ANTIBIOTIC MUCIDIN, A NEW ANTIMYCIN A-LIKE INHIBITOR OF ELECTRON TRANSPORT IN RAT LIVER MITOCHONDRIA

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SUMMARY: Mucidin at concentration 1 /ug/mg mitochondrial protein completely inhibited the oxidation of succinate and NADH-linked substrates in rat liver mitochondria. In succinate oxidizing mitochondria mucidin induced a crossover point between cytochromes b and c + c_1. Under these conditions mucidin had no effect on the ATPase activity as well as on the phosphorylation efficiency of rat liver mitochondria measured with TMPD plus ascorbate as substrate. These properties of mucidin resemble those of other inhibitors of mitochondrial electron transport such as antimycin A and HQNO.

Mucidin has recently been reported as an antifungal antibiotic produced by the basidiomycete Oudemansiella mucida (1). This antibiotic has been isolated, crystallized and some of it biological as well as physico-chemical properties were described (2,3). Moreover, we found that antibiotic specifically inhibited the respiration of intact mold and yeast cells (4). In this communication we report the effects of mucidin on oxidative phosphorylation in rat liver mitochondria and conclude that the mode of action of mucidin is very similar to that of antimycin A.

Abbreviations: TMPD, N,N,N',N'- tetramethyl-p-phenylenediamine
DNP, 2,4 dinitrophenol
EGTA, ethyleneglycol-bis(β-aminoethyl ether)-N,N'-tetraacetic acid

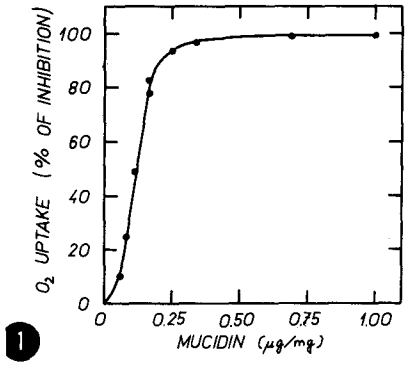
MATERIAL AND METHODS

Rat liver mitochondria were prepared by the method of Johnson and Lardy (5), except that a medium of 250 mM sucrose, 20 mM Tris-HCl and 1 mM Tris-EGTA (final pH 7.4) was used throughout the preparation, and also for the final suspension of mitochondria. The uptake of oxygen was measured polarographically with a vibrating gold electrode (6). The time course and extent of oxidation and reductions of the flavoproteins and cytochromes were measured with a dual wavelength spectrophotometer (Hitachi Perkin-Elmer, model 356) (7). Mitochondrial ATPase activity was determined by measuring the release of inorganic phosphate (8). Stoichiometry of oxygen uptake and phosphate esterification in the presence of TMPD in rat liver mitochondria were determined manometrically as described by Chamalaun and Tager (9). Mitochondrial protein was determined by the biuret procedure (10). Mucidin was used as a solution in absolute ethanol.

RESULTS AND DISCUSSION

Mucidin at concentration 1 μg/mg mitochondrial protein completely inhibited the oxygen uptake in rat liver mitochondria measured in the presence of NADH-linked substrates (pyruvate plus malate, α-ketoglutarate plus malate) as well as succinate. Results presented in Fig. 1 show that half-maximal inhibition of DNP-uncoupled respiration is obtained with about 0.125 μg mucidin/mg mitochondrial protein. On the other hand, the oxidation of TMPD plus ascorbate was unaffected by mucidin (1 to 50 μg/mg protein).

Dual wavelength spectrophotometer recordings of the oxidation--reduction behaviour of flavoproteins and cytochromes $\underline{b}$ and $\underline{c} + \underline{c}_1$ in mucidin-treated rat liver mitochondria, presented in Fig. 2, clearly show a crossover point between cytochromes $\underline{b}$ and $\underline{c} + \underline{c}_1$.



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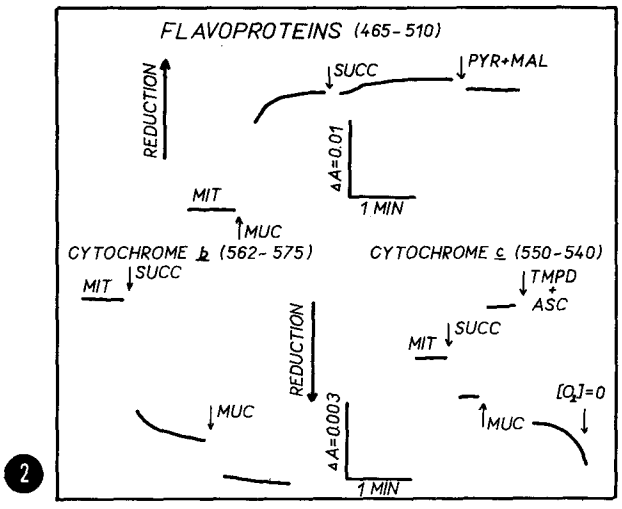

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Fig. 1. Effect of mucidin on the oxidation of succinate in rat liver mitochondria. The reaction mixture contained in 1 ml : 0.25 M mannitol, 10 mM potassium phosphate, 10 mM Tris-HCl, 10 mM potassium succinate, 0.1 mM DNP, mitochondria (2 to 4 mg protein) and mucidin (as indicated in the Fig.) ; final pH 7.4. Temperature 30°C. The values are means of 4 experiments.

Fig. 2. Dual wavelength spectrophotometer recordings of the effect of mucidin on the oxidation-reduction behavior of flavoproteins and cytochromes $\underline{b}$ and $\underline{c}$ + $\underline{c}_1$ in rat liver mitochondria. The light path was 1 cm. Concentrations were: MIT = 2.2 mg/ml mitochondrial protein, MUC = 2 μg/mg mitochondrial protein, SUCC = 10 mM succinate, PYR = 10 mM pyruvate, MAL = 5 mM malate, TMPD = 0.2 mM N,N,N',N'- tetramethyl-p-phenylenediamine dihydrochloride, ASC = 7.5 mM ascorbate. Reactions were carried out in 3.0 ml medium (0.25 M mannitol, 10 mM potassium phosphate, 10 mM Tris-HCl, 10 mM KCl, final pH 7.4) at room temperature.

In this respect, no qualitative difference was observed in the effect of antimycin A and mucidin in rat liver mitochondria oxidizing succinate or pyruvate plus malate.

As is shown in Fig. 3, mucidin at concentrations sufficient to prevent the electron transport through the respiratory chain had no effect on DNP-induced ATPase activity of rat liver

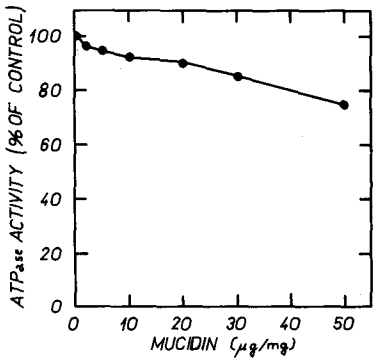

Fig. 3. Effect of mucidin on DNP-induced ATPase activity of rat liver mitochondria. The reaction mixture contained in 1 ml: 0.25 M mannitol, 25 mM sucrose, 30 mM KCl, 10 mM Tris-HCl, 6 mM ATP, 0.2 mM DNP, mucidin (at concentrations indicated in Fig.) and mitochondria (1 mg protein), final pH 7.4. After 10 minutes of incubation at 37°C the liberated inorganic phosphate in the trichloroacetic acid extract was determined. The values are means of three experiments.

mitochondria. Only at relatively high concentrations of mucidin a significant inhibition of ATPase activity was observed. The ATPase activity of mitochondria measured in the absence of DNP was only slightly affected by mucidin (1 to 10 μg/mg mitochondrial protein).

Table I presents oxidative phosphorylation data obtained with mitochondria with TMPD plus ascorbate as substrate. Under the experimental conditions used, mucidin (0.62 to 12.5 μg/mg protein) had no effect on the stoichiometry of the oxygen uptake and the phosphate esterification indicating that mucidin does not uncouple or inhibit the energy transduction in rat liver mitochondria.

Hydroxynaphthoquinones (11,12), 2,3 dimercaptopropanol (13,14) and antibiotics such as antimycins (15,16) and hydroxyquinoline-N-oxides (17) represent the group of specific inhibitors of the mitochondrial electron transport between

TABLE I

Effect of mucidin on oxidative phosphorylation with TMPD plus ascorbate in rat liver mitochondria. The main compartment of the Warburg vessels contained in 2 ml: 50 mM sucrose, 15 mM KCl, 2 mM EDTA, 50 mM Tris-HCl, 10 mM potassium phosphate, 0.5 mM ATP, 25 mM glucose, 5 I.U. hexokinase (Sigma,Typ III) , 1 µg rotenone, 0.1 per cent bovine serum albumin, 0.2 mM TMPD, 7.5 mM ascorbate, DNP or mucidin (as indicated in the Table) and mitochondria (3.2 mg protein); final pH 7.4. The central well contained 0.2 ml 2 M KOH and a piece of fluted paper. The measurements commenced, after 7 min of termal equilibration, by the addition of the glucose and hexokinase from the side arms and after 20 min of additional incubation at 30°C were terminated with 5 per cent trichloroacetic acid. Inorganic phosphate was determined in the supernatant by the methods of Sumner (8). Each values is the mean of two incubations.

Mucidin (µg/ml)	DNP (mM)	ΔO (µgatoms)	ΔP_i (µmoles)	P/O
-	-	3.49	3.28	0.94
-	0.25	4.01	0	0
1	-	3.39	3.08	0.91
2	-	3.21	2.86	0.89
10	-	3.30	3.04	0.92
20	-	3.21	2.95	0.92

cytochromes b and c. Thus, at presence, mucidin is the third antibiotic interacting with the b.c region of the respiratory chain. Taking into account the chemical formula of mucidin $C_{16}H_{18}O_3$ (3), this antibiotic might be one of the derivates of the hydroxy-naphtoquinone. However, recent results obtained about its structure ruled out such a possibility (18).

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