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CHLORAMPHENICOL-INDUCTION OF A SECOND CYANIDE- AND AZIDE-INSENSITIVE MITOCHONDRIAL PATHWAY IN *USTILAGO MAYDIS*

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Summary

Growth of wild type *Ustilago maydis* in presence of chloramphenicol results in increased resistance of cyanide-insensitive respiration to hydroxamic acid derivatives. The biphasic nature of the Dixon plots for hydroxamate inhibition of mitochondrial alternative respiration suggested involvement of two cyanide-insensitive systems. This hypothesis was confirmed by the analysis of kinetic data. One of the two systems exhibits normal sensitivity to hydroxamates and appears similar to that present in mitochondria from cells grown in control medium. In contrast, the inducible system is characterised by resistance to hydroxamates and lower substrate affinity. With excess substrate and AMP in the reaction mixture the two systems have equal participation in the cyanide-insensitive respiration of chloramphenicol-treated mitochondria. In the absence of AMP, however, the inducible oxidase seems to exhibit much higher affinity for oxygen. The new system appears responsible also for changes in sensitivity of alternative respiration to low temperatures. It differs from the hydroxamate resistant systems recently reported for other fungi in its high activity and its complete insensitivity to azide and cyanide.

Introduction

Some organisms are known to constitutively form an alternative mitochondrial electron transport pathway which is insensitive to cyanide and other inhibitors of the cytochrome chain. Wild type strains of the corn smut fungus *Ustilago maydis* belong to this category [1,2]. In other organisms, normally sensitive to cyanide, the formation of an alternative pathway may be induced

by changes in the conditions of cultivation. Growth in presence of chloramphenicol, the known inhibitor of mitochondrial translation, is one of the most popular methods for this induction [3—5].

Sensitivity to hydroxamic acid derivatives appears to be a common feature of the constitutive and the inducible cyanide-insensitive systems [6]. When, however, we examined the respiration of *U. maydis* sporidia, grown for 24 h in chloramphenicol-containing medium we observed high rates of substrate oxidation in presence of high concentrations of both KCN and benzhydroxamic acid. This paper will describe experiments showing that while in mitochondria from *U. maydis* cells grown in control medium cyanide-insensitive respiration is mediated by one, in mitochondria from chloramphenicol-treated cells such respiration is mediated by two distinct pathways. The main differences between the two systems will be described.

Part of this work has been presented in preliminary form at a scientific meeting [7].

Materials and Methods

In all experiments the wild type strain ATCC 14826 of *U. maydis* was used. This strain was maintained on slants of the complete medium of Holliday [8] and was subcultured every 2 months. To obtain sporidia active inoculum was prepared by growing the fungus for 20 h in 250-ml shake flasks containing 50 ml of a glucose medium [9] supplemented with 0.1% yeast extract. Such 50-ml inocula were transferred to 2-l flasks containing 500 ml of the same medium with or without chloramphenicol (10.0 mM). The antibiotic was added to the autoclaved medium from a stock solution in methanol. In all cases cultures were incubated on a rotary shaker at 30°C and 180 rev./min. Sporidia were harvested after 24 h of incubation by centrifugation at 4000 × *g* and 4°C for 5 min. Washing and desruption of cells to obtain mitochondria was done by the method of White and Thorn [10] with minor modifications. In a previous paper [11] we have described the behavior of mitochondria obtained by this method from sporidia grown in control medium.

Chloramphenicol, antimycin A, and rotenone were purchased from Sigma Chemical Co., benzhydroxamic acid from Kodak, salicyl hydroxamic acid from Aldrich Chemical Company, KCN from Merck, and sodium azide (NaN_3) from Riedel-de Haen AG. Stock solutions of chloramphenicol, hydroxamates, antimycin A, and rotenone were prepared in methanol and of KCN and NaN_3 in water. Methanol concentration in the reaction mixture was 1.0% (v/v) in treated and control samples. All chemicals, not specifically mentioned here were reagent grade from either Calbiochem or Sigma Chemical Co.

Growth and whole cell respiration measurements were made using sporidia from logarithmic phase cultures and glucose 1.0% (w/v) substrate at 30°C as described by Georgopoulos and Sisler [1]. Mitochondrial respiratory activities were also determined polarographically using 0.1 ml mitochondrial preparation and 1.9 ml of a reaction mixture containing phosphate, EDTA, bovine serum albumin, cytochrome *c*, KCl, and $MgCl_2$ [11]. Unless otherwise specified, in experiments with mitochondria either 1.5 mM NADH or 25.0 mM sodium succinate were used as the respiratory substrates, measurements were conducted

at 25°C, and the initial rates of O_2 consumption were recorded. Alternative oxidase activities were measured in presence of 1.0 mM KCN and, unless otherwise stated, of 1.5 mM AMP which has been found [12] to play an important role in the cyanide-resistant respiration of *U. maydis* mitochondria. Respiration rates (V) were calculated as reported [11].

Results

Effect of chloramphenicol on growth

Addition of chloramphenicol (10.0 mM) to the medium does not cause the growth of wild type *U. maydis* to cease. However, the doubling time during the logarithmic phase was increased from 3 to 5 h and the final yield of cells was reduced to approx. 70% of the untreated control. Somewhat similar effects of chloramphenicol have been observed with other eucaryotic microorganisms [13].

Inhibitor sensitivity

The chloramphenicol treatment has little effect on the response of cell respiration of *U. maydis* to addition of KCN but renders the cyanide-insensitive O_2 uptake totally resistant to a benzhydroxamic acid concentration causing practically complete inhibition in the case of cells grown in control medium (Table I). That this hydroxamate resistance is a property of the chloramphenicol-treated mitochondria is shown by the results of the in vitro experiments also included in Table I. With both exogenous NADH and succinate as

TABLE I

SENSITIVITY OF WHOLE CELL AND MITOCHONDRIAL RESPIRATION OF *USTILAGO MAYDIS*, GROWN IN CONTROL OR CHLORAMPHENICOL (CAP)-CONTAINING MEDIUM, TO CYANIDE, BENZHYDROXAMIC ACID (BHAM), AND AZIDE

The respiration rate is given in nmoles O_2/min per mg dry weight in the case of whole cells and per mg protein in the case of mitochondria

Inhibitors	Respiration rate	
	Control	CAP
Whole cells oxidizing glucose		
None	107	114
1.00 mM KCN	161	132
1.00 mM KCN + 2.00 mM BHAM	7	126
Mitochondria oxidizing NADH		
None	321	381
1.00 mM KCN	146	339
1.00 mM KCN + 0.05 mM rotenone	45	116
1.00 mM KCN + 2.00 mM BHAM	1	106
1.00 mM KCN + 2.00 mM BHAM + 2.50 mM NaN_3	—	99
Mitochondria oxidizing succinate		
None	110	155
1.00 mM KCN	62	124
0.01 mM antimycin A	65	127
1.00 mM KCN + 2.00 mM BHAM	6	71
1.00 mM KCN + 2.00 mM BHAM + 2.50 mM NaN_3	—	74

the substrate, high rates of cyanide-insensitive, hydroxamate-resistant respiration are obtained and this respiration is insensitive to high concentrations of azide. The increased hydroxamate resistance of the treated mitochondria cannot be due to higher amounts of hydroxamate-interacting components because dilution 1 : 10 of the preparation from the chloramphenicol treatment did not proportionally affect hydroxamate sensitivity.

As in the case of control mitochondria, the cyanide-insensitive succinate oxidation of the chloramphenicol-treated mitochondria is not affected by antimycin A (Table I), indicating that the site of action of this inhibitor is bypassed. The chloramphenicol treatment is also without effect on the rotenone sensitivity of the alternative NADH oxidation (Table I).

Fig. 1 shows the results of benzhydroxamate titration experiments in the form of V_T vs. $g(i)$ plots [14]. The linearity of these plots indicates that the rate of flux through the cytochrome pathway is not affected by the inhibition of the alternative pathway, as has also been found with plant mitochondria [15]. It is also shown in Fig. 1 that while with control mitochondria of *U. maydis* oxygen uptake in the absence of cyanide is mediated only by the cytochrome pathway ($V_T = V_{cyt}$), this is not the case with chloramphenicol-treated mitochondria. The slope $\rho = 0.6$ obtained with the latter mitochondria indicates that in the absence of inhibitors the alternative respiration is operating at 60% of its maximum rate [15]. Appropriate experiments, on the other hand, showed that benzhydroxamic acid inhibition of cyanide-insensitive oxidation of succinate and NADH by *U. maydis* mitochondria is noncompetitive (results not shown).

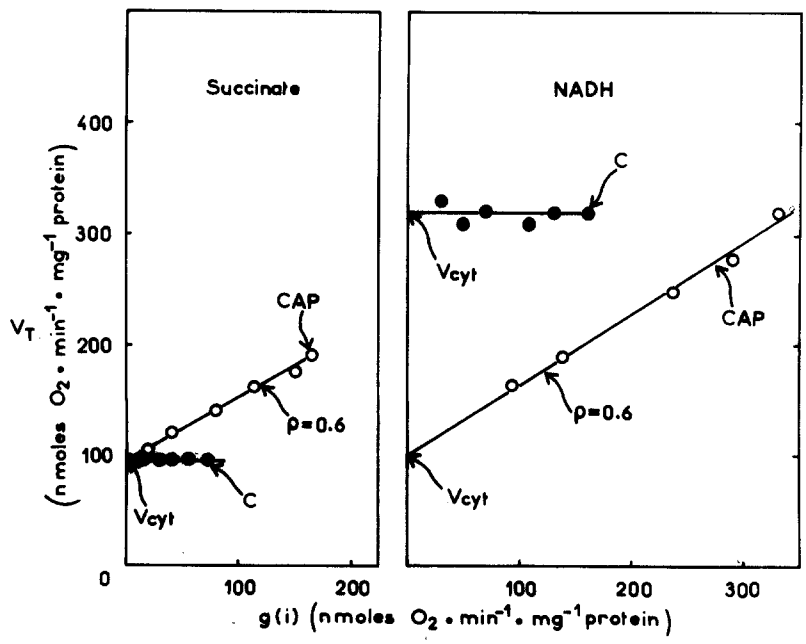

Fig. 1. V_T (total respiratory rate) as a function of $g(i)$ (rate of cyanide-insensitive respiration) at various benzhydroxamic acid concentrations. *U. maydis* mitochondria from cells grown in control (C) or chloramphenicol(CAP)-containing medium.

Dosage response experiments have shown that growing wild type cells of *U. maydis* in chloramphenicol-containing medium results in a 15-fold increase of resistance of cyanide-insensitive respiration to both hydroxamic acid derivatives in vivo (Fig. 2). Representative data from experiments with mitochondria are shown in Fig. 3 in the form of a Dixon plot. Characteristic of the behavior of the treated mitochondria is the biphasic nature of the Dixon plots for hydroxamate inhibition as compared to the linear plots obtained with control mitochondria (Fig. 3). This observation suggested that two cyanide-insensitive systems, differring in hydroxamate sensitivity, are present in mitochondria from chloramphenicol-treated cells. In three different experiments with NADH the apparent K_i values for the benzhydroxamate-resistant system of treated mitochondria ranged from 400 to 600 μM as compared to 65—90 μM for the sensitive system of control mitochondria. With succinate as the substrate the K_i values were 1000—1300 and 250-300 μM respectively. At high hydroxamate concentrations most of the cyanide-insensitive respiration of treated mitochondria must, therefore, be mediated by the resistant system.

Substrate affinity

Figs. 4 and 5 show that the double reciprocal plots relating reaction velocity in presence of cyanide to substrate concentration are linear in case of control mitochondria of *U. maydis*. The approximate K_m values, calculated from the intercept with the $1/S$ axis, in four experiments were found to be 0.07—0.12 mM and 0.3—0.7 mM for NADH and succinate, respectively. The corresponding maximal rates (V) were 200—300 and 60—100 nmol O_2/min per mg protein. By contrast, the plots obtained with chloramphenicol-treated mitochondria characteristically deviate from linearity. Deviation is more clearly seen in the Scatchard plots (Figs. 4 and 5). This difference can be explained if the cyanide-insensitive respiration of treated mitochondria is mediated by more

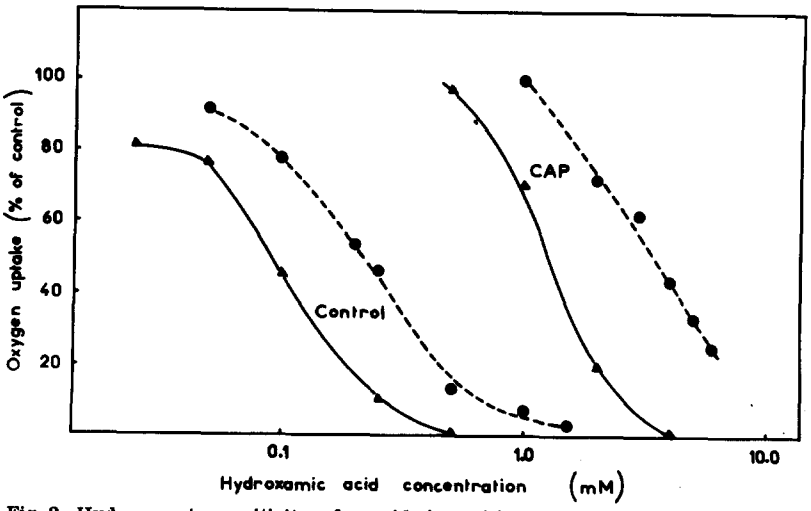

Fig. 2. Hydroxamate sensitivity of cyanide-insensitive respiration of whole cells of *U. maydis*, grown in control or chloramphenicol(CAP)-containing medium. ▲———▲, inhibition by salicyl hydroxamic acid; ●- - - - -●, inhibition by benzhydroxamic acid.

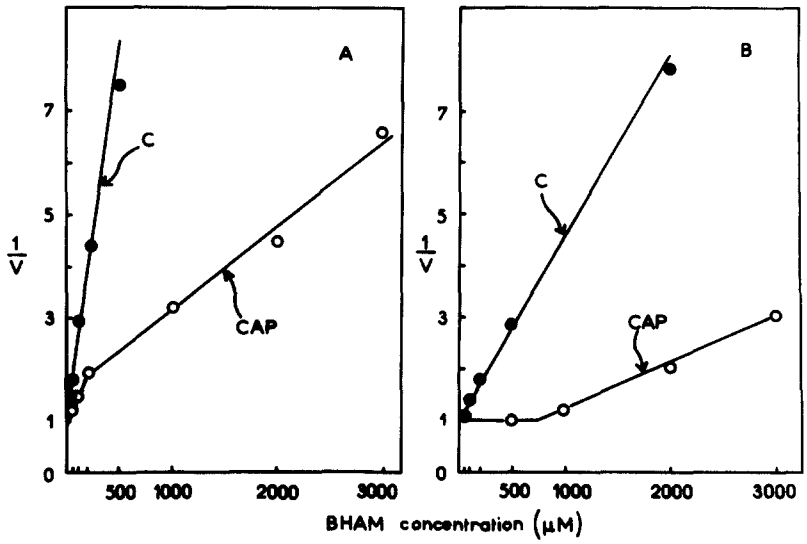

Fig. 3. Dixon plots for benzhydroxamic acid (BHAM) inhibition of the cyanide-insensitive oxidation of NADH (A) and succinate (B) by *U. maydis* mitochondria from cells grown in control (C) or chloramphenicol(CAP)-containing medium.

than one system [16]. The data were analyzed by applying the method described by Reid et al. [17]. This analysis showed that the experimental data fit the hypothesis that mitochondria from cells grown in chloramphenicol containing medium possess two cyanide-insensitive systems, one of high and one of low substrate affinity. The apparent kinetic constants for the two systems are given in Table II. With both substrates the apparent K_m values dif-

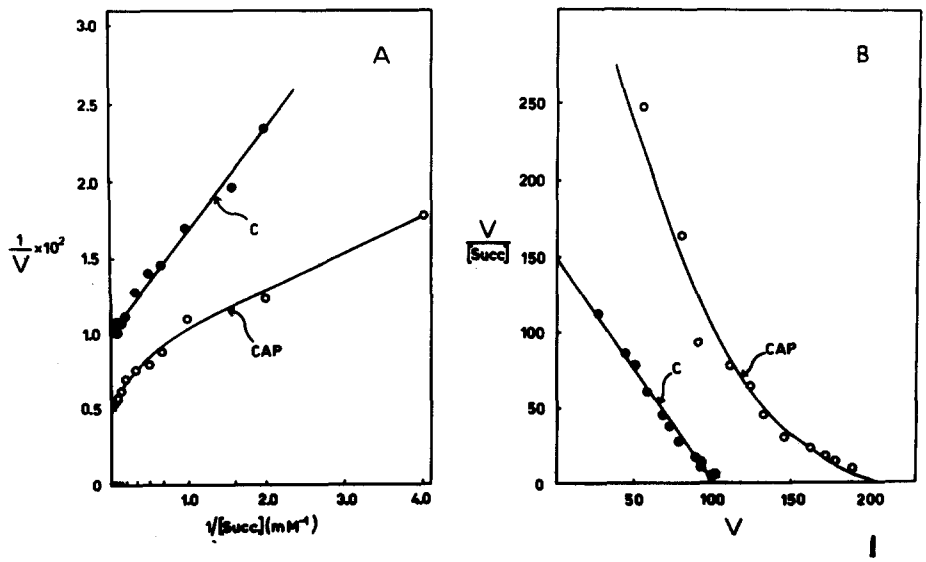

Fig. 4. Double reciprocal (A) and Scatchard (B) plots for cyanide-insensitive succinate oxidation by *U. maydis* mitochondria from cells grown in control (C) or chloramphenicol(CAP)-containing medium.

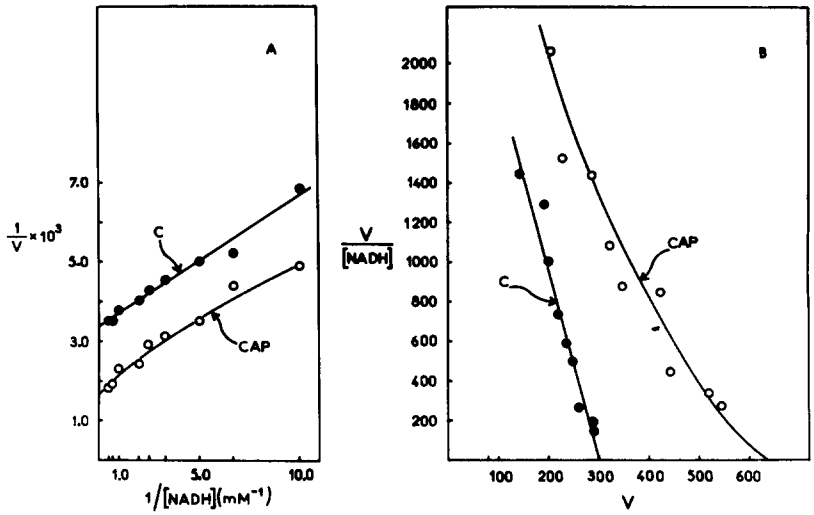

Fig. 5. Double reciprocal (A) and Scatchard (B) plots for cyanide-insensitive exogenous NADH oxidation by *U. maydis* mitochondria from cells grown in control (C) or chloramphenicol(CAP)-containing medium.

fer characteristically and both the K_m and the V values for the high affinity system of treated mitochondria are in good agreement with those of the constitutive system of control mitochondria, which is sensitive to hydroxamates. The effect of the chloramphenicol treatment must, therefore, be due to the induction of a new cyanide-insensitive pathway, of low substrate affinity, which is hydroxamate resistant. The similarity of the V values for the two systems of treated mitochondria suggests that when excess substrate is available and AMP is present in the reaction mixture (see below) each system is responsible for about 50% of the cyanide-insensitive respiration of these mitochondria.

Response to temperature changes

Temperature effects on alternative respiration were assayed by measuring the rates of O_2 reduction in presence of 1.0 mM KCN at temperatures ranging from 5 to 55°C. Fig. 6 shows Arrhenius plots of the data. Independently

TABLE II

APPARENT KINETIC CONSTANTS FOR CYANIDE-INSENSITIVE OXIDATION OF EXOGENOUS NADH AND SUCCINATE BY THE HIGH- AND THE LOW-AFFINITY SYSTEM OF *USTILAGO MAYDIS* MITOCHONDRIA FROM CELLS GROWN IN CHLORAMPHENICOL-CONTAINING MEDIUM

Substrate	High-affinity system		Low-affinity system	
	K_m (mM)	V *	K_m (mM)	V *
NADH	0.1	300	0.5	310
Succinate	0.25	100	4.0	105

* In nmol O_2/min per mg protein.

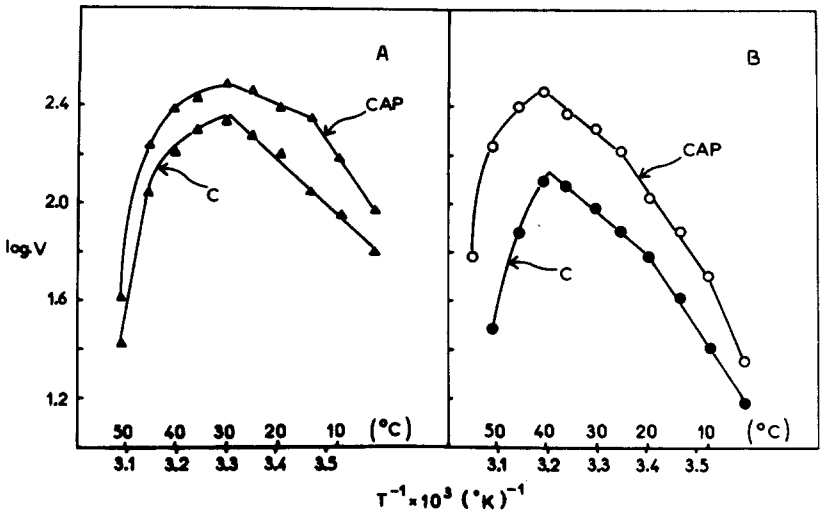

Fig. 6. Arrhenius plots of rates of cyanide-insensitive oxidation of exogenous NADH (A) and succinate (B) by *U. maydis* mitochondria from cells grown in control (C) or chloramphenicol(CAP)-containing medium.

of the chloramphenicol treatment, rates increased with increasing temperature up to 30 and 40°C with exogenous NADH and succinate respectively. Thus growth in presence of chloramphenicol does not affect the transition temperature of high temperature inactivation. It does, however, have definite effects on the response to low temperatures. With exogenous NADH as the electron donor a linear slope over the temperature range from 30 to 5°C is obtained in the case of control mitochondria but a low temperature inactivation, manifested as a sharp decrease in rate, beginning at about 15°C is observed with treated mitochondria (Fig. 6A). In addition, the treatment has caused a change in the activation energy, taken from the linear region of the Arrhenius plot, from 8.2 to 3.6 kcal/mol. Both effects support the view of involvement of some new system in the cyanide-insensitive NADH oxidation of mitochondria from cells grown with chloramphenicol. The treatment has also affected the general appearance of the Arrhenius plot of cyanide-insensitive succinate oxidase activity (Fig. 6B).

Requirement for AMP

In a previous communication [12] we reported that satisfactory and fairly constant rates of cyanide-insensitive respiration of *U. maydis* mitochondria from cells grown in control medium, are obtained only in presence of purine nucleotides which dramatically increase the affinity of the alternative oxidase for oxygen. A requirement of the untreated mitochondria for AMP is also shown in Fig. 7 which, in addition, indicates a striking effect of the treatment in this regard. With both substrates, control mitochondria require AMP even at the highest O_2 concentration that could be tested (approx. 90% air-saturation of the reaction mixture), while AMP starts to have an effect only when most of the oxygen has been reduced in the case of chloramphenicol-treated mitochondria. This difference is easily explainable on the assumption of a chloram-

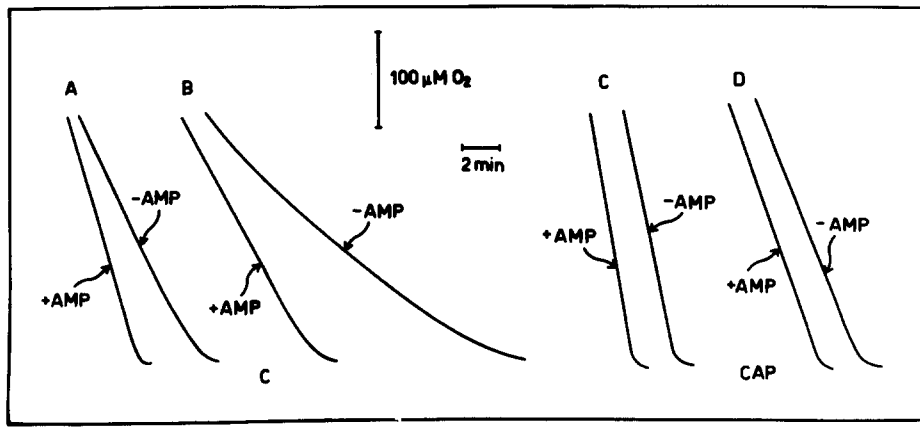

Fig. 7. Oxygen electrode traces showing the effect of AMP (1.5 mM) on cyanide-insensitive exogenous NADH (A,C) and succinate (B,D) oxidation by *U. maydis* mitochondria from cells grown in control (A,B) or chloramphenicol (C,D)-containing medium.

phenicol-induced alternative oxidase of high O_2 affinity in the absence of purine nucleotides. When such nucleotides are not present most of the cyanide-insensitive respiration of mitochondria from treated cells must then be mediated by the inducible system, hence the much lower sensitivity to O_2 tension shown in Fig. 7.

Discussion

The data of Table I show that addition of cyanide increases O_2 uptake by whole cells of *U. maydis* but has an inhibitory effect in experiments with mitochondria. Our method of cell disruption, however, yields non-phosphorylating mitochondria [11] and when the respiration of whole *U. maydis* cells was uncoupled by addition of carbonyl cyanide *m*-chlorophenylhydrazone, KCN reduced O_2 uptake (data not shown) to approximately the same extent as in the in vitro experiments of Table I. As has been suggested [2], the stimulation of respiration of whole cells caused by the inhibitors of the cytochrome pathway must result from bypassing two phosphorylation sites.

High rates of hydroxamate-insensitive, cyanide-sensitive respiration of mitochondria from cells grown with chloramphenicol are shown by the data of Fig. 1. This may appear curious in view of the known inhibitory effect of the antibiotic on the synthesis of important components of the cytochrome chain [18]. What may be suggested is that either the inhibition of synthesis is not complete or the inhibited components are working at very high efficiency. The electron flux through the cyanide-sensitive system can be determined from the V_T intercept where $g(i) = 0$ in Fig. 1 [14]. With NADH as the substrate the value for treated mitochondria is indeed lower, approx. 30% of that for control mitochondria. This, however, is not the case with succinate where the function of the cytochrome system appears unaffected by the chloramphenicol treatment, while total succinate oxidase acitivity is substantially increased Fig. 1). Apparently, the acitivity of succinate dehydrogenase rather than cyto-

chrome oxidase is the limiting factor for the oxidation of succinate by control mitochondria and the treatment somehow increases this acitivity. This view is also supported by the results of experiments with mitochondria from an antimycin-sensitive mutant which will be described in a following paper.

It might be argued that the effect of the chloramphenicol treatment on the hydroxamate sensitivity of alternative respiration (Table I) may be due only to a modification of the known, hydroxamate-sensitive, system. In such a case, however, the Dixon plots for hydroxamate inhibition should remain linear, while our measurements gave biphasic plots (Fig. 3). The involvement of two systems is clearly shown by the substrate affinity data (Figs. 4 and 5). The apparent kinetic constants given in Table II prove that the treatment does not affect the characteristics of the known cyanide-insensitive system but induces the formation of a second one. Therefore, wild type cells of *U. maydis* possess the genes coding for two cyanide-insensitive mitochondrial pathways. The phenotype of the first of these pathways appears in cells grown under normal conditions while that of the latter is induced by inhibition of mitochondrial translation. Whether such induction can be achieved by other means has not so far been investigated. The work with the antimycin-sensitive mutant, to be described in a following paper, also proves the existence of the genetic information for an inducible system in *U. maydis*.

The inducible system is distinctly less sensitive to hydroxamates (Figs. 2 and 3) and of a lower substrate affinity (Table II) than the constitutive system of *U. maydis* which was known from previous reports [1,2,11]. In the presence of AMP the participation of the two systems in cyanide-insensitive respiration is determined by the substrate concentration, but in its absence most of this respiration must be mediated by the inducible system which appears to exhibit a higher O_2 affinity (Fig. 7). The experiments on temperature effects also differentiate between chloramphenicol-treated and control mitochondria (Fig. 6). The effect of the treatment on the sensitivity of cyanide-insensitive respiration to low temperatures may well be explained by the induction of the new system suggested by the rest of the data.

It was assumed until recently that the alternative electron transport pathways described in eucaryotic microorganisms, whether constitutive or inducible, are similar and, perhaps, identical with the intensively studied higher plant system [19]. A comparison of the hydroxamate sensitivities reported for various systems did, in fact, support this view. It is only since 1977 that high resistance to hydroxamates has been reported for some new alternative systems. We shall briefly refer to these reports and examine similarities and differences between the respective systems and the one recognised in *U. maydis*.

Edwards and Unger [20] report that in a strain of *Neurospora crassa* which does not form an alternative pathway when grown in control medium, two such pathways are induced by growth in presence of chloramphenicol, one of high and one of low hydroxamate sensitivity. In experiments with whole cells the activity of the latter system was very low, not exceeding 5 nmol O_2/min per mg dry weight, while in vitro it approached the limit of the resolution of the equipment used. The system is characterised by sensitivity to azide (K_i = 200 μM). Even if mitochondrial, therefore, the *N. crassa* system is distinctly

different from the inducible system of *U. maydis* which not only exhibits high activity both in vivo and in vitro, but is also insensitive to high concentrations of azide as well as cyanide (Table I).

For the same reasons the *U. maydis* system is different from the hydroxamate-resistant and azide-sensitive system which has been recognised in *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, and other yeasts and the appearance of which is accompanied by changes in the redox states of cytochromes (Refs. 21, 22 and Goffeau, A., personal communication).

Mitochondrial oxidase systems insensitive to hydroxamates and azide have been described in *Acanthamoeba castellanii* [23] and *Paramecium tetraurelia* [24]. Both of these are sensitive to cyanide and, therefore, also distinctly different from the inducible system of *U. maydis* which exhibits high activity in the presence of all three inhibitors, KCN (1.0 mM), NaN_3 (2.5 mM), and hydroxamate (2.0 mM) as shown in Table I. A report on an azide- and hydroxamate-insensitive system of trypanosomes [25] gives no information on cyanide-sensitivity. This system supports approx. 20% of cellular respiration, is sensitive to CO and probably utilizes cytochrome *o* as the terminal oxidase. No information on the presence or absence of cytochrome *o* in *U. maydis* is available and since we have not tested the CO sensitivity of the inducible system here described we can not say whether it is similar with the one occurring in trypanosomes.

Some of the still unsolved problems in the study of branched electron transport chains are the nature of alternative oxidase(s), the exact point of interaction of the cytochrome and the alternative pathway and the control mechanisms which modulate the apportionment of electron flux [26]. Undoubtedly the recognition of a second alternative system in the mitochondria of *U. maydis* complicates these problems. Nothing is known about the new oxidase except that it does not require purine nucleotides to exhibit good affinity for O_2 in vitro. With respect to the branching point, ubiquinone is considered the most likely component of interaction in systems with two pathways [27]. Perhaps, a modification of the respiratory kinetic model of the mobile ubiquinone pool [28] could provide a basis for the study of the three-pathway chain recognised in the present work.

References

1 Georgopoulos, S.G. and Sisler, H.D. (1970) J. Bacteriol. 103, 745—750
2 Sherald, J.L. and Sisler, H.D. (1972) Plant Cell Physiol. 13, 1039—1052
3 Lambowitz, A.M. and Slayman, C.W. (1971) J. Bacteriol. 108, 1087—1096
4 Hanssens, L., D'Hondt, E. and Verachtert, H. (1974) Arch. Microbiol. 98, 339—349
5 Duriew-Trautman, O. and Tavlitzki, J. (1976) J. Bacteriol. 127, 1063—1069
6 Henry, M.F. and Nyns, E.J. (1975) Sub. Cell. Biochem. 4, 1—65
7 Ziogas, B.N. and Georgopoulos, S.G. (1978) Abstr. 3rd Int. Congr. Plant Pathology, p. 113, Deutsche Phytomedizinische Gesellschaft, Göttingen
8 Holliday, R. (1961) Genet. Res. 2, 204—230
9 Coursen, B.W. and Sisler, H.D. (1960) Am. J. Bot. 47, 541—549
10 White, G.A. and Thorn, G.D. (1975) Pestic. Biochem. Physiol. 5, 380—395
11 Ziogas, B.N. and Georgopoulos, S.G. (1979) Pestic. Biochem. Physiol. 11, 208—217
12 Ziogas, B.N. and Georgopoulos, S.G. (1980) Phytopathol. Mediterr. 19, 191—194
13 Turner, G. and Lloyd, D. (1971) J. Gen. Microbiol. 67, 175—188
14 Bahr, J.T. and Bonner, W.D., Jr. (1973) J. Biol. Chem. 248, 3441—3445
15 Bahr, J.T. and Bonner, W.D., Jr. (1973) J. Biol. Chem. 248, 3446—3450
16 Segel, I.H. (1976) Biochemical Calculations, 2nd edn., pp. 237—241, John Wiley and Sons, New York

17 Reid, K.G., Utech, N.M. and Holden, J.T. (1970) J. Biol. Chem. 245, 5261—5272
18 Schatz, G. and Mason, T.L. (1974) Annu. Rev. Biochem. 43, 51—87
19 Lloyd, D. and Edwards, S.W. (1978) in Functions of Alternative Terminal Oxidases (Degn, H., Lloyd, D. and Hill, G., eds.), pp. 1—10, Pergamon Press, Oxford
20 Edwards, D.L. and Unger, B.W. (1978) J. Bacteriol. 133, 1130—1134
21 Labaille, F., Colson, A.-M., Petit, L. and Goffeau, A. (1977) J. Biol. Chem. 252, 5716—5723
22 Goffeau, A. and Crosby, B. (1978) in Pure and Applied Biochemistry of Yeasts (Horecker, B.L. and Bacilla, M., eds.), pp. 81—96, Academic Press, New York
23 Lloyd, D., Edwards, S., Kristensen, B. and Degn, H. (1979) Biochem. J. 182, 11—15
24 Doussiere, J., Sainsard-Chanet, A. and Vignais, P.V. (1979) Biochim. Biophys. Acta 548, 236—252
25 Hill, G.C. (1978) in Functions of Alternative Terminal Oxidases (Degn, H., Lloyd, D. and Hill, G., eds.), pp. 67—77, Pergamon Press, Oxford
26 Solomos, T. (1977) Annu. Rev. Plant Physiol. 28, 279—297
27 Storey, B.T. (1976) Plant Physiol. 58, 521—525
28 De Troostembergh, J.C. and Nyns, E.-J. (1978) Eur. J. Biochem. 85, 423—432