THE EFFECT OF RIFAMYCIN ON RNA SYNTHESIS IN THE RAT LIVER MITOCHONDRIA

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Received October 15, 1969

There exists a special system of protein synthesis in mitochondria which differs from that in the cytoplasm by the character of ribosomes, sRNA and aminoacyl-sRNA synthetases. In some features it is close to the system of protein system in bacteria: mitochondrial ribosomes as well as bacterial ones have the sedimentation constant at 70S; protein synthesis in them is inhibited by chloramphenicole and is not inhibited by cycloheximide whereas protein synthesis in the cytoplasm of animal cells is not inhibited by the former and is inhibited by the latter. A question arises, whether the resemblance between mitochondria and bacteria bears only on the system of protein synthesis or other systems of macromolecular synthesis, i. e. RNA synthesis as well.

Isolated mitochondria of animal cells and microorganisms are capable of independent RNA synthesis (Wintersberger, I964; Luck and Reich, I964; Neubert and Helge, I965). Saccone et al. (I967) established that this synthesis is catalyzed by RNA-polymerase localized inside mitochondria and cannot be related to contaminations of the nuclear enzyme. In this respect it would be very interesting to establish whether the mitochondrial RNA-polymerase differs from the nuclear enzyme and its resemblance with the bacterial RNA-polymerase can be detected.

In order to solve this question the sensitivity of RNA synthesis to rifamycin in mitochondria and nuclei has been studied. Some authors (Wehrli et al., I968; Austin etal., I969) believe that this antibiotics is a very active inhibitor of RNA-polymerase in bacteria but slightly influences the activity of RNA-polymerase in animal cells.

METHODS

Mitochondria were isolated from the liver of rat males afetr the 24 hours' starvation, according to Schneider and Hogeboom (I950). Nuclei were isolated after Chaufeau in the modification of Gvozdev (I960). Conditions of incubation are given in Tables. ^{I4}C-ATP and ^{I4}C-uridine were used as radioactive precursors of RNA. After incubation the samples were fixed with trichloroacetic acid (TCA). Acid-insoluble residues were washed 3 times with 5% TCA, alcohol and alcohol-ether(3:I)

RESULTS AND DISCUSSION

In Table 1 the data on the effect of rifamycin upon ^{I4}C-

TABLE 1

THE EFFECT OF RIFAMYCIN UPON THE INCORPORATION OF ^{I4}C-URIDINE AND ^{I4}C-ATP IN RNA UNDER THE INCUBATION OF RAT LIVER NUCLEI

Composition of the medium: in 0.5 ml (μmoles) - tris, pH 7.4 I0; $MgCl_2$ 5; KCl 20; EDTA o.5; phosphoenole pyruvate 2; pyruvate kinase I0γ . Incubation 20 min. at 30°. Control - fixation at 0' incubation

Composition of samples	^{I4}C-uridine**		^{I4}C-ATP***	
	imp/min in sample	inhibition,%	imp/min in sample	inhibition,%
Complete *	2I36	-	2942	-
+ actinomycin	802	6I.8	0	I00
+ rifamycin I0	2I9I	0	2892	I.I
+ rifamycin 20	2859	0	2945	0
+ rifamycin 50	2732	0	2747	6.6
+ rifamycin I00	2786	0	2593	II.4

*Content of antibiotics is given in γ/ml.

**In the sample: ATP 1 μmole, GTP and CTP 0.2 μmoles both, ^{I4}C-uridine 2.6γ (specific activity 2I5 mC/mM),protein 2.I2 mg. Control 5I5 imp/min per sample.

***In the sample: CTP, GTP and UTP 0.2 μmoles all three, ^{I4}C-ATP I4 (specific activity 25725 imp/min/γ),protein 2.96 mg. Control II53 imp/min per sample.

ATP and ^{14}C-uridine incorporation in RNA of rat liver nuclei are given. The results obtained have shown that even very high concentrations of the antiotics (100 γ/ml) either do not inhibit the incorporation in RNA at all or inhibit it to an insignificant degree. These findings coincide with the data of Austin et al. (1969) on the absence of an inhibiting effect of rifamycin upon RNA synthesis in the calf thymus nuclei.

In the mitochondria preincubated in 0.1 M phosphate (to increase permeability for substrates) ^{14}C-ATP incorporates in the acid-insoluble fraction. This incorporation can be considered as RNA synthesis since it is completely inhibited and radioactivity is completely removed by the extraction with hot TCA. The data in Table 2 show that the incorporation of ^{14}C-ATP in mitochondria is inhibited by rifamycin. A significant inhibition of incorporation (by 37.5%) is observed already at the concentration of 0.5 2 γ of the antibiotics induce an almost complete inhibition of RNA synthesis (by 76-88%). In intact mitochondria, non-preincubated in phosphate, RNA synthesis also occurs but the incorporation of ^{14}C-ATP is much lower. This incorporation was completely inhibited by rifamycin at the concentration of 10-20 γ/ml but proved to be insensitive to actinomycin at the concentration of 100 γ/ml.

The inhibition of RNA synthesis in mitochondria by rifamycin can hardly de compared with the effect of rifamycin upon bacteria because of different permeability of these objects. The activity of purified RNA-polymerase from E.coli is inhibited by 70-80% already by 0.1% rifamycin (Khesin et al.,1969). A lesser sensitivity of the enzyme in mitochondria appears to be due to their low permeability for the antibiotics. The data obtained permit to draw a conclusion on the resemblance between the effect of rifamycin upon the bacterial RNA-polymerase and the enzyme in mitochondria of animal cells. Hence it can be seen that the mitochondrial RNA-polymerase differs from the nuclear one and is, in some ways, close to the bacterial enzyme.

Thus the resemblance between the mitochondria and bacteria is revealed not only in the ring DNA structure and the character of protein biosynthesis but also in the properties of the enzyme synthesizing RNA. This finding rises the question on the site of the mitochondrial RNA-polymerase synthesis.RNA-

TABLE 2

THE EFFECT OF RIFAMYCIN UPON THE INCORPORATION OF ^{14}C-ATP IN RNA UNDER THE INCUBATION OF RAT LIVER MITOCHONDRIA

Conditions of experiments as in Table 1; incubation 30 min. at 30°

Composition of samples	Experiment I*		Experiment II**	
	imp/min in sample	inhibition,%	Imp/min in sample	inhibition,%
Complete	535	-	120	-
+ actinomycin 20	-	-	5	95.4
+ actinomycin 60	0	100	0	100
+ rifamycin 0.5	-	-	75	37.5
+ rifamycin 1	-	-	45	62.5
+ rifamycin 2	128	76.2	15	87.5
+ rifamycin 5	88	83.6	-	-
+ rifamycin 10	54	91.0	0	100
+ rifamycin 20	0	100	-	-
Complete, extracted with hot TCA	0			

*Protein 8.7 mg. Control 218 imp/min in the sample.
**Protein 7.9 mg. Control 252 imp/min in the sample.

polymerase is a complex high-molecular enzyme. Therefore three possibilities are open: (1) RNA-polymerase is synthesized inside mitochondria on the account of information in their DNA. We believe this possibility to be rather improbable because of a small amount of information stored in the mitochondrial DNA. (2) The synthesis of the mitochondrial RNA-polymerase is coded by the nuclear genome, this enzyme, as well as some others, being incorporated in mitochondria in the course of their formation. (3) Inside mitochondria is synthesized only that polypeptide which is responsible for the reaction of RNA-polymerase with rifamycin and, possibly, for the formation of catalytic site (Khesin et al., 1969). To solve this question further experiments are required which are now in progress in our laboratory.

Acknowledgements. The author is greatly indebted to Prof. R.B. Khesin for his continuous interest to the work and valuable advices and to Mrs T. S. Kharitonova for technical assistance.

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