Effects of Chloramphenicol on the Mitochondrial Respiratory Chain in the Wild Strain and in a Cytoplasmic Chloramphenicol-Resistant Mutant of *Tetrahymena pyriformis*

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The effects of chloramphenicol (CAP) on mitochondrial respiratory activity in the wild strain (ST) and in a cytoplasmic CAP-resistant mutant (STR₁) of *Tetrahymena pyriformis* were studied by determining oxygen consumption, by spectrophotometry, and by cytochemistry. In the absence of CAP both strains had the same respiration capacity, and the low-temperature spectra of their isolated mitochondria were similar. Furthermore, the mitochondria of both strains showed a positive reaction with diaminobenzidine, denoting a similar cytochrome oxidase activity. However, when cells were grown in CAP for 24 or 48 h, the peaks of cytochrome oxidase and cytochrome *b* were almost absent in the wild type. In this type the oxygen consumption was greatly decreased, and the mitochondria were no longer stained by diaminobenzidine. In the mutant, the peaks of cytochrome oxidase and cytochrome *b* were decreased only; respiration was less affected than in the wild type, and cytochrome oxidase activity was still disclosed by the diaminobenzidine reaction. These results show that CAP inhibits the synthesis of two cytochromes (*b* and oxidase) which are partially translated into the mitochondria of *T. pyriformis*. In the mutant, CAP reduces only the mitochondrial translation, resulting in reduced mitochondrial activity and reduced growth rate of the cell. These results are compared with the nucleo-mitochondrial regulation mechanisms discussed in our previous works.

The respiratory chain of *Tetrahymena pyriformis* ST is well known (9, 11, 22). This chain differs from those of yeasts and higher organisms in many respects (see reference 9 for a review), one of which is the presence of abundant cytochrome *a*₂, which absorbs at 617 nm. A cytoplasmic mutant (STR₁) resistant to chloramphenicol (CAP), a specific inhibitor of mitochondrial protein synthesis, has been isolated from strain ST of *T. pyriformis* (14). This mutant grows at the same rate as the wild strain in normal medium, but its growth is slowed in media supplemented with 250 or 500 µg of CAP per ml (3). Cytoplasmic mutations conferring resistance to antibiotics induce modifications of the respiratory chain. These modifications have been clearly established in *Paramecium tetraurelia* (1). All of the cytoplasmic mutants observed, whether resistant to erythromycin or to CAP, have a longer generation time than that of the wild type and show a decreased amount of cytochrome oxidase, increased levels of cyanide-insensitive respiration, and a lowered P/O ratio. These results are similar to those obtained with *Neurospora* (6, 10, 21) and with a CAP-resistant mutant of *Podospora* (2).

In *T. pyriformis*, the situation would seem to

be different because both the mutant and wild types have the same generation time. Therefore, we compared the respiratory chain of both strains to disclose possible defects in the mutant.

The effect of CAP on growth and mitochondrial function in *T. pyriformis* was studied by Turner and Lloyd (22). These authors showed that growth of the cells is completely inhibited within a few generations by 500 µg of CAP per ml and that after 72 h of CAP treatment the cells are respiratory deficient. More recently, in the ST strain of *T. pyriformis* grown in the presence of CAP, an important decrease in the mitochondrial contents of cytochrome *b* and cytochrome oxidase was observed (16, 17). Therefore, the question may be raised whether CAP treatment of mutant cells (STR₁) also causes modifications in the respiratory chain.

In the present work, the composition of the respiratory chain was studied by low-temperature spectrophotometry of reduced isolated mitochondria. The diaminobenzidine (DAB) reaction (12, 19) revealed the cytochrome oxidase activity of the mitochondria in situ. Results are discussed on the basis of nucleo-mitochondrial regulation mechanisms in relation to our previ-

ous works (3, 4, 14; R. Perasso, Thèse d'Etat, Université de Paris-Sud, Orsay, France, 1980).

MATERIALS AND METHODS

Strains and culture conditions. Two strains were used for these experiments: the wild strain ST of *T. pyriformis*, and its cytoplasmic mutant STR₁, which is resistant to CAP (14). Cells were grown as previously described (14).

Respiration of whole cells. The cells were concentrated by slow centrifugation (800 rpm for 3 min with a GGT centrifuge). A 0.2-ml portion of the pellet was transferred to a chamber containing 1.5 ml of 10^{-2} M Tris-hydrochloride buffer (pH 7.2). Some experiments were performed without concentration of the cells. The inhibition tests were done with a solution of 1 M KCN in 1 M morpholinepropanesulfonic acid buffer (pH 7.4) and with a solution of 1 M salicylhydroxamic acid in dimethyl formamide. KCN and salicylhydroxamic acid were added in the chamber to a final concentration of 1 to 2 mM. Oxygen uptake was measured polarographically with a Clarke oxygen electrode.

Evaluation of cell concentration. The evaluation of the number of cells per milliliter was done with a Coulter A Counter (Coultronics, Paris, France) on samples of 1 or 2 ml.

Isolation of mitochondria. Mitochondria were isolated by the procedure previously described (3).

Determination of protein contents. All protein determinations were carried out by the biuret method, with crystalline bovine serum albumin as a standard.

Spectrophotometry. The mitochondria were suspended in a medium containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris-hydrochloride (pH 7.4). The mitochondrial suspensions were adjusted to contain 100 mg of protein per ml. Sodium hydrosulfite (dithionite) was used as the reducing agent. Low-temperature spectra were obtained with a Cary model 20 spectrophotometer.

Extraction of cytochrome c. A pellet of mitochondria was suspended in distilled water to obtain 2 mg of protein per ml. After homogenization with a Potter homogenizer, the mitochondria were allowed to stand for 5 min at 0°C, and then crystallized KCl was added to obtain a final concentration of 0.25 M. After a second homogenization, the mitochondria were allowed to stand for 15 min at 0°C. The suspension was centrifuged for 20 min at $30,000 \times g$. The supernatant contained cytochrome c. Another extraction of cytochrome c with KCl was carried out by the same process on the pellet.

The two supernatants were pooled and dialyzed overnight at 4°C against distilled water. Centrifugation for 2 h at $100,000 \times g$ was then carried out to obtain a solution of cytochrome c devoid of insoluble particles. The supernatant was lyophilized. The cytochrome c was dissolved in phosphate buffer (10 mM, pH 7.3) and reduced with dithionite.

Cytochemistry and microscopy. Cytochemistry and microscopy were performed by the method of Stelly et al. (20).

RESULTS

Respiration of whole cells. (i) **Cells grown without CAP (controls).** Both strains were sensitive

to KCN. A complementary inhibition after the addition of salicylhydroxamic acid was never observed.

The oxygen uptake was similar for both strains grown in normal medium for 24 or 48 h (Fig. 1). The consumption of oxygen ranged from about 5.6 to 6.5 $\mu\text{M O}_2 \text{ min}^{-1}$ per 10^5 cells.

(ii) **Cells grown with CAP.** Modifications in the respiratory activity were observed in both strains (Fig. 1). In the wild strain, this activity decreased continuously as a function of the duration of CAP treatment. In the mutant strain, this activity decreased more slowly after 24 h of treatment. After 48 h, respiration increased but remained below that of the control.

Spectrophotometry. (i) **Cells grown without CAP (controls).** The absolute spectra of dithionite-reduced mitochondria from ST cells (Fig. 2) were similar to those obtained by several authors (9, 11, 23). The extracted cytochrome c of *T. pyriformis* absorbed at 551 nm (Fig. 2). Therefore, the peak at 553 nm probably corresponded to the composition of two absorbances: that of cytochrome c_{551} and that of cytochrome b_{556} .

The low-temperature spectra of mitochondria of strain ST compared with those of strain STR₁ showed no differences. Moreover, we did not observe any modification in these spectra related to the phase of the culture.

(ii) **Cells grown with CAP.** When cells were grown in CAP-containing medium for 24 or 48 h, we observed important modifications in the spectra of the mitochondria from the wild type (Fig. 2). The peak $\alpha_{617 \text{ nm}}$ and the shoulder

FIG. 1. O₂ consumption of whole cells grown for 24 and 48 h in the absence (controls) or presence of CAP. ST and STR₁ are, respectively, the wild strain and the CAP-resistant mutant.

FIG. 3. DAB reactivity in the mitochondria of strain ST (a) and strain STR₁ (b). (a1 and b1) Mitochondrial staining in DAB-H₂O₂ medium; both kinds of mitochondria are stained. (a2 and b2) Controls in DAB-H₂O₂-KCN medium; full inhibition of staining has occurred. (a3, a4, b3, and b4) DAB reactivity in mitochondria of CAP-treated *T. pyriformis* (CAP treatment, 250 μ g/ml for 48 h). (a3 and b3) Mitochondrial staining in DAB-H₂O₂ medium; only the mitochondria of STR₁ are stained. (a4 and b4) Controls in DAB-H₂O₂-KCN medium; full inhibition of staining has occurred. ($\times 30,000$)

resistant mutant of *T. pyriformis*, isolated by Roberts and Orias (15), which shows a reduced growth rate and cytoplasmic inheritance.

In a previous study, we showed that in the mutant grown for 24 h in CAP-containing medium, the mitochondria display the same morphological alterations as those of the wild type, but recover an almost normal morphology after 48 h of CAP treatment (3). The data shown in Fig. 1 relating to the oxygen uptake by the mitochondria of the mutant grown under the same conditions are in agreement with this point. After 24 h of treatment, when their morphology was greatly altered, the mitochondria had a low capacity of respiration (63% of the control), whereas after 48 h they had recovered a morphology and respiratory activity which were almost normal (82% of the control).

The resistance to CAP of the STR₁ mutant is due to a modification in the protein contents of the mitoribosomes (4). These mitoribosomes bound less to CAP than those of the wild strain did (4). We can explain the mitochondrial behavior of the mutant in terms of nucleo-mitochondrial regulatory mechanisms, with the following hypothesis. In the first step, the antibiotic causes a lack of balance between the decreased translation in the mitochondria and the unchanged translation in the cytoplasmic background. Then, a new balance between the activity of the two translational systems is established, due to an increase in the number of mitoribosomes (4) and to a decrease in the growth rate of the cell which is, in turn, controlled by the rate of ATP production by the mitochondria (5, 13).

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