SENSITIVITY TO ERYTHROMYCIN OF MITOCHONDRIAL PROTEIN SYNTHESIS IN ISOLATED FLIGHT MUSCLE MITOCHONDRIA OF THE BLOWFLY LUCILIA

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Received 2 March 1972

1. Introduction

Studies of the mitochondrial genome have revealed phylogenetic differences between the mitochondrial DNA [1–4], ribosomes [5, 6], and ribosomal RNA's [5, 6] of yeast and animals. However, the requirements for protein synthesis *in vitro* [7] as well as the type of protein synthesized [4, 8] are apparently similar in all mitochondria studied. While it seems clear that chloramphenicol inhibits the incorporation of amino acids into the protein of all mitochondria studied and that cycloheximide does not [4, 5], the effect of some other antibacterial inhibitors is less clear. Thus it is generally agreed that yeast mitochondria are sensitive to the macrolide antibiotic erythromycin [2], but the effect of this inhibitor on animal mitochondria is still in doubt. It has been claimed [9, 10] that mitochondria from rat liver, rabbit liver and cat liver are all resistant to erythromycin and that this reflects a difference between the mitochondrial ribosomes of yeast and animals; however, both Kroon and De Vries [11] and Beattie [12] believe that resistance to erythromycin in rat liver is due to a permeability barrier at the mitochondria membrane.

Since the claim by Firkin and Linnane [9] raises the possibility of phylogenetic differences in inhibitor sensitivity between mitochondria of different organisms it is thought of interest to examine the erythromycin sensitivity of mitochondria isolated from developing flight muscles of the blowfly *Lucilia cuprina*.

2. Methods

Flight muscle mitochondria were isolated from newly emerged axenic adults of Lucilia as described previously [13], except that the second centrifugation was decreased from 20,800 $g \times 10$ min to 9,200 $g \times 10$ min, and the microbiuret [14] rather than the biuret [15] protein estimation was used. No experiments reported here were contaminated to a greater extent than 50 bacteria/incubation. Mitochondria (approx. 1 mg protein) were incubated at 30° for 15 min in 1 ml of a medium (pH 7.4) containing 65 mM KCl, 10 mM potassium phosphate, 1 mM EDTA, 18 mM $MgSO_4$, 10 mM Hepes buffer, a mixture of 19 amino acids (without leucine) each 0.05 mM, ATP generating system (4 mM ATP, 5 mM phosphoenolpyruvate, 20 μg pyruvate kinase) and 0.5 μCi U-^{14}C-leucine (specific activity 331 mCi/mmole, Amersham).

^{14}C-Leucine incorporation into mitochondrial protein was estimated as described previously [13] except that both an amino acyl tRNA extraction (15 min × 85–90° in TCA) and lipid extraction (ethanol, ethanol/ether, ether washes) were included routinely. Counting efficiency was 80–84%. Either erythromycin (Sigma) or erythromycin gluceptate (Lilley) was used.

3. Results and discussion

Although there is evidence [13, 16, 17] that insect mitochondria isolated in KCl are much more permeable

Table 1
The effect of erythromycin on incorporation *in vitro* of ^{14}C-leucine into protein of flight muscle mitochondria of Lucilia.

Erythromycin (μg/ml)	Incorporation of control mitochondria (%)		
	Intact mitochondria	15 min pre-inc. at 30°	30 min pre-inc. at 30°
50	99 (3)	102 (3)	–
100	98 (3)	103 (1)	–
150	94 (2)	95 (1)	105 (1)
200	108 (2)	98 (1)	–
300	107 (3)	97 (4)	87 (1)

Values in parenthesis represent the number of separate experiments. Intact mitochondria were pre-incubated for 5 min at 30°. All experiments were initiated by adding ^{14}C-leucine. Control incorporation was 4,200 cpm/mg protein/15 min for intact mitochondria.

Table 2
The effect of erythromycin on incorporation *in vitro* of ^{14}C-leucine into protein of flight muscle mitochondria resuspended in water.

Erythromycin (μg/ml)	Incorporation of control mitochondria (%)		
	Mitochondria resuspended in H_2O (1)	On ice in H_2O 15 min (2)	On ice in H_2O 30 min (3)
20	103 (1)	–	–
50	107 (1)	97 (4)	97 (1)
100	117 (1)	–	–
150	–	–	88 (1)
300	124 (1)	106 (4)	99 (1)
500	–	100 (2)	–
1000	–	99 (2)	–

Results of typical experiments showing the effect of H_2O resuspension and incubation on ice; the number of separate experiments is shown in parenthesis. Control incorporations were 4,700 (1), 4,600 (2), 3,900 (3) cpm/mg proteins/15 min.

than those isolated in sucrose, the KCl preparations used in these experiments showed no sensitivity to erythromycin (50–300 μg/ml) (table 1). Preincubation (for up to 0.5 hr) with erythromycin (50–300 μg/ml) before addition of the ^{14}C-leucine still produced no inhibition (c.f. Beattie [12]).

When mitochondria were finally resuspended in water and stood on ice for periods of 1–30 min before being added to the incubation medium, no inhibition by erythromycin (20–1000 μg/ml) was observed (table 2.; cf. Kroon and De Vries [11]), despite the fact that physical disruption of the mitchondria could be detected by interference microscopy. Water resuspension usually had little effect on the level of incorporation, although after 30 min on ice some decrease was observed. However, on a few occasions there was complete loss of incorporation (see also [18, 19]); it seems likely that this resulted from excessive homogenisation of the water-lysed pellet.

When mitochondria were sonicated for 5 sec in 0.15 M KCl + 1 mM EDTA, erythromycin (50–300 μg/ml) still produced no inhibition, although incorporation was decreased to about 65% of the control. Mitochondria finally resuspended in water and stood on ice for 10 min before sonication (5 sec) were still capable of some incorporation (about 6% of control), but there was no inhibition with erythromycin (50–1000 μg/ml).

The experiments with erythromycin reported here gave somewhat variable results (range 85–125% of controls), but pronounced or consistent inhibition was *never* observed.

Using the criteria of Kroon and De Vries [11, 19] and Beattie [12] it seems that resistance to erythromycin by Lucilia mitochondria is not due to a permeability barrier at the mitochondrial membrane. It must be stressed that the incorporation observed was typically mitochondrial [7, 13], in that it was unaffected by cycloheximide (even at 500 μg/ml) and was inhibited substantially by chloramphenicol [5, 12]. Lack of inhibition by cycloheximide eliminates any possibility of a contribution from an extra-mitochondrial component to the synthesis.

Except with water-lysed, sonicated mitochondria, levels of incorporation were extremely high (25–30 pmole/mg protein/hr). When saturating amounts of leucine are used, much greater incorporation is observed (approx. 120 pmole/mg protein/hr [13]).

In contrast to the findings of Kroon and De Vries [11], it seems that an ATP generating system is most satisfactory as the energy source for these *in vitro* experiments [7]. Using such a system has the advantage of avoiding ambiguities due to possible effects

of inhibitors on ATP generation by oxidative phosphorylation.

The results in this report are consistent with the view that erythromycin resistance in Lucilia mitochondria is due to a change in the nature of the mitochondrial ribosomes which must be different to those of yeast mitochondria; unambiguous definition of this difference awaits the development of methods for the preparation of mitochondrial ribosomes from Lucilia active in protein synthesis, which can be tested for erythromycin sensitivity.

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