition	nmol ATP hydrolyzed/min per mg protein	
		Control animals	Cloramphenicol-treated animals
Intact mitochondria	–	296±14 (20)	310±21 (20)
Sonicated mitochondria	–	530±15 (20)	497±23 (20)
	Dio-9 (300 μg/mg protein)	88± 3 (10)	87± 3 (10)

bition of ATPase activity by various concentrations of added oligomycin, triethyltin and venturicidin is shown in Fig. 1. Though the extent of inhibition varies somewhat with increasing inhibitor concentration, approx. 1/3 of the ATPase in chloramphenicol-induced megamitochondria appears to be absolutely insensitive to oligomycin or triethyltin. No significant reduction of the inhibitor-resistant portion of ATPase was found up to 100 μg oligomycin or 400 nmol triethyltin per mg mitochondrial protein. Inhibition of ATPase in chloramphenicol-induced mitochondria by venturicidin increases slightly at very high levels of added inhibitor, but never attains control values. Results shown in Fig. 1 were obtained on sonicated mitochondria. Sonication has no influence on the inhibitor-sensitivity of the ATPase complex, since

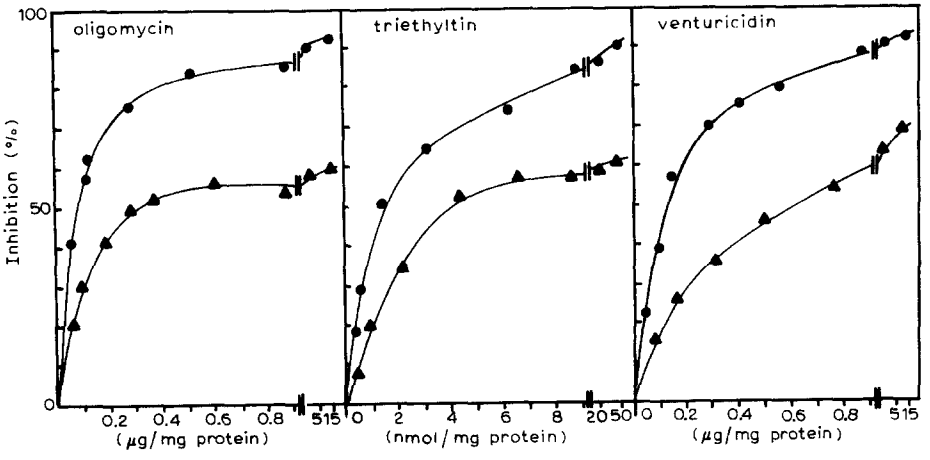

Fig. 1. Comparison of the inhibitory effect of oligomycin, triethyltin and venturicidin on ATPase activity in liver mitochondria from controls and chloramphenicol-treated mice. Experiments were performed on submitochondrial level at 25 °C. Details are described in Methods. ●—●, control; ▲—▲, chloramphenicol.

the portion of inhibitor-resistant ATPase in intact mitochondria is the same as on submitochondrial level.

These findings indicate substantial alterations of the ATPase complex with respect to the action of the tested inhibitors. Resistance of the mitochondrial ATPase to excess of inhibitor suggests that one or more of the subunits, mediating inhibitor-sensitivity are partially missing after chloramphenicol-treatment. The remaining portion of oligomycin-sensitive ATPase (defined as amount of ATPase which is completely inhibited by 10 μg oligomycin/mg mitochondrial protein) reveals the same inhibitor-sensitivity as ATPase in control organelles.

Since after chloramphenicol-treatment about one third of the total ATPase activity has lost its characteristic response to the inhibitors, which interact with the membrane sector of the complex, it appeared interesting to check the binding properties of this altered portion of the ATPase complex to the mitochondrial inner membrane. For this purpose mitochondrial membranes and soluble contents were separated either by sonication of the mitochondrial suspension or by treating the organelles with low concentration of the non-ionic detergent Lubrol WX as described in Methods. Both experiments resulted in a release of 6–7 % of the total ATPase activity from the membrane of chloramphenicol-induced megamitochondria and of 4–6 % from control mitochondria. This indicates that the ATPase complex portion, which is altered in chloramphenicol-induced mitochondria with respect to functional changes of its membrane sector is still tightly associated with the inner membrane of the organelles.

To characterize further the influence of chloramphenicol on functional properties of the ATPase complex, reactions connected with the energy conserving system, i.e. ATP synthesis and ATP-P_i exchange, were tested. As demonstrated in Table II, the rate of respiration-driven ATP production is considerably smaller in megamitochondria than in control mitochondria. This was also stated using sonicated mitochondria, thus excluding a restriction of the adenine nucleotide carrier as a rate limiting factor after chloramphenicol-treatment. Since respiration under state 3 conditions is diminished proportionally to ATP synthesis, P/O ratios are not significantly changed in the intact mitochondria. This indicates that oxidative phosphorylation, though limited in rate, remains tightly coupled after chloramphenicol-treatment of the animals. This is also emphasized by the finding that state 3 respiration retains its full sensitivity to inhibition by oligomycin (Table V). Results presented in Table II further prove that the decreased phosphorylation rate in chloramphenicol-induced megamitochondria, respiring various substrates, is not limited by the functional capacity of the respiratory chain. Whereas megamitochondria show a 48 % reduction of succinate oxidation, when coupled to ATP formation, oxygen consumption released by FCCP is practically the same as in control mitochondria. Also with palmitoylcarnitine and α-oxoglutarate as respiratory substrates FCCP-released oxidation is about 30–40 % higher than respiration under state 3 conditions. It becomes evident from these experiments that oxidative phosphorylation by megamitochondria is limited by the diminished capacity of the ATP-synthesizing system.

In view of the results presented in Table II it nevertheless appears interesting to check functional capacities of the respiratory chain in chloramphenicol-induced megamitochondria. Even though ATP synthetase is rate limiting with respect to the oxygen uptake during state 3 respiration, a reduced respiratory chain activity might

TABLE II

PARAMETERS OF RESPIRATION AND OXIDATIVE PHOSPHORYLATION IN LIVER MITOCHONDRIA FROM CONTROLS AND CHLORAMPHENICOL-TREATED MICE

Mitochondria from 2 livers were pooled for each experiment. Uncoupled respiration was measured in the presence of 5 μM FCCP and state 3 respiration after addition of 2 mM ADP; the concentration of respiratory substrates was 2 mM. Other experimental conditions are described in the methods section. Results are given as mean values ±S.E. from 8–10 experiments.

Substrate		Uncoupled respiration (natom O/min per mg protein)	State 3 respiration (natom O/min per mg protein)	$^{32}P_i$ uptake (nmol/min per mg protein)	P/O
Succinate (+ 8 μM rotenone)	control	148± 4.7	159±7.1	251±16	1.58
	chloramphenicol	143± 4.4	82±2.4	126± 6.5	1.54
Palmitoylcarnitine (+0.5 mM malate)	control	121±12	114±3.9	262±14	2.30
	chloramphenicol	98± 4.9	70±5.2	160± 9.2	2.29
α-Oxoglutarate	control	118± 4.1	121±5.0	306±18	2.53
	chloramphenicol	88± 4.5	69±5.0	165±13	2.39

TABLE III

SPECIFIC ACTIVITIES OF RESPIRATORY CHAIN ENZYMES IN MITOCHONDRIA FROM CONTROLS AND CHLORAMPHENICOL-TREATED MICE

Enzyme activities were assayed on sonicated mitochondria, as described in Methods, at 25 °C, with exception of succinate dehydrogenase activity, which was determined at 38 °C. The antimycin titer presents the amount of antimycin (nmol/mg mitochondrial protein) required for complete inhibition of succinate-cytochrome *c* reductase activity. Enzyme activities are expressed as nmol substrate utilized/min per mg mitochondrial protein, cytochrome oxidase activity is given as natom O/min per mg mitochondrial protein. Figures present mean values ±S.E. from 10 experiments.

Enzyme	Control animals	Chloramphenicol-treated animals
NADH-cytochrome *c* reductase (Rotenone sensitive)	98± 3.7	43± 2.7
NADH-duroquinone reductase (Rotenone sensitive)	77± 2.7	40± 1.3
Succinate-cytochrome *c* reductase	148± 5.6	157± 5.8
Succinate dehydrogenase	471±37	974±45
Cytochrome oxidase	1588±89	302±14
Antimycin titer	0.14	0.06

explain why the FCCP-released respiration of palmitoyl-carnitine and α-oxoglutarate measured with megamitochondria, is significantly lower than with control organelles. This reduction of uncoupled respiration, which is not observed with succinate, may have various causes, but it could be due to a restrained capacity of NADH-cytochrome *c* reductase.

Results compiled in Table III show a 55 % decrease of NADH-cytochrome *c* reductase activity in mitochondria from chloramphenicol-treated animals, when compared with the control group. This decrease is most likely caused by an alteration of NADH-dehydrogenase, since the electron flow from the dehydrogenase to cytochrome *c* is not impaired by low ubiquinone contents (unpublished results) nor by the capacity of the bc_1-segment. The latter conclusion results from the rate of succinate-cytochrome *c* reductase, measured in megamitochondria (cf. below), which is considerably higher than NADH-cytochrome *c* reductase activity. Exclusion of the bc_1-segment by using the artificial electron acceptor duroquinone, which reacts on the oxygen side of ubiquinone, also indicates a significant reduction of NADH oxidation in chloramphenicol-induced mitochondria.

In accordance with succinate respiration (FCCP-released), both classes of mitochondria show little difference in the activity of succinate-cytochrome *c* reductase. However, the amount of antimycin necessary for complete inhibition of succinate-cytochrome *c* reductase is reduced by 54 % in chloramphenicol-induced mitochondria. This suggests that the subunit of the bc_1-segment which carries the antimycin binding site is present in subnormal amounts. The activity of succinate dehydrogenase, primary enzyme of the complex, is even higher in megamitochondria when tested by means of an artificial electron acceptor (phenazine methosulfate).

The capacity of cytochrome oxidase is reduced by about 80 % in chloramphenicol-induced mitochondria. However, with respect to the high activity of cytochrome oxidase in control mitochondria this decrease seems not to be rate limiting on respiration; the rate of FCCP-stimulated succinate respiration, which is unaffected by chloramphenicol influence, remains obviously within the range of the restricted complex activity.

It is certainly problematic to compare oxygen consumption by intact mitochondria and the activities of respiratory chain fragments, measured on submitochondrial level. However, according to our results it seems possible that the decreased capacity of NADH oxidation in chloramphenicol-induced mitochondria limits the FCCP-released oxidation of NAD-specific respiratory substrates; on the other hand, alterations of the respiratory enzyme assembly caused by chloramphenicol-treatment of the animals appear not responsible for reduced oxidative phosphorylation in the mitochondria.

The decrease of oligomycin-sensitive ATPase and of ATP synthetase in liver mitochondria of chloramphenicol-treated mice, led us to measure the ATP-P_i exchange, which is generally considered to be a "partial reaction" of oxidative phosphorylation independent of respiration [19]. As shown in Table IV this exchange is diminished in chloramphenicol-induced mitochondria, although the decrease is somewhat smaller than the decrease of ATP synthetase activity. In contrast to ATPase, but similarly to ATP synthetase, the ATP-P_i exchange system retains its full sensitivity to specific inhibitors of the ATPase complex such as oligomycin, triethyltin or venturicidin. Venturicidin (8μg/mg protein) inhibits only about 80 % of the ATP-P_i ex-

TABLE IV

INFLUENCE OF INHIBITORS AND FCCP ON THE ATP-P_i EXCHANGE SYSTEM OF MITOCHONDRIA FROM CONTROLS AND CHLORAMPHENICOL-TREATED MICE

Activities are expressed as nmol P_i exchanged/min per mg mitochondrial protein. Experimental conditions are described in Methods. Results are given as mean values ± S.E from 8 experiments.

Addition	Control animals		Chloramphenicol-treated animals	
	Specific activity	% Inhibition	Specific activity	% Inhibition
None	86±3.6	–	59±2.5	–
Oligomycin (4 μg/mg)	3.4±0.33	96	3.0±0.28	95
Venturicidin (8 μg/mg)	17.0±1.3	80	11.5±1.2	80
Triethyltin (10 nmol/ mg)	6.8±0.43	92	5.8±0.40	90
FCCP (2μM)	1.9±0.16	98	1.8±0.17	97

change in both species of mitochondria, thus confirming results by Lardy and coworkers [20], who found that pathways of oxidative phosphorylation are less sensitive to venturicidin than mitochondrial ATPase. There was also no difference with respect to the uncoupling effect of FCCP.

Table V presents functional parameters of the ATPase complex given as percent of control activity and compared with respect to the sensitivity to oligomycin. Though the specific acitivity of ATPase is practically unchanged, the oligomycin-

TABLE V

COMPARISON OF FUNCTIONAL PARAMETERS OF THE ATPase COMPLEX IN MITOCHONDRIA FROM CONTROLS AND CHLORAMPHENICOL-TREATED MICE

Data presented are from typical experiments, performed with samples from the same preparation of mitochondria, pooled from three livers from controls and chloramphenicol-treated mice respectively. ATP-synthetase and state 3 respiration were measured with succinate as respiratory substrate. The concentration of added oligomycin was 4 μg/mg protein; temperature was 25 °C. Experimental details are described in Methods. Values are related to activities measured on control mitochondria (100 %).

	ATPase		ATP synthetase		State 3 respiration		ATP-P_i exchange	
	% activity	% activity*	% activity	% activity*	% activity	% activity*	% activity	% activity*
Control	100	91	100	94	100	90	100	97
Chloramphenicol	97	52	51	49	52	49	63	59

* Oligomycin sensitive.

sensitive portion of the enzyme is strongly reduced after chloramphenicol-treatment of the animals; differently from ATPase, the other parameters are considerably cut down in chloramphenicol-induced mitochondria, but their inhibitor-sensitivity is virtually the same as in control mitochondria. The oligomycin-sensitive portion of ATPase activity in mitochondria from mice treated with chloramphenicol corresponds strikingly to ATP synthetase activity and state 3 respiration; the limitation of the ATP-P_i exchange measured here is somewhat smaller. Nevertheless, a correlation of the activity of intact, i.e. oligomycin-sensitive ATPase and the capacity of the oxidative phosphorylation system in the mitochondria appears obvious.

DISCUSSION

Specific inhibitors of mitochondrial protein synthesis, such as chloramphenicol, tetracycline and others, have been successfully employed to study mitochondrial protein synthesis in procaryotes and lower eucaryotes [2], and in HeLa cells [21, 24] and mammalian cells of other origin [22, 23]. However, results obtained from these studies could not be satisfactorily reproduced in mammalian tissues under in vivo conditions, since the applied inhibitors, such as chloramphenicol, produce very little effect, when used with adult laboratory animals [25]. This may be due to a cell turnover too slow with respect to the capacity of mitochondrial protein synthesis, still remaining in the presence of the employed inhibitor concentration, which is limited by the toxicity of the antibiotic, when used under in vivo conditions. Only in regenerating tissues, e.g. after partial hepatectomy [25, 26, 27] and in other tissues with a high proliferation rate [28] does mitochondrial translation show a significant response to inhibitor influence. But even in these tissues the period of accelerated cell turnover appears too short to allow inhibitor action on mitochondrial genome expression to its full extent.

Striking alterations of mitochondria in livers of weanling mice, fed a diet containing chloramphenicol [3], strongly support this assumption. The turnover of liver cells should be considerably increased in these animals, which normally triple their liver weight within 10 days. Liver mitochondria from weanling mice, treated with chloramphenicol, were characterized as matrix-enriched megamitochondria with reduction of cristae; the diameter of some of the larger organelles exceeds 10 μm, approaching the size of the nucleus [3]. Specific biochemical changes in these mitochondria indicate a significant inhibition of the mitochondrial translation system.

This efficient experimental model was here employed to gain information on the biogenesis of the ATPase complex in mammalian mitochondria. Experiments reveal almost no alteration of F_1-ATPase activity in liver mitochondria by the influence of chloramphenicol, whereas a drastic decrease of the oligomycin-sensitivity of the ATPase complex is observed, thus confirming results by Kroon et al. [23, 28], who reported a somewhat diminished oligomycin-sensitivity of ATPase in cultured rat heart cells and cells of the intestinal tract after chloramphenicol- or oxytetracyclin-treatment. Differently from mitochondria from yeast grown in the presence of chloramphenicol [30] which reveal an oligomycin resistance exceeding 90 % of the total ATPase activity, chloramphenicol-induced megamitochondria lose only about 40 % of their inhibitor-sensitivity. Since the decrease in oligomycin sensitivity of ATPase corresponds to the inability of the mitochondria to carry out oxidative phosphory-

lation (cf. below), this difference can be explained from the ability of yeasts to shift energy production to the glycolytic pathway, whilst weanling mice are dying when the chloramphenicol supply exceeds a certain limit.

Tzagoloff et al. [29] demonstrated that oligomycin-sensitive ATPase from yeast mitochondria contains four hydrophobic polypeptides, which are tightly associated with the inner mitochondrial membrane and are considered to be synthesized on mitochondrial ribosomes. Oligomycin [29] as well as venturicidin and triethyltin, which act in a mode similar to oligomycin [20, 17], are supposed to react with hydrophobic subunits of the ATPase complex [18]. Studies by Griffith and coworkers [31, 32, 33] on single-gene yeast mutants showed that at least two mitochondrial genes and perhaps non-mitochondrial genes as well [34], are responsible for oligomycin-, venturicidin- and triethyltin-sensitivity in yeast and that the site of action of these inhibitors is possibly located on different polypeptides. The complete resistance of approx. 40 % of the ATPase activity to all three inhibitors, observed with chloramphenicol-induced mitochondria, can therefore be interpreted in a reduction of inhibitor-sensitivity conferring membrane components of the enzyme complex. This confirms that also in mammalian cells mitochondrial translation products are required for the formation of these lipoprotein subunits. Chloramphenicol reduces the sensitivity to all three inhibitors to an almost equal degree; this may indicate that the formation of polypeptides mediating inhibitor-sensitivity is depressed to a similar extent. This appears noteworthy, since chloramphenicol reveals a clearly variable effect on the activity of other mitochondrial enzymes, also depending on protein subunits synthesized by the mitochondria, e.g. cytochrome oxidase.

Whereas F_1-ATPase is able to catalyze ATP hydrolysis without being connected to the rest of the enzyme complex, catalysis of energy conservation, i.e. ATP synthesis, as well as the ATP-P_i exchange function require full integration of ATPase into the inner membrane [35]. Although chloramphenicol treatment does not appear to affect the binding of the ATPase to the mitochondrial membrane, the reduced capacity of the oxidative phosphorylation system in megamitochondria may also reflect an alteration of the membrane sector in the ATPase complex. Two points should be considered in this context: firstly, all tested functions of the energy conserving system, still active after chloramphenicol treatment, are fully sensitive to the tested inhibitors. Secondly, the decreased functional capacity, observed with megamitochondria, corresponds closely to the activity of the remaining inhibitor-sensitive ATPase portion in the mitochondria (Table V). It appears tempting to conclude that the inhibitor-resistant portion of the ATPase complex cannot participate in the pathways of oxidative phosphorylation and is only unspecifically associated with the inner mitochondrial membrane; subunits conferring inhibitor sensitivity are required for the functional integrity of the enzyme complex. It further appears worth noting that the inhibitor-resistant portion of the ATPase complex reveals no increased ATPase activity, thus seeming to uncouple oxidative phosphorylation.

Numerous studies on the substructure of mitochondrial ATPase indicate no significant ultrastructural difference of the enzyme complex in lower organisms and higher eucaryotic systems [35]. It is therefore not surprising that properties of the ATPase complex in chloramphenicol-induced megamitochondria of liver parenchymal cells are partly similar to such found on mitochondria from extrachromosomal inherited yeast mutants or from yeast grown on a medium containing chloramphenicol

[30, 31, 32]; an analogous mechanism in biogenesis of mitochondrial ATPase in yeast and mammals seems possible.

Results obtained on changes of electron transport activities in megamitochondria lead to a resembling view with respect to certain respiratory chain segments. Notwithstanding an obvious contrast of our results with respect to findings on yeast should be mentioned. Since no significant reduction of NADH dehydrogenase activity was stated in yeast grown in the presence of chloramphenicol [36], formation of respiratory chain complex I was not attributed to the mitochondrial protein synthesizing system. The capacity of this respiratory chain segment appears strikingly reduced in chloramphenicol-induced megamitochondria, when compared with controls. However, it cannot be decided from the experimental data presented here, if the decrease in enzyme activity observed in megamitochondria is actually caused by blocking of the mitochondrial translation or if it is a secondary effect. It is known that chloramphenicol inhibits NADH dehydrogenase, when applied in concentrations 10–20 times higher than required to inhibit mitochondrial protein synthesis [37]. Although it appears unlikely that the level of chloramphenicol in isolated megamitochondria attains these concentrations, it cannot be rigorously excluded that permanent treatment with chloramphenicol of the animals causes an alteration of the functional properties of complex I independently from the specific action of chloramphenicol on the mitochondrial translation.

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