Purification and Properties of Malate-NAD+ Dehydrogenase of Moraxella Iwofi (N.C.I.B. 8250)

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Many strict aerobes have a particulate (non-NAD-linked) malate dehydrogenase (Jurtshuk et al., 1969), whereas many facultative anaerobes have only a soluble (NAD-linked) malate dehydrogenase. Moraxella Iwofi, like Micrococcus lysodeikticus (Cohn, 1958) and Acetobacter xylinium (Benziman & Galanter, 1964), has both a particulate and a soluble dehydrogenase (Jones, 1969; Jones & King, 1972).

In this communication we report the purification and study of the NAD-dependent malate dehydrogenase of Moraxella Iwofi (N.C.I.B. 8250). Malate dehydrogenase (EC 1.1.1.37) was assayed by measuring the initial rate of decrease in $E_{\text{110}}$ resulting from the oxidation of NADH during the reduction of oxaloacetate or by the increase in $E_{\text{110}}$ resulting from the reverse reaction.

Moraxella Iwofi was grown aerobically on a semi-synthetic medium with glutamate as carbon source to late exponential phase. Cells were disrupted by passage through a French pressure cell at $1.4 \times 10^9$ Pa. The cell-free extract was separated into a particulate fraction and a supernatant fraction by centrifugation at 365000g at 4°C. The NAD+-dependent malate dehydrogenase was present in the supernatant fraction, and this fraction was used for subsequent purification.

The purification procedure involved ion-exchange chromatography with DEAE-cellulose, preparative polyacrylamide-gel electrophoresis, followed by stepwise elution on a second DEAE-cellulose column. The preparation yielded a pure enzyme (single protein bands on 7.5, 10 and 15 % polyacrylamide gels coincident with malate dehydrogenase activity, demonstrated by tetrazolium staining on replicate gels).

The mol.wt. of the malate dehydrogenase was estimated as 61000 (by gel filtration on Sephadex G-100) or 60300 (by ultracentrifuge).

Kinetic studies for the determination of the $K_m$ for oxaloacetate ($3.87 \times 10^{-5}$M) and NADH ($1.35 \times 10^{-5}$M) were carried out at pH8.0 and those for malate ($1.74 \times 10^{-4}$M) and NAD$^+$ ($4.35 \times 10^{-4}$M) were carried out at pH10.5. The Michaelis constants for the above substrates are given in parentheses.

The malate dehydrogenase displayed strict substrate specificity for oxaloacetic acid and showed no activity with a number of keto acids. However, the enzyme showed low activity towards (+)-tartaric acid, (-)-tartaric acid, meso-tartaric acid and tartronic acid.

$\text{Mg}^{2+}$, $\text{Ca}^{2+}$ and $\text{Co}^{2+}$ had little inhibiting effect at concentrations below 10 mM. However, $\text{AgNO}_3$ (0.1 mM) was found to inhibit the enzyme by 58%. It was relatively insensitive to $\text{HgCl}_2$ (inhibition 63% at 1 mM). Substrate inhibition by oxaloacetate and NADH was also observed.