EFFECT OF OXYGEN TENSION ON THE ALDOLASES OF *MYCOBACTERIUM TUBERCULOSIS* H_{37} Rv

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1. Introduction

Fructose-1,6-diphosphate aldolase (EC 4.1.2.13) is widely distributed in nature [1]. Most of the bacteria contain a class II type aldolase. However, in *Escherichia coli* class II type aldolase is found in glucose grown cells, but both class I and class II type aldolases are present in pyruvate or lactate grown cells [2]. Photoautotrophically grown *Euglena gracilis* has the class I aldolase whereas under heterotrophic growth conditions class II type aldolase predominates [3].

In this communication it is reported that in the human pathogenic strain *Mycobacterium tuberculosis* H_{37}Rv, oxygen tension affects the synthesis of the two types of aldolases.

2. Experimental

M. tuberculosis H_{37}Rv (NCTC 7416) was grown at 37°C for 3 weeks in Youmans and Karlson medium [4] containing 2% glycerol, either in a fermentor or as surface culture.

Cell-free extracts were prepared by disruption of a 20% cell suspension, in 0.05 M phosphate buffer, pH 6, containing 1 mM 2-mercaptoethanol, using a Raytheon sonic oscillator, 10 KHz for 30 min and subsequent centrifugation at 20 000 *g* for 30 min. After precipitation of the nucleic acids with protamine sulphate, the supernatant was loaded on a DEAE-cellulose column and the two aldolases separated using 0.1–0.5 M NaCl gradient. Each enzyme was of FDP; 0.3 μmole further purified by Sephadex G-150.

FDP aldolase activity was estimated enzymatically by the method of Blostein and Rutter [5]. The activity was measured at 30°, in cuvettes, containing, in 1 ml; 50 μmoles of Tris–HCl, pH 7.5; 2 μmoles of NADH; 8 IU glycerophosphate dehydrogenase; 32 IU triosephosphate isomerase and the enzyme. The rate of reaction of aldolase was estimated by following the decrease in extinction at 340 nm. Correction was made for the NADH oxidase activity of the crude extract.

Proteins were estimated by the method of Warburg and Christian [6].

3. Results

Cell-free extracts from *M. tuberculosis* H_{37}Rv, grown as surface culture contain a class II type aldolase. However, the aldolase in the sonic extract of fermentor grown cells is found to be insensitive to EDTA. Chromatography of the extract on DEAE-cellulose results in two peaks of aldolase activity. The effects of varying concentrations of EDTA on these two aldolases is shown in fig. 1. High levels of EDTA do not affect the activity of the first enzyme, whereas 1 mM EDTA completely abolishes the activity of the second enzyme.

Table 1 shows the effect of $NaBH_4$ on the aldolases of *M. tuberculosis* H_{37}Rv. The first enzyme is completely inactivated on reduction with $NaBH_4$ in

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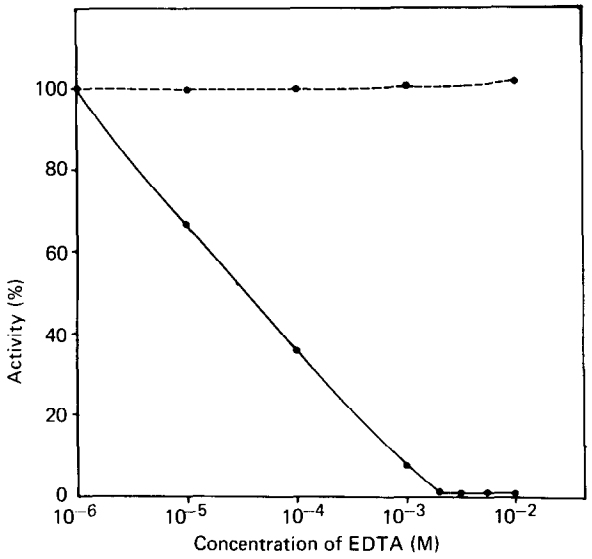

Fig. 1. Effect of EDTA on *M. tuberculosis* H_{37}Rv aldolases. EDTA at concentrations indicated was added to the assay mixture and reaction started by the addition of the substrate: (•- - - - •) activity of the first enzyme; (•——•) activity of the second enzyme.

presence of FDP, but similar treatment is found to have no effect on the second enzyme. This indicates the Schiff-base forming nature of the first enzyme, a property shared by all class I aldolases.

The data of kinetic studies with FDP and F-1-P

Table 1
Effect of $NaBH_4$ on *M. tuberculosis* H_{37}Rv aldolases in presence of FDP

Treatment	First enzyme		Second enzyme	
	Residual* activity	% activity	Residual activity	% activity
None	10.05	100	0.12	100
FDP	13.00	130	0.12	100
$NaBH_4$	8.04	80	0.11	92
$NaBH_4$ + FDP	0.402	4	0.11	92

* μmoles of FDP cleaved/min/mg protein.
Aldolase (1 mg) in 1 ml of Tris-acetate buffer, pH 6.7, containing 3.68 mM FDP, was reduced by the addition of 10 μl of 1M $NaBH_4$ solution over a period of 20 min. and the reaction stopped by the addition of 5 μl of acetone. Samples were diluted in 0.1 M Tris–acetate (pH 6.7) and assayed for residual aldolase activity.

Table 2
Kinetic parameters, K_m and V_{max}, for *M. tuberculosis* H_{37}Rv aldolases

Parameter	First enzyme	Second enzyme
K_m FDP	1.5×10^{-5} M	5×10^{-4} M
K_m F-1-P	1×10^{-2} M	–
V_{max} FDP	10.05	0.12
V_{max} F-1-P	0.603	–
FDP:F-1-P	17	–

Aldolase activity was estimated at varying concentrations of FDP and F-1-P at pH 7.5 using the coupled assay. K_m values are calculated from reciprocal plots. V_{max} values are μmoles FDP cleaved/min/mg protein.

as substrates are summarised in table 2. The first enzyme shows an FDP:F-1-P ratio of 17. On the other hand, the second enzyme does not cleave F-1-P. The V_{max} values are 10 μmoles of FDP cleaved/min/mg protein and 0.12 μmole of FDP cleaved/min/mg protein for the first and second enzymes respectively. The first enzyme is purified 250-fold and characterised. After DEAE-cellulose chromatography the second

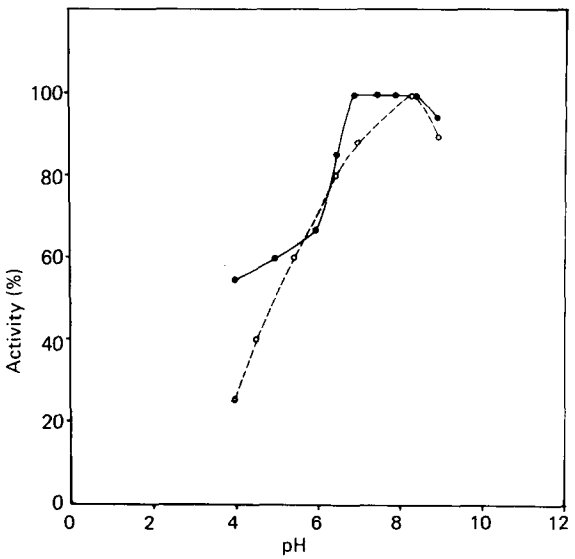

Fig. 2. Effect of pH on cleavage activity of *M. tuberculosis* H_{37}Rv aldolases. The two aldolases were resolved on DEAE-cellulose and cleavage activity assayed in 0.01 M Tris–malonate buffers: (•——•), pH profile of the first enzyme; (o- - - - o) pH profile of the second enzyme.

enzyme accounts for 10% of the total aldolase activity.

Fig. 2 displays the pH profile of the two enzymes. In contrast to the first enzyme which has a broad pH optimum (7–10), the second enzyme has a sharp optimum pH (8.5).

The above criteria, the inactivation of the first enzyme by carboxypeptidase A and activation of the second enzyme by K^+ point out that the first aldolase peak from the DEAE-cellulose column is a class I type and the second aldolase peak a class II type (classification according to Rutter [7]).

4. Discussion

A switch from aerobiosis to anaerobiosis alters several of the glycolytic and tricarboxylic acid cycle enzymes in *E. coli* [8]. In stationary grown cells (low pO_2) the EMP pathway would necessitate the synthesis of more of class II aldolase since this enzyme is reported to be functional for glycolysis. A change from surface culture (low pO_2) to growth in a fermentor (high pO_2) would result in the enhancement of tricarboxylic acid cycle with a concomitant increase in gluconeogenesis for which more of class I aldolase is synthesised [2]. It is of interest to note that in cells grown at high oxygen tension both class I and class II aldolases are present in a proportion 90:10, but in surface grown cells only class II aldolase could be detected. Hence the oxygen tension in the growth medium appears to regulate the synthesis of FDP aldolase. Class I enzyme plays a predominant role in cells grown at high oxygen tension and class II aldolase is of importance under conditions of low oxygen tension.

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