CONTRIBUTION OF MITOCHONDRIAL PROTEIN SYNTHESIS TO THE FORMATION OF CYTOCHROME OXIDASE IN *LOCUSTA MIGRATORIA*

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1. Introduction

It has been shown recently that one polypeptide of a cytochrome oxidase preparation from *Neurospora crassa* was labeled by the *in vivo* incorporation of radioactive leucine in the presence of cycloheximide. This polypeptide was considered to be a product of the mitochondrial protein synthesis [1]. In this paper the mitochondrial contribution to the formation of cytochrome oxidase in the flight muscle of *Locusta migratoria* was investigated. It is well known that the size of the mitochondrial DNA is considerably smaller in higher animals than in fungi [2]. Hence one may ask, whether or not products of the mitochondrial protein synthesis have been eliminated during evolution. The experiments presented here suggest that in the case of *Locusta migratoria* one polypeptide of cytochrome oxidase is also synthesized by mitochondrial ribosomes. The electrophoretic mobility of this polypeptide was found to be similar to the corresponding polypeptide from cytochrome oxidase of *Neurospora crassa.*

2. Methods

Locusta migratoria flight muscle mitochondria were prepared as described elsewhere [3].

Mitochondrial membrane protein was labeled according to the procedure described by Sebald [4]. 60 locusts were injected two days after the imaginal moult with 0.25 mCi L-[^{3}H]leucine and 50 μg cycloheximide each, in 20 μl saline. Cycloheximide was reinjected three times during the following 70 min and then the locusts were sacrificed. In control experiment a total of 5 mCi L-[^{3}H]leucine was injected in the absence of cycloheximide.

Cytochrome oxidase was isolated by means of chromatography on oleyl–polymethacrylic acid resin [5, 1

Sodium dodecylsulfate gel electrophoresis was performed as reported recently [1] modified by using 15% polyacrylamide gels.

The spectra of cytochrome oxidase were recorded with a Beckman DK 1 A spectrophotometer.

Cytochrome oxidase activity was measured by following the decrease of absorbance at 550 nm in a solution containing 7 μM ferrocytochrome *c*, 0.1 M Tris-acetate, pH 7.5 and 0.5% Tween 80 [6].

Protein and radioactivity were determined as described previously [1].

3. Results and discussion

3.1. *Absorption spectra and enzymatic activity of cytochrome oxidase preparation*

The absolute spectra of the air oxidized and dithionite reduced forms of cytochrome oxidase preparation from *Locusta migratoria* are shown in fig. 1. The ratio of absorbance at 278 nm (ox.)/441 nm (red.) is 2.5, a value similar to that reported for cytochrome oxidase isolated from beef heart [7]. Cytochrome oxidase preparations from locusts displayed absorption maxima at 599 nm and 421 nm in the oxidized form, and at 601 nm and 441 nm in the reduced form, differing from analogous cytochrome oxidase preparations from *Neurospora crassa* with maxima at 604 nm, 428 nm, 605 nm, and 443 nm [1]. The pre-

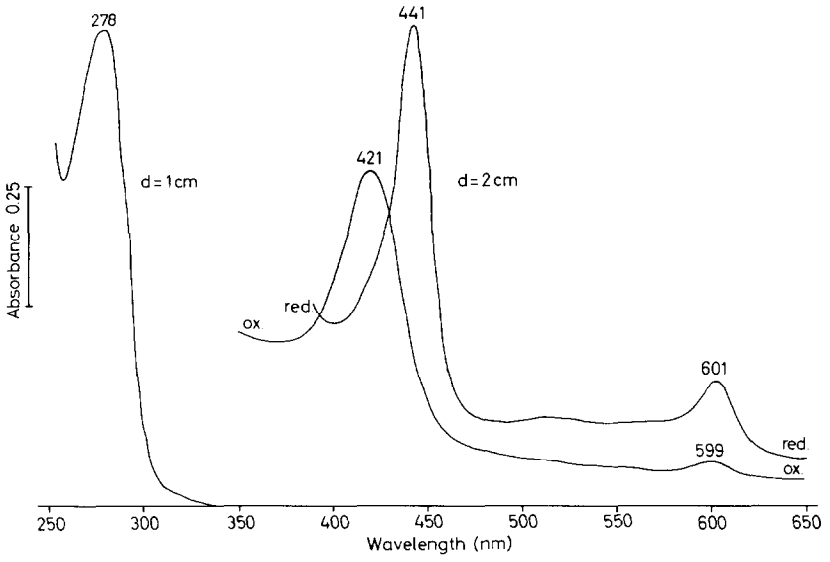

Fig. 1. Absolute spectra of the air oxidized and dithionite reduced cytochrome oxidase preparation.

parations from locusts were enzymatically active: Turnover rates of 2–4 moles ferrocytochrome c × mmole cytochrome aa_3^{-1} × min^{-1} were measured with isolated cytochrome oxidase and of 5–10 moles ferrocytochrome c × mmole cytochrome aa_3^{-1} × min^{-1} with whole mitochondrial membrane protein.

3.2. *Mitochondrially synthetized polypeptide of cytochrome oxidase*

The protein moiety of cytochrome oxidase was labelled by *in vivo* incorporation of [^{3}H]leucine in the presence of cycloheximide (table 1). Its specific ^{3}H-radioactivity greatly exceeded the specific ^{3}H-radioactivity of cytoplasmic proteins. In control experiments in the absence of cycloheximide, [^{3}H]leucine was incorporated similarly into the proteins of cytoplasm and mitochondria.

By means of gel electrophoresis in sodium dodecylsulfate medium the protein of the cytochrome oxidase preparation was separated into 7 main fractions (fig. 2). By calibration of the gel with proteins of known molecular weights, the bands were attributed to polypeptides with apparent molecular weights of 8 000, 10 000, 12 500, 14 500, 19 000, 24 000 and 38 000. All bands contained about the same amount of label when [^{3}H]leucine was incorporated into cytochrome oxidase in the absence of cycloheximide. However, with cycloheximide present, only the polypeptide at the molecular weight region of 19 000 incorporated significant radioactivity. This band is considered to be a product of mitochondrial protein synthesis.

The electrophoretic pattern of the protein of the whole mitochondrial membrane showed a great number of bands (fig. 3). This is reflected by protein staining as well as by determination of radioactivity incorporated in the absence of cycloheximide. When labeling was performed in the presence of this inhibitor only 3 radioactive peaks were detected. It is

Table 1
Distribution of [^{3}H]leucine incorporated in the presence and absence of cycloheximide.

Addition	Specific radioactivity of			
	100 000 g Supernatant of cytoplasm	Mitochondrial matrix	Mitochondrial membrane	Isolated cytochron oxidase
	(counts × min^{-1} × μg $protein^{-1}$)			
None	200	155	165	96
Cycloheximide	1	2	40	37

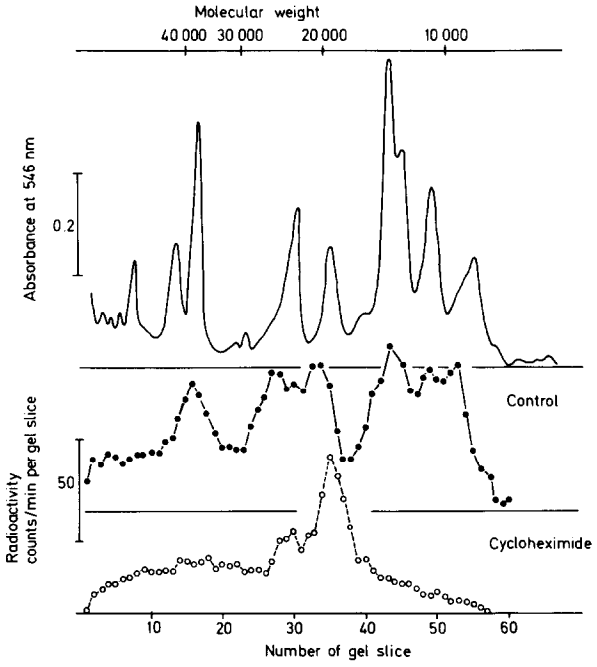

Fig. 2. Electrophoretic pattern of cytochrome oxidase preparation. (———) Absorbance at 546 nm of the stained protein bands; (●——●——●) radioactivity incorporated in the absence of cycloheximide; (○– – –○– – –○) radioactivity incorporated in the presence of cycloheximide.

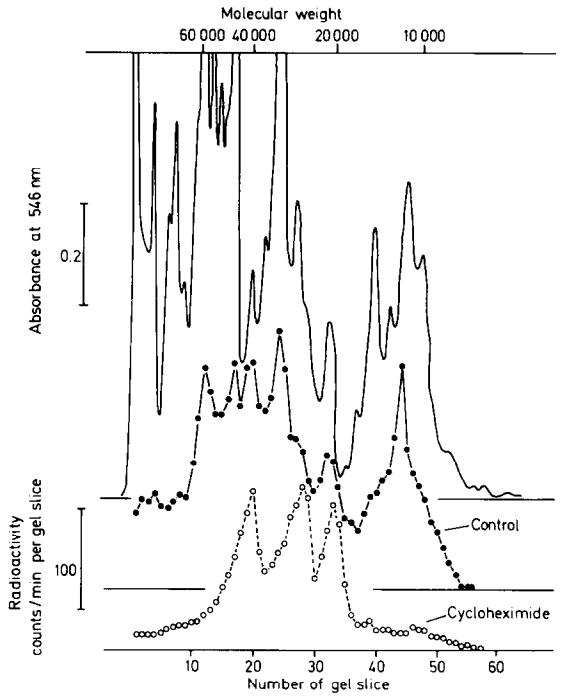

Fig. 3. Electrophoretic pattern of whole mitochondrial membrane protein. (———) Absorbance at 546 nm of the stained protein bands; (●——●——●) radioactivity incorporated in the absence of cycloheximide; (○——○——○) radioactivity incorporated in the presence of cycloheximide

noteworthy that one out of these appeared at the 19 000 weight region.

The results obtained with *Locusta migratoria* are in good agreement with data recently presented for *Neurospora crassa.* In both organisms a small number of polypeptides of the mitochondrial membrane are synthetized by the mitochondria. One with a molecular weight of approx. 19 000 appears to be a component of cytochrome oxidase. This agreement between organisms from different kingdoms may be more general, suggesting a universal mitochondrial contribution to the formation of cytochrome oxidase.

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References

[1] H. Weiss, W. Sebald and Th. Bücher, European J. Biochem. 22 (1971) 19.
[2] M. Ashwell and T.S. Work, Ann. Rev. Biochem. 39 (1970) 251.
[3] W. Kleinow and W. Neupert, FEBS Letters 15 (1971) 359
[4] W. Sebald, Th. Hoffstötter, D. Hacker and Th. Bücher, FEBS Letters 2 (1969) 177.
[5] H. Weiss and Th. Bücher, European J. Biochem. 17 (1970 561.
[6] L. Smith and H. Conrad, Arch. Biochem. Biophys. 63 (1956) 403.
[7] T. Yonetani, J. Biol. Chem. 236 (1961) 1680.