PARTICULATE MALATE OXIDATION IN STRICTLY AEROBIC BACTERIA: THE RESPIRATORY CHAIN OF *MORAXELLA LWOFFI*

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1. Introduction

Studies on the oxidation of certain Krebs cycle intermediates showed [1] that whilst disintegrated cells of a number of strictly aerobic bacteria showed oxygen uptake rates with fumarate equal to, or exceeding, the rates obtained with succinate, similar preparations from facultatively aerobic bacteria, whilst oxidising succinate readily, gave very low rates of oxygen uptake with fumarate. This was correlated with a difference in the malate oxidising systems of the two types of organisms. The facultative organisms oxidised malate through a soluble NAD^+-linked dehydrogenase: the strict aerobes employed a "particulate" malate oxidase associated with the membrane-bound electron transport system. *Moraxella lwoffi*, however, possessed in addition to the membrane-bound malate dehydrogenase, a very active "soluble" dehydrogenase (cf. [3]). In this paper we show that the two are easily separable, and as a preliminary step towards elucidating their respective roles, we report on the cytochrome system of this organism as revealed by difference spectroscopy at room temp and at 77° K (liquid N_2).

2. Materials and methods

Moraxella lwoffi (N.C.I.B. 8250) were grown in 1 l of nutrient broth (Oxoid No. 2) in 2 l flasks on a rotary shaker for 16 hr or in 10 l batches and a medium providing mineral salts with 0.3% casein hydrolysate, 0.2% glutamic acid (adjusted to pH 7.4) in a fermenter unit under stirring and vigorous aeration at 30° for 8 hr. Cells were harvested, washed and resuspended (20 mg dry wt/ml) in 0.05 M phosphate pH 7.4 and disintegrated by passing twice through a French pressure cell. Coarse debris was removed by centrifugation for 15 min at 10,000 *g*, and the disintegrated cells then separated into "soluble" and "particulate" fractions by centrifugation for 90 min at 140,000 *g*.

Malate dehydrogenase in the particulate fraction was determined by following decrease in absorbance at 600 nm in a system containing particulate fraction (10 mg/ml); dichloroindophenol (0.027%), phenomethazine sulphate (0.033%), KCN (5×10^{-3} M) and L-malate (0.2 M) in 0.05 M phosphate buffer pH 7.4 (cf. [2]). The dehydrogenase in the soluble fraction was assayed by following the decrease at 340 nm in a system containing "soluble fraction" (0.1 → 1.0 mg/ml), NADH (5×10^{-2} M), oxaloacetate (0.1 M) in 0.05 M phosphate buffer pH 7.4.

Differential spectroscopy was performed in a Perkin-Elmer model 356 instrument: the suspension of disintegrated cell material was placed in both reference and experimental cuvettes (3 mm light path). A small quantity of H_2O_2 was added to the former, and $Na_2S_2O_4$ to the latter, to ensure complete oxidation and reduction, respectively. For low-temperature spectra the liquid nitrogen attachment was used (light path 3 mm).

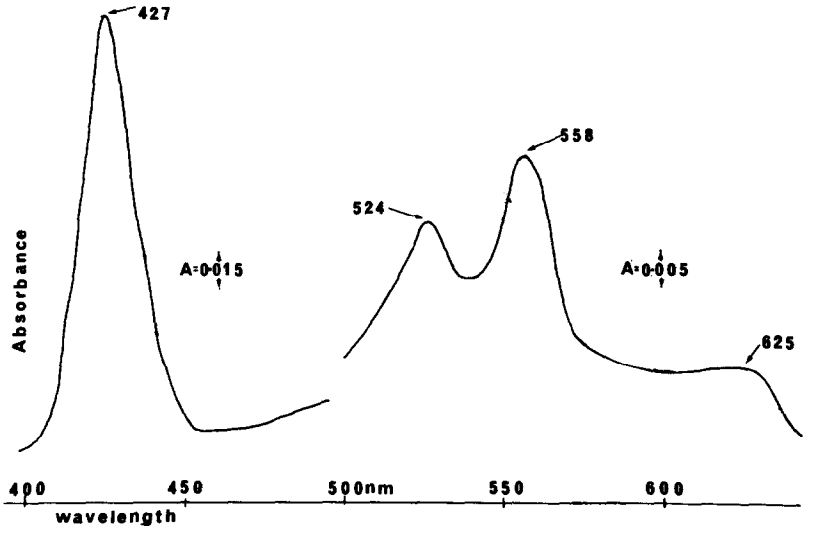

Fig. 1. Room temp (22°) difference spectra of *M. lwoffi* disintegrated cells, reduced ($Na_2S_2O_4$) minus oxidized. 11.0 mg dry wt/ml. Light path 3 mm.

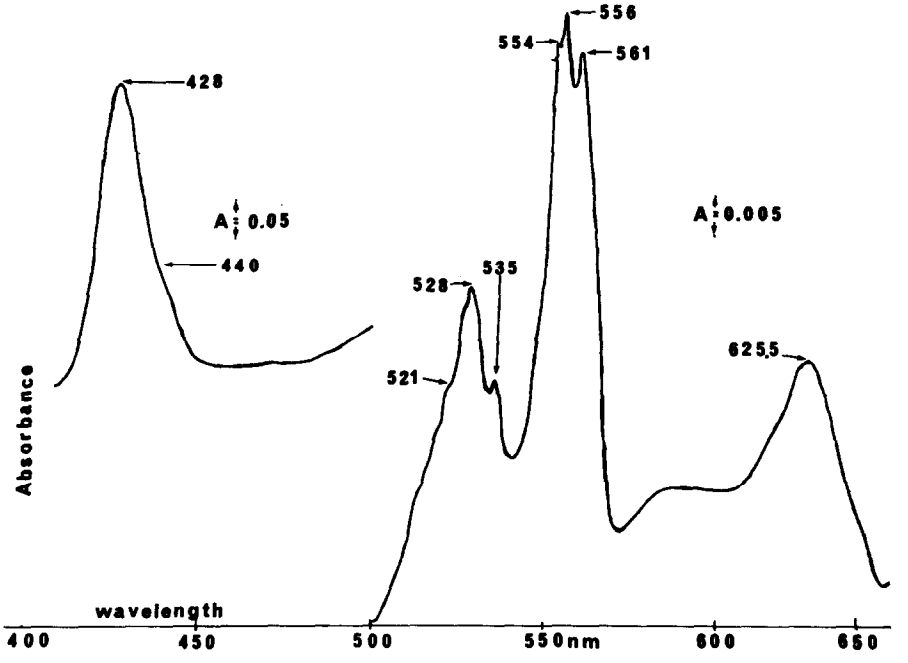

Fig. 2. Liquid nitrogen (77° K) difference spectra of *M. lwoffi* disintegrated cells, reduced ($Na_2S_2O_4$) minus oxidized. 11.0 mg dry wt/ml. Light path 3 mm.

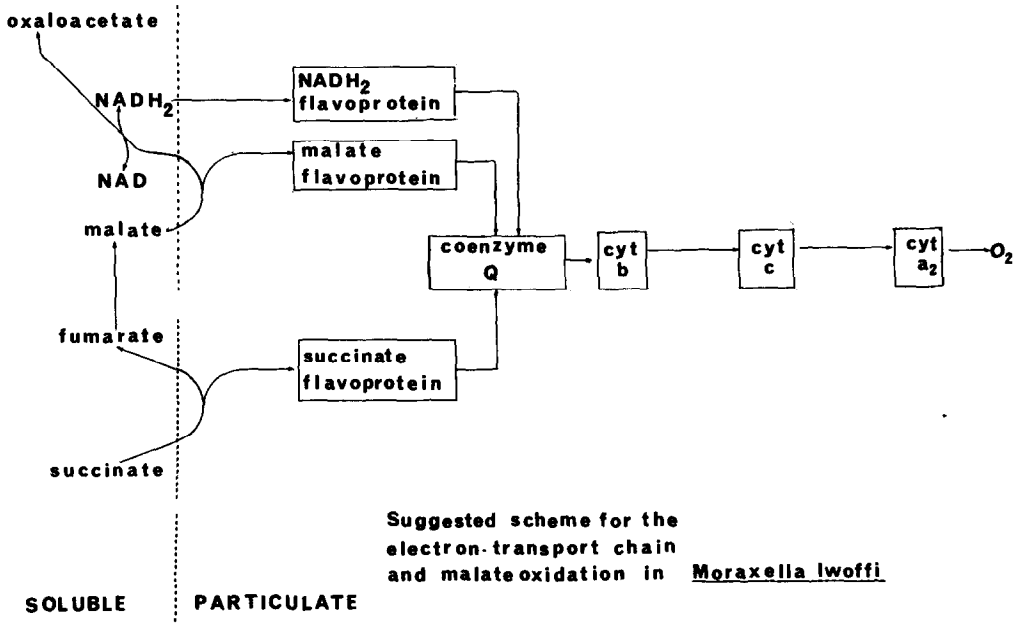

Fig. 3. Suggested scheme for the electron transport chain and malate oxidation in *M. lwoffi.*

3. Results and discussion

3.1. *Separation of particulate and soluble malate dehydrogenases*

5 ml of particulate fraction was placed on a 2.5 X 30 cm column of Sephadex G-200 and eluted with 0.02 M phosphate buffer pH 7.4. 5 ml fractions were collected and assayed for L-malate/DCIP activity. The only active fractions were no. 9–11, coinciding with the void volume of the column as determined by blue-dextran. No fraction showed oxalacetate/NADH activity.

When the experiment was repeated using 5 ml of supernatant fraction, oxaloacetate/NADH activity was recovered in a single wide peak running through fraction 34 to 44. No activity was found for the malate/DCIP system. The two malate dehydrogenase systems are thus clearly separable, and apparently distinct.

3.2. *Cytochromes of M. lwoffi*

Fig. 1 shows the reduced-minus-oxidised spectrum of disintegrated cells, at 22°, and fig. 2 the corresponding spectrum at 77° K (Liquid nitrogen) on the sample. This is a well-marked "a-2" band at 625.5 nm, but we found no indication of an "a" component (cf. Whittaker [4]); notably, there was no increase in absorbance in this region in the 77° K as compared with the room temp spectrum.

The low-temperature spectrum shows three distinct components in the 550 and 530 nm regions. It is not possible, on this spectroscopic evidence only, to classify these as "b" or "c" types. Investigation of their chemical properties and enzymic role is now being undertaken to establish their position in the scheme (fig. 3) which we propose as a working hypothesis for the oxidative metabolism of this organism.

References

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[4] P.A. Whittaker, Microbios. 4 (13) (1970) 65.