A STUDY OF PROTEIN SYNTHESIS IN DAMAGED MITOCHONDRIA

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We showed in a previous paper [1] that biosynthesis of protein takes place in the mitochondria,* isolated and damaged by lysis in water, from rat liver cells, and we also studied the effect of certain enzyme poisons (dinitrophenol and sodium fluoride) on this process.

In the present communication we give a further account of protein biosynthesis in the damaged mitochondria.

Isolation of the mitochondria, the mode of inflicting damage and the method of conduction of the experiments on protein biosynthesis were described by us before [1].

TABLE 1

The Effect of Cofactor Obtained From Damaged Mitochondria on Protein Synthesis in Intact Mitochondria

Increase in the content of protein (in mg) obtained by addition of cofactor from	
intact mitochondria	damaged mitochondria
+ 0.26	+ 0.13
+ 0.17	+ 0.26
+ 0.14	+ 0.14
+ 0.20	+ 0.20

TABLE 2

The Effect of Cofactor Obtained From Damaged Mitochondria on Protein Synthesis in the Same

Increase in the content of protein (in mg) obtained by addition of cofactor from	
intact mitochondria	damaged nitochondria
+ 0.18	0
+ 0.20	+ 0.07
+ 0.18	0
+ 0.16	– 0.20
+ 0.26	+ 0.06

EXPERIMENTAL METHOD AND RESULTS

It is known that the process of biosynthesis of protein requires a cofactor, which is elaborated by mitochondria during incubation [7, 3]. This factor evidently provides the necessary energy for synthesis.

* The cell fraction investigated in both the previous [1] and the present research actually consisted of a mixture of lightly staining large granules and of mitochondria themselves, and for convenience we will refer to this fraction conventionally as mitochondria.

TABLE 3

The Effect of Treatment with Potassium Chloride Solutions on Protein Synthesis by Damaged Mitochondria

System of damaged mitochondria	Increase in protein (in mg) residues treated with	
	0.14 M KCl	0.4 M KCl
+ 0.08	0	0
+ 0.27	0	0

We have shown [1] that the structural integrity of the mitochondria is not essential for protein synthesis, but at the same time it is still not known to what extent it is necessary for production of cofactor by the mitochondria. Experiments were accordingly carried out to provide information on this problem.

In these experiments, cofactor, obtained by suitable incubation of mitochondria destroyed by lysis in water [7, 3] was added to both intact and damaged mitochondria. As a control we used cofactor obtained by incubation of intact mitochondria under the same conditions. The results are shown in Tables 1 and 2.

As may be seen from Table 1, synthesis of protein in intact mitochondria takes an identical course after addition of cofactor from both intact and damaged mitochondria.

TABLE 4

The Effect of Ribonuclease on Protein Synthesis in Intact Mitochondria

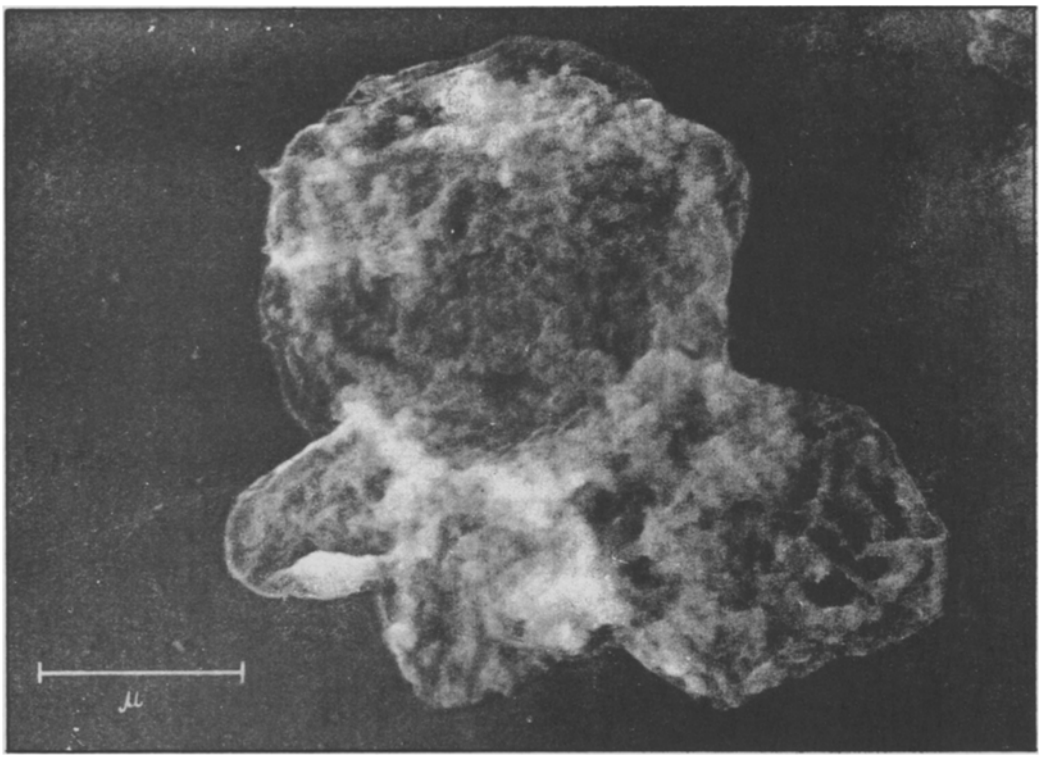
Without ribonuclease		In the presence of ribonuclease	
Increase of protein			
in mg	as a % of the original	in mg	as a % of the original
+ 0.21	+ 12.7	0	0
+ 0.13	+ 11.2	0	0
+ 0.24	+ 8.7	− 0.15	− 5.5
+ 0.15	+ 8.1	− 0.27	− 14.5
+ 0.07	+ 2.9	− 0.08	− 3.3

TABLE 5

The Effect of Ribonuclease on Protein Synthesis in Damaged Mitochondria

Without ribonuclease		In the presence of ribonuclease	
Increase of protein			
in mg	as a % of the original	in mg	as a % of the original
+ 0.15	+ 9.0	− 0.02	− 1.2
+ 0.41	+ 25.0	+ 0.02	+ 1.2
+ 0.36	+ 20.0	+ 0.18	+ 10.0
+ 0.15	+ 6.1	+ 0.06	+ 2.4

A different picture is seen in the synthesis of protein in the damaged mitochondria. It follows from Table 2 that protein synthesis takes place after addition of cofactor from intact mitochondria, whereas on addition of cofactor from damaged mitochondria no increase in the protein content is observed. The question arises: why



Membranes of destroyed mitochondria, treated with solutions of potassium chloride (0.4 μ). Magnification x 27,000.

after the addition of cofactor from damaged mitochondria does protein synthesis take place in the intact granules but not in the damaged ones? Is not cofactor produced by intact mitochondria spontaneously during incubation? For the elucidation of this problem special experiments were carried out in which instead of ready-made cofactor, we added to intact and to damaged mitochondria adenosinetriphosphate, succinic acid, glucose and cytochrome C, i.e. all the essential compounds for the production of active cofactor during incubation of intact mitochondria [7, 3].

It was found that in this case intact mitochondria are able to synthesize protein, but no protein synthesis is observed in the damaged mitochondria, i.e. it appears that the above assumption which we made is correct.

Ribonucleic acid is known to be of essential importance in the biosynthesis of protein [9, 6]. This was shown to be so for various protein-synthesizing objects.

In order to examine the role of ribonucleic acid in the synthesis of protein by isolated mitochondria, experiments were performed in which ribonucleic acid was removed from the system by extraction with saline. For this purpose a suspension of mitochondria, lysed in water, was centrifuged at 7500 g during cooling. One part of the residue was then extracted three times with 0.14 M potassium chloride solution and the remaining part with 0.4 M potassium chloride. At the conclusion of the last centrifugation the residue was suspended in the medium used in the experiment and its protein-synthesizing power was investigated. As a control we used a complete system of damaged granules.

The results of the experiments are shown in Table 3.

As we can see, under these experimental conditions protein synthesis ceased completely.

Examination of the residue,* obtained as described above, with the electron microscope showed that it is composed of membranes from mitochondria, largely free from contents (see Figure). In these experiments it is

* The electron-microscopic study was carried out in the electron microscopy room of the Institute of Experimental Biology by Scientific Assistants A. P. Pekhov and V. N. Reingol'd, to whom we express our deep gratitude.

evident that the action of the salt removed part of the proteins as well as the ribonucleic acid, and so it is hardly possible to ascribe the disappearance of the protein-synthesizing power exclusively to the removal of ribonucleic acid.

Experiments were then carried out in which ribonucleic acid was removed from the system by means of ribonuclease. For this purpose, before the beginning of incubation, 20 μg of crystalline ribonuclease was added to part of the experimental samples in addition to all the other components necessary for protein synthesis. As controls we used samples to which no ribonuclease was added.

The results obtained are shown in Tables 4 and 5.

As may be seen from these tables, decomposition of ribonucleic acid by ribonuclease leads to suppression of the process of protein synthesis in both intact and damaged mitochondria. Evidently the mitochondrial membranes do not hinder the action of ribonuclease.

Experiments on the production of cofactor from damaged mitochondria showed that the integrity of the mitochondria is a necessary condition for its production by these structures. Hence in order that the damaged mitochondria should be capable of protein synthesis in vitro, cofactor is necessary, which is produced by intact mitochondria. It must be assumed that during destruction of mitochondria there is disturbance of oxidative phosphorylation processes which are essential for the production of cofactor. At the same time, isolated and intact mitochondria have the power to synthesize proteins without the addition of ready-made cofactor, which is elaborated by them spontaneously in the process of protein synthesis.

Reports have recently appeared [5, 8, 4] that despite damage to the structure of mitochondria, the oxidative phosphorylation processes within them are preserved, but nevertheless it must be remembered that an essential factor here is the method of damaging the mitochondria. In the papers cited [4, 5, 8] this was done by means of digitonin and not by lysis in distilled water as was done in our own investigation.

As the investigation with the electron microscope showed, the residue from the suspension of mitochondria, damaged by treatment with saline, consists mainly of mitochondrial membranes, and moreover this preparation does not synthesize proteins. The reasons why the protein-synthesizing power of these preparations is disturbed are not yet known. On the one hand we can hardly ascribe to the membrane the power to synthesize protein, since it presumably does not contain ribonucleic acid [2]; on the other hand, the treatment carried out largely removes from the preparation not only the contents of the mitochondria but also, besides ribonucleic acid, certain saline-soluble proteins and extractives, so that the membranes obtained in this experiment and used in the experiments on biosynthesis of proteins differ considerably in their composition and possibly also in their structure from natural membranes.

The experiments with crystalline ribonuclease show that ribonucleic acid plays a direct part in protein synthesis by both intact and damaged mitochondria.

SUMMARY

Experiments were performed with mitochondrias lyzed in distilled water. It was demonstrated that mitochondrias have to be intact for formation of the cofactor required for the protein biosynthesis in vitro. Isolated intact mitochondrias are able to synthesize protein without any addition of a previously obtained cofactor.

Destructed mitochondrias as are able to synthesize protein only with the addition of a cofactor obtained from intact mitochondrias. Treatment with crystalline ribonuclease results in the inhibition of the protein synthesis both by intact and destructed mitochondrias.

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