Lack of Amino Acid Incorporation by Isolated Mitochondria from Respiratory-Deficient Cytoplasmic Yeast Mutants

Isolated mitochondria are capable of incorporating amino acids into mitochondrial proteins. This ability probably reflects the existence of a functioning protein synthetizing system within mitochondria (for review see ref. [1]). A role of mitochondrial DNA in this process has been implicated [2–4].

A mutation of mitochondrial DNA in yeast results in hereditary respiratory deficiency [5]. This mutation is accompanied by morphological changes of the mitochondria [6,7], loss of cytochromes *a*, a_3, *b*, c_1 (ref. [8]), insensitivity of mitochondrial ATPase towards oligomycin [9,10] and changes in the properties of mitochondrial 'structural protein' [11,12]. The same modifications of yeast mitochondria have been found to occur in wild-type yeast cells grown in the presence of antibiotics [7,10–14] known as a potent inhibitor of protein synthesis in isolated mitochondria: chloramphenicol [2,15], lincomycine and antibiotics of the macrolide group [15].

Analogies in the properties of mitochondria of the cytoplasmic respiratory deficient mutants and the wild-type yeast cells grown in the presence of the above-mentioned drugs suggests that the cytoplasmic mutation to respiratory deficiency in yeast could alter the protein synthetizing activity of the mitochondria [7]. Moreover, such a possibility may be considered on the basis of the finding of WINTERSBERGER [16], who observed that mitochondria of cytoplasmic respiratory-deficient yeast mutants lack the RNA components typical of wild-type mitochondria. The present paper investigates the possibility of the altered protein synthetizing activity of mitochondria of cytoplasmic respiratory-deficient yeast mutants by comparing the incorporation of ^{14}C-leucine by isolated mitochondria from these mutants and from wild-type yeast cells.

The *Saccharomyces cerevisiae* Hansen DTXII (ϱ^+), diploid, was obtained from brewery Trenčín. The corresponding respiratory-deficient mutant DTXII A (ϱ^-), diploid was prepared in our institute from DTXII by acriflavine treatment. Strain D-243-2B-116 (ϱ^-), highly suppressible haploid, was kindly provided by Dr. H. JAKOB, Centre de Génétique Moléculaire, Gif-sur-Yvette (France). Cultivation of the cells, preparation of spheroplasts and mitochondria were performed according to KOVÁČ et al. [9,17]. In every single experiment all strains were tested simultaneously. Equilibrium centrifugation [18] revealed identical positions of mitochondria from the wild-type and respiratory-deficient mutants in sucrose gradient. Sterile solutions were used for preparation of mitochondria. The mitochondria were suspended in 0.65 *M* mannitol-1 m*M* EDTA, pH 7.6 and centrifuged twice for 10 min at 1500 g. They were then sedimented by 15 min centrifugation at 28,000 *g*, washed once with 0.65 *M* mannitol-1 m*M* EDTA, pH 7.6 and suspended in the same solution to the concentration of 10 mg protein/ml. Aliquots (0.1 ml) of this suspension were added to 20 ml Erlenmayer flasks containing the following components (cf. ref. [15]) in a volume of 0.8 ml: 40 µmoles *Tris*-HCl (pH 7.4), 5 µmoles KH_2PO_4, 100 µmoles KCl, 8 µmoles $MgCl_2$, 1 µmole ATP, 10 µmoles phosphoenolpyruvate, 30 µg pyruvate kinase and chloramphenicol or cycloheximide in concentrations indicated in the Table. After 3 min incubation at 30 °C, 0.4 µC of ^{14}C-leucine (24 µC per µmole) in 0.1 ml was added. After 20 min incubation with shaking at 30 °C the incorporation was stopped and proteins precipitated by the dropwise addition of 5 m 10% trichloroacetic acid containing 1 mg ^{12}C-leucine/ml. The precipitate was washed once with the same solution of trichloroacetic acid and treated further according to LAMB et al. [15]. Radioactivity was determined in a Packard Tri Carb scintillation counter with a counting efficiency of 25–30%.

The conditions described above were found to be optimal for the incorporation of ^{14}C-leucine into mitochondria isolated from wild-type yeast cells. Under these conditions mitochondria from wild-type yeast incorporated about 70 pmoles of ^{14}C-leucine/mg protein per 20 min into trichloroacetic acid-insoluble fraction. In the absence of exogenous ATP or ADP plus oxidizable substrate incorporation was at the limit of detection. Oligomycin stimulated the incorporation driven by exogenous ATP. Incorporation was severely inhibited by chloramphenicol and almost unaffected by cycloheximide. This observation shows that under our conditions the contribution of contaminating cytoplasmic microsomes to the total incorporation was negligible. All these findings are in agreement with those described by LAMB et al. [15].

Incorporation of ^{14}C-leucine into mitochondria isolated from wild-type and cytoplasmic respiratory-deficient mutant yeast cells

Strain	Inhibitor	pmoles of ^{14}C-leucine incorporated per mg protein in 20 min	Inhibition (%)
DTXII	none	77	–
	0.5 m*M* Chloramphenicol	4.5	94.3
	3×10^{-5}% Cycloheximide	73.5	4.6
DTXII A	none	0	–
D-243-2B-116	none	0	–

Results of a typical experiment are presented.

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Under conditions optimal for the incorporation of ^{14}C-leucine into wild-type mitochondria, mitochondria from cytoplasmic respiratory-deficient mutants did not incorporate detectable amounts of radioactivity.

The inability of isolated mitochondria from cytoplasmic respiratory-deficient mutants to incorporate ^{14}C-leucine into trichloroacetic acid-insoluble fraction under conditions where wild-type mitochondria were active could be most simply explained as a direct result of the mutation of mitochondrial DNA. Even though other possibilities, e.g. greater susceptibility of mutant mitochondria to damage or a loss of some auxiliary components during isolation have not been completely excluded, it seems to be very likely that mitochondria of cytoplasmic respiratory-deficient mutants lack the protein synthetizing activity in situ as well. The finding thus underscores the importance of the product of mitochondrial protein synthesis for structural and functional state of mitochondria[19].

Zusammenfassung. Die Mutation der mitochondrialen DNA, die in den atmungsdefekten Hefen resultiert, führt zu dem Verlust der Fähigkeit der isolierten Mutantenmitochondrien, ^{14}C-Leuzin in die TES-unlösbare Fraktion zu inkorporieren.

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Effect of Dichloro Diphenyl Trichloro-Ethane (DDT) on Leghemoglobin Content of Root Nodules of *Phaseolus aureus* (Green Gram)

Pesticidal chemicals in increasing quantities are finding their way into soil. Some pesticides are known for their long persistence in soil[1], being inhibitors of biological system of one sort they may also exert deleterious effect on soil microbial population and their activities which are of importance for soil fertility.

The formation of nodules on the roots of legumes is due to symbiosis between *Rhizobium* and the host plant. There are reports that there is a relationship between leghemoglobin content of nodules and nitrogen fixation[2–6]. This communication deals with the effect of DDT on the leghemoglobin content of root nodules of green gram plants (*Phaseolus aureus*).

Materials and methods. Different levels of the insecticides-0, 1, 5, 10, 40, 100 and 1000 ppm were mixed with an alluvial soil (2.5 kg each), sandy loam in nature, in pots. Organic carbon, total nitrogen and pH of the soil were 0.53, 0.053% and 7.8 respectively. Effect of organic matter was also studied in respect of reduction of toxicity of higher doses of the insecticide. Moisture was adjusted to $^1/_3$ of the water holding capacity of the soil; healthy seeds of the crop (variety Pusa basakhi, a short duration crop which matures in 75 days) were inoculated with 48 h old culture of specific *Rhizobium* and were sown. Plants were uprooted after 4 and 7 weeks of growth, nodules were separated, weighed and the leghemoglobin content was determined by Benzidine hydrogen peroxide method[7].

Results. The results (Table) showed that the insecticide up to 40 ppm was not toxic to the leghemoglobin content of the nodules of the crop. Moreover, DDT at the level of 1, 5 and 10 ppm increased the leghemoglobin content and the increases over control with the above concentrations were 12.2, 37.2 and 27.2% in the first and 59.7, 57.9 and 30.5% in the second uprooting respectively. With higher concentrations (100 and 1000 ppm) it could not be estimated due to the absence of root nodules, except in 100 ppm DDT+ organic matter treatment. It is worth noting that in the case of 100 ppm of the insecticide, 1% organic matter application to soil neutralized the toxic effect of the insecticide remarkably.

Effect of DDT on nodulation and leghemoglobin content of *Phaseolus aureus*

Level of DDT (ppm)	After 4 weeks of growth		After 7 weeks of growth	
	Wt. of nodules (g)	Leg-hemoglobin mg/g nodules	Wt. of nodules (g)	Leg-hemoglobin mg/g nodules
Control (0)	0.027	20.05	0.295	41.32
1	0.026	22.50	0.367	66.00
5	0.031	27.00	0.371	65.23
10	0.026	25.50	0.244	53.92
40	0.027	18.00	0.157	42.09
100	Nil	–	Nil	–
100 + 1% farm yard manure	0.017	15.25	0.056	39.66
1000	Nil	–	Nil	–
1000 + 1% farm yard manure	Nil	–	Nil	–
Significant C.D. at 5%	0.01		0.05	3.24

Zusammenfassung. Geringe Mengen von DDT im Boden fördern die Bildung von Wurzelknöllchen bei Leguminosen und erhöhen den Gehalt an «Leghämoglobin» in den Knöllchen.

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