PHYLOGENETIC DIFFERENCES IN THE SENSITIVITY OF MITOCHONDRIAL PROTEIN SYNTHESISING SYSTEMS TO ANTIBIOTICS

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1. Introduction

The inhibitory action of chloramphenicol on the incorporation of amino acids into protein by isolated mammalian mitochondria (rat liver, beef heart) has been clearly demonstrated in a number of laboratories [1–4]. More recently we have shown that the inhibitory action of chloramphenicol on the mitochondrial protein synthesising system of mammalian tissues also occurs *in vivo*, and results in the selective inhibition of the synthesis of the mitochondrial cytochromes *a*, a_3, *b* and c_1 in HeLa cells and regenerating rat liver [5,6]. It has also been established that the mitochondrial protein synthesising system of yeast is sensitive *in vivo* and *in vitro* not only to chloramphenicol but also to lincomycin and the macrolide antibiotics such as erythromycin [7,8], so that its sensitivity to antibiotics resembles that of the bacterial ribosome, a finding which suggested that mitochondrial protein synthesising systems in general may be sensitive to this wide range of antibiotics. However, this communication reports that significant differences exist in the antibiotic sensitivity of the mitochondrial protein synthesising systems of yeast and mammalian mitochondria. Although chloramphenicol inhibits amino acid incorporation into protein by mitochondria from the mammalian species investigated (rat, rabbit and cat), the incorporation is insensitive to both erythromycin and lincomycin. The results are interpreted to suggest that in the course of the evolution of the mammalian mitochondrial protein synthesising system an intrinsic change in the system has occurred which reflects a change in cytoplasmic genetic information.

2. Methods

Liver mitochondria were prepared by the method of Hogeboom [9], using autoclaved equipment and solutions which had been sterilised by millipore filtration. Bacterial counts in the final incubation mixtures were routinely fewer than 50 bacteria per ml. Thrice-washed mitochondria were incubated for 30 min at 37°C. In a medium consisting of 100 mM sucrose, 100 mM KCl, 13 mM $MgCl_2$, 0.5 mM EDTA, 50 mM Tris buffer pH 7.5, 2 mM ATP, 5 mM phosphoenolpyruvate, 5 E.U. pyruvate kinase and 0.3 $\mu C(^{14}C)$ leucine (specific activity 96 $\mu C/\mu Mole$). The incorporation of (^{14}C) leucine into the mitochondrial protein was determined by the method of Lamb et al. [8].

3. Results and discussion

Chloramphenicol was found to inhibit the incorporation of (^{14}C) leucine into the protein of mitochondria isolated from yeast and the livers of the rat, rabbit and cat (table 1). Mammalian mitochondria had a lower activity than yeast mitochondria and were generally somewhat less sensitive to the antibiotic, the maximum inhibitions observed being about 55% compared with 70% for yeast. In contrast to the chloramphenicol effect, erythromycin and lincomycin did not significantly inhibit amino acid incorporation by the mitochondria from the three mammalian species, while the yeast system was inhibited (table 1).

It was considered that the insensitivity of mammalian mitochondrial protein synthesis to erythromycin and lincomycin *in vitro* could result either from

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Table 1

The *in vitro* effect of chloramphenicol, erythromycin and lincomycin on the incorporation of (^{14}C) leucine into protein by yeast and mammalian mitochondria.

Antibiotic (μg/ml)	Percentage inhibition of (^{14}C) leucine incorporation into protein of isolated mitochondria			
	Mitochondrial source			
	S. cerevisiae (diploid strain)	Rat liver	Rabbit liver	Cat liver
Chloramphenicol				
20	52	38	15	35
50	65	50	41	48
100	70	55	45	56
Erythromycin				
10	57	0	0	0
100	55	0	0	0
500	53	7	0	5
1000	60	5	–	–
Lincomycin				
50	57	0	0	0
200	76	7	0	10
500	–	9	–	–

The results cited are of a typical experiment in a series and are expressed as the % inhibition of (^{14}C) leucine incorporation into protein of isolated mitochondria in the presence of the antibiotics as indicated. The specific activities of the total mitochondrial proteins in absence of antibiotics in the different systems were 5240, 212, 580 and 117 cpm/mg protein for the yeast, rat liver, rabbit liver and cat liver systems, respectively.

Table 2

The effect of antibiotics on the incorporation of (^{14}C) leucine into protein by intact and sonicated rat liver mitochondria.

	Type of mitochondria	
	Intact	Sonicated
ATP regenerating system	Specific activity of mitochondrial protein (cpm/mg)	
present	186	234
omitted	164	21
Antibiotic (μg/ml)	Percentage inhibition by antibiotics	
Chloramphenicol 100	55	80
Erythromycin 500	7	0
Lincomycin 500	9	9

Isolated rat liver mitochondria were assayed as prepared, and also after 5 sec sonication in a 60 W M.S.E. sonic oscillator. The values are the means of the results obtained from 3 experiments. The ATP regenerating system consisted of 2 mM ATP, 5 mM phosphoenolpyruvate and 5 E.U. pyruvate kinase. The effects of the antibiotics were determined in the presence of the ATP regenerating system.

differences in the mammalian mitochondrial protein synthesising system *per se*, or from the failure of these antibiotics to penetrate the mitochondrial membranes. Consequently, we have examined the effects of the antibiotics after the mitochondria had been briefly sonicated in order to damage the mitochondrial membranes and the associated permeability barriers as previously described by Wheeldon et al. [3]. Sonication of rat liver mitochondria for 5 sec almost completely inhibited their capacity to support the incorporation of amino acids into protein while utilising endogenous substrates. On the other hand, the system incorporating amino acids into protein is not inactivated by the brief sonication, as in the presence of added ATP and an ATP regenerating system the overall activity is about 25% greater than in intact mitochondria (table 2). This change in the properties of the system can be interpreted as evidence of a general increase in membrane permeability, which is supported by the observed increase in the sensitivity of amino acid incorporation to chloramphenicol in the sonicated mitochondria (table 2). Despite the increased inhibition by chloramphenicol, amino acid incorporation into protein by sonicated rat liver mitochondria remained insensitive to erythromycin and lincomycin (table 2). Similar results were obtained with rabbit and cat liver mitochondria so that the basis for the insensitivity to erythromycin and linomycin appears to be a property of the actual mammalian mitochondrial protein synthesising system.

It has been suggested that the action of chloramphenicol on mitochondria from higher organisms is primarily the inhibition of NADH oxidase and that effects on protein synthesis are secondary to this [10,11]. This conclusion is not supported by our recently reported findings which show that at low levels, chloramphenicol does not affect the respiration

of HeLa cells while cytochrome *a*, a_3, *b* and c_1 synthesis is inhibited [5]. In our present experiments we found that none of the antibiotics up to a concentration of 1.5 mg/ml of reaction medium inhibited the oxidation of succinate by the isolated mammalian mitochondria. On the other hand, chloramphenicol at high levels did inhibit the oxidation of NAD linked substrates (33% inhibition at 1.0 mg/ml), but had no effect below a concentration of 300 μg/ml. Erythromycin and lincomycin, even at concentrations of 1.5 mg/ml, had no effect on the respiration of NAD linked substrates. It can be concluded that chloramphenicol has a primary effect on the mitochondrial protein synthesising system, and its effect on mitochondrial respiration requires high concentrations and is separate from its action on mitochondrial protein synthesis.

Although many important characteristics of the mitochondrial protein synthesising system appear to be common to both yeast and mammals, such as its role in the synthesis of the mitochondrial cytochromes *a*, a_3, *b* and c_1 [5,6], we have now demonstrated certain specific differences between the mitochondrial systems of these different phyla. This difference is apparently located in a component of the mitochondrial system which results in the mammalian system becoming insensitive to the inhibitory action of erythromycin and lincomycin. The difference between the yeast and mammalian mitochondrial protein synthesising systems has been shown to extend also to the response to the aminoglycoside antibiotics, paramomycin and the neomycins B and C, which inhibit the isolated yeast mitochondrial system but do not inhibit the mammalian mitochondrial system *in vitro* [12]. This suggests that there may be differences at several sites in the mitochondrial protein synthesising system, as the aminoglycoside antibiotics in bacteria interfere with protein synthesis by affecting the 30S subunit of the bacterial ribosome, whereas chloramphenicol, erythromycin and lincomycin react with the 50S subunit [13].

The genetic basis of the phylogenetic difference in the response of the mitochondrial protein synthesising systems to erythromycin and lincomycin is suggested by the demonstration in our laboratory that in the yeast the mutation of a cytoplasmic gene can result in the loss of the sensitivity of the mitochondrial system to erythromycin and lincomycin, but not to chloramphenicol [14]. The resulting mutant yeast strain therefore has a mitochondrial protein synthesising system whose sensitivity to these antibiotics resembles that of mammalian mitochondria. Thus, in the evolution of the mammal an analogous cytoplasmic mutation may have been responsible for the loss of the erythromycin and lincomycin sensitivity of the mitochondrial protein synthesising system.

Chloramphenicol produces a number of toxic effects in man, such as inhibition of bone marrow activity, which may be accounted for by its effect on mitochondrial protein synthesis [5,6]. On the other hand, the lack of an inhibitory effect of erythromycin and lincomycin on the mammalian mitochondrial protein synthesising system appears to explain why these antibiotics do not produce comparable toxic effects in man.

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