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Purification and characterization of erythritol dehydrogenase from Mycobacterium smegmatis

Oczyszczanie i charakterystyka dehydrogenazy erytrytolowej z *Mycobacterium smegmatis*

Erythritol, a four-carbon polyol (polyalcohol), is widely distributed in nature. It has 60–80% of the sweetness of sucrose [4]. Erythritol is a low-caloric, noncariogenic sweetener that is safe for diabetics [10]. This polyol has a very high negative heat capacity, providing a strong cooling effect when dissolved [4].

Considering the data from literature, all metabolic reactions of polyols (more of erythritol) belong to two types: oxidoreduction or phosphorylation. The redox reactions are catalyzed by two types of enzymes:

1) reductases of aldoses (alditol:NADP 1-oxidoreductases, polyols 1-oxidoreductases), which coenzymes are $NADP^+$/NADPH:

$$\text{polyol} + NAD(P)^+ \leftrightarrow \text{aldose} + NAD(P)H + H^+$$

An example of this type reaction is erythrose conversion to erythritol by NAD(P)H-dependent erythrose reductase (EC 1.1.1.21) from *Candida magnoliae* KFCC 11023 [6].

2) reductases of ketoses (polyols 2-oxidoreductases, polyols dehydrogenases), coenzymes (NAD^+/NADH, rarely $NADP^+$/NADPH, sometimes both these systems) dependent.

$$\text{polyol} + NAD(P)^+ \leftrightarrow \text{ketose} + NAD(P)H + H^+$$

For example, the oxidation of erythritol with NAD^+ as coenzyme by 2,3-cis-polyol (DPN) dehydrogenase (C3-5) (xylitol:NAD^+ 2-oxidoreductase (D-xylulose forming), EC 1.1.1.9) from *Aerobacter aerogenes* gave L-erythrulose [5].

The phosphorylation of polyols is catalyzed by phosphotransferases (kinases) giving phosphates esters of polyols as reaction products.

$$\text{polyol} + ATP \rightarrow \text{polyol-phosphate} + ADP$$

An example of this type reaction is turning of erythritol in *Brucella abortus* with ATP-dependent erythritol kinase (ATP:erythritol 4-phosphotransferase, EC 2.7.1.27) into L-erythritol-4-phosphate (D-erythritol-1-phosphate) [7].

Fast growing species of mycobacteria are capable to utilize poyols as an energy and carbon source for their growth [3]. In *Mycobacterium smegmatis (M. smegmatis)* ATCC 20 a new catabolic pathway of erythritol was described: erythritol ↔ L-erythrulose → L-erythrulose-1-phosphate ↔ HCHO + phosphodihydroxyacetone [12, 14]. The enzyme catalyzing the initial reaction of the pathway was found to be erythritol dehydrogenase (EDH) (EC 1.1.1.56), which showed an inductive character [13]. Erythritol, xylitol were a decreasing order in the best stimulating agents for EDH biosynthesis in *M. smegmatis* ATCC 20 [14].

This report describes preliminary results of the isolation, purification and characterization of

catalytic and physicochemical properties of the EDH from *M. smegmatis* ATCC 20. However, further research is needed to confirm and develop the already performed research.

EXPERIMENTAL PROCEDURES

Bacterial strains and culture conditions. The studied organism was *Mycobacterium smegmatis* ATCC 20, which was maintained as Löwenstein slant culture. *M. smegmatis* ATCC 20 was also grown in a mineral salt medium containing as follows: glutamic acid (1% w/v); citric acid (0.2%); KH_2PO_4 (0.05%); $MgSO_4 \cdot 7H_2O$ (0.05%); ferric ammonium citrate (0.005%); $ZnSO_4 \cdot 7H_2O$ (0.0025%) and erythritol (2.0% w/v). The pH of the medium was adjusted to 7.0 with KOH before autoclaving. The bacterial strains were cultured at 37°C.

Reagents and materials. All reagents were of analytical quality and, unless stated otherwise, supplied by Sigma Chemical Co., Fluka Chemie or ICN Biomedicals INC. 3-(cyclohexyloamino)-propane-sulfonic acid (CAPS) was obtained from Calbiochem, USA. Blue Dextran 2000, Sephadex G-200, Chelating Sepharose Fast Flow, Polybuffer exchanger (PBE 94) and Polybuffer 74 were purchased from Pharmacia Fine Chemicals AB, Sweden. Bio-Rad protein reagent was obtained from Bio-Rad Laboratories. DEAE-cellulose (DE-52 cellulose) was purchased from Whatman Ltd, England. N-Brom-succinimid (NBS) was obtained from Schuchardt, München, Germany. Diaflo YM-100 (cutoff, 100 kDa) ultrafiltration membranes were purchased from Amicon, Inc., USA.

Assay of EDH activity. The enzymatic activity of EDH was measured at room temperature (RT) by monitoring NAD^+ reduction or NADH oxidation at 340 nm, using a Cecil 5501 spectrophotometr (Cecil Instruments, England). The first assay mixture (1.2 mL) contained: 240 μmol glycine/NaOH buffer, pH 9.7, 40 μmol erythritol, 1.32 μmol NAD^+ and enzyme preparation. The second assay mixture (1.2 mL) contained: 240 μmol citrate/Na_2HPO_4 buffer, pH 6.5, 20 μmol L-erythrulose, 0.5 μmol NADH and enzyme preparation. One unit (U) of EDH activity was defined as the amount of enzyme catalyzing 1 μmol of NAD^+ reduction and 1 μmol of NADH oxidation per min. in erythritol oxidation and L-erythrulose reduction, respectively. The specific activity was defined as units of enzyme activity per mg of protein.

Purification of EDH activity. *M. smegmatis* ATCC 20 was grown in 1 L Roux flasks containing 0.2 L medium for 4–5 days. The cells were harvested by filtration through a gauze, washed with redistilled water, and stored as a paste in aluminum foil at -18°C until used. All purification steps were performed at 4°C unless otherwise specified.

Step 1: preparation of crude extract. One hundred grams of cell paste was suspended in 500 mL of ice-cold 25 mM potassium phosphate buffer pH 7.4 containing 5 mM β-mercaptoethanol (buffer A). The cells were disrupted by sonic oscillation (3 x 5 min, 22 kHz, 180 W) and centrifuged for 45 min at 17,000 *g*. To the supernatant, DNase (EC 3.1.21.1) was added (1 U/1 mL) and incubated overnight at 4şC.

Step 2: ammonium sulfate fractionation. The liquid, which was designated "crude extract", was placed in an ice bath on a magnetic stirrer and solid $(NH_4)_2SO_4$ was added, slowly to 40% saturation and the precipitate appeared was removed by a conventional centrifugation. The supernatant liquid was then brought to 70% saturation by the slow and continual addition of solid $(NH_4)_2SO_4$. The precipitate was collected by centrifugation, dissolved in a minimal volume of buffer A and dialyzed overnight against the same buffer (AS-fraction).

Step 3: DEAE-cellulose chromatography. The AS-fraction was applied to a column of DEAE cellulose (2.3 x 18.5 cm) that had been equilibrated with buffer

A. The column was washed thoroughly with the same buffer (150 mL) and the wash, which did not contain the activity of EDH, was discarded. The EDH was eluted from the column with 0.2 M NaCl in the buffer A (250 mL). Fractions containing the active enzyme were pooled and concentrated on the Amicon filtration apparatus (Millipore, Billarica, MA) using a YM-100 membrane.

Step 4: Phenyl-Sepharose CL-4B chromatography. EDH containing 5% ammonium sulfate (SA) was applied to Phenyl Sepharose CL-4B (1.6 x 12.5 cm). After application of a sample, the column was washed with buffer A containing, first, 5% SA (200 mL) and, second, 1% SA (150 mL). The EDH activity was eluted from the hydrophobic column with buffer A (200 mL). Most active fractions were pooled and concentrated by ultrafiltration.

Step 5: IMAC-Zn(II). To make the adsorbent to immobilized metal affinity chromatography (IMAC) the gel Chelating Sepharose Fast Flow was packed in the column (1.0 x 12.5 cm). Then, it was washed as follows (by 50 mL): 50 mM ethylene-diaminetetraacetic disodium salt (EDTA) solution, redistilled water, $ZnSO_4 \cdot 7H_2O$ (5 mg/mL) solution and citrate/Na_2HPO_4 buffer, pH 7.4, which contained 1 M NaCl. The enzyme after the above step of purification was adjusted to 1 M NaCl, and applied to a column Chelating Sepharose Fast Flow-Zn(II). The ballast proteins were eluted with citrate/Na_2HPO_4 buffer, pH 7.4 with 1 M NaCl (70 mL). The activity of EDH was eluted with a reverse linear gradient of pH from 7.4 to 4.0 in citrate/Na_2HPO_4 with 1 M NaCl (total volume of gradient 300 mL). Most active fractions were pooled, desalted immediately by ultrafiltration and kept frozen in buffer A.

Other methods. Protein concentration was measured by the method of Lowry et al. [8] and with Bio-Rad protein reagent using bovine serum albumin as standard [1] or spectrophotometrically at 280 nm. Native polyacrylamide gel electrophoresis (PAGE) was performed on 7.0% (w/v) slab gel by the method described by Davis [2] on Mini-PROTEAN®3 Cell apparatus (Bio-Rad). Gel was stained with amido black 10B in 7% acetic acid at RT for 30 min. For assays at different pH values, the reactions were performed at RT in the following buffers (240 μmol) and pH values: citrate/Na_2HPO_4, pH 5.6 to 7.7 with L-erythrulose as substrate; Tris(hydroksymethyl)-aminomethane/HCl (TRIS/HCl), pH 8.0 to 9.0; glycine/NaOH, pH 9.3 to 10.5; and CAPS, pH 9.7 to 10.5 with erythritol as substrate. For the determination of the effects of divalent metal ions on activity of EDH, 1 mM concentrations of these ions were preincubated with enzyme in (N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) (HEPES) buffer, pH 7.0 at RT for 5 min. before assaying enzyme activity. The substrate specificity of EDH was measured in both directions. The possibility of using available polyols and estimating K_m values for this in the oxidative reaction was tested in glycine/NaOH buffer, pH 9.7 and NAD^+ as coenzyme. The substrate specificity and K_m values for ketoses and aldoses in the reductive reaction were examined in citrate/Na_2HPO_4 buffer, pH 6.5 and NADH as coenzyme. The specificity of characterized dehydrogenase towards coenzymes NAD^+/$NADP^+$ and NADH/NADPH was measured in glycine/NaOH buffer, pH 9.7 with erythritol as substrate and in citrate/Na_2HPO_4 buffer, pH 6.5 with L-erythrulose as substrate, respectively. To examine the effects of inhibitors thiols groups and NBS (compound for modification of tryptophan) on the activity EDH, they were preincubated with enzyme in HEPES buffer, pH 7.0 at RT for 15 min. before assaying enzyme activity. The chelators (except TRIS) were preincubated with enzyme in citrate/Na_2HPO_4 buffer, pH 5.6 at RT for 15 min. before assaying enzyme activity. The molecular mass of the native EDH was estimated by gel filtration on Sephadex G-200 (1.6 x 95 cm). Molecular mass standards included: β-amylase from potato (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa) and carbonic anhydrase (29 kDa). Isoelectric point (pI) the EDH was determined by chromatofocusing. The enzyme was applied to a column of Polybuffer exchanger (PBE 94) (Pharmacia: 1.0 x 25 cm) equilibrated with 25 mM imidazole/HCl buffer, pH 7.4. Elution was performed with 400 mL eights times diluted Polybuffer

74/HCl, pH 4.0. Fractions were collected and analyzed for protein, enzymatic activity and pH. The thermal stability of EDH was tested at pH 7.4 in buffer A. Preparations were incubated for 0.5 min. at 22, 37, 40, 55, 60, 70, 80, 90 and 95°C and next the enzymes activities were assayed.

RESULTS

PURIFICATION OF THE M. SMEGMATIS ATCC 20 EDH

The steps in the purification included three liquid chromatography techniques: ion-exchange chromatography on DEAE-cellulose, hydrophobic chromatography on Phenyl-Sepharose CL-4B and IMAC on Chelating Sepharose Fast Flow-Zn(II) matrix. The erythritol dehydrogenase was purified 311 times with a yield of 22% from the cytosolic extract of *M. smegmatis* ATCC 20 using the procedures outlined in Table 1. The homogeneity of the purified enzyme after IMAC-Zn(II) was examined by PAGE. Figure 1 shows one protein band at this stage of purification EDH preparation.

Table 1. Purification of erythritol dehydrogenase from *M. smegmatis* ATCC 20

Purification step	Total protein (mg)	Total activity* (U)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Crude extract	2303	83.0	0.036	-	100
AS-fraction	1050	53.0	0.050	1.4	64
DEAE-cellulose	465	52.0	0.111	3.1	62
Phenyl-Sepharose CL-4B	13	25.6	1.970	54.7	31
IMAC-Zn(II)	1.65	18.5	11.200	311	22

* The enzyme activity was assayed in glycine/NaOH buffer, pH 9.7, by measuring increase of absorbance NADH using erythritol as the substrate

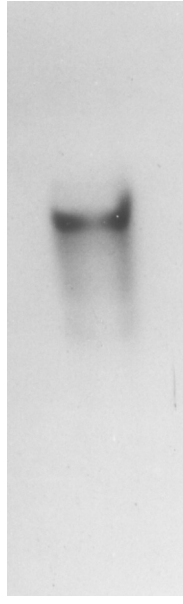

Fig. 1. PAGE of EDH from *M. smegmatis* ATCC 20 after IMAC-Zn(II) column. The lane was loaded with 5 μg protein

PROPERTIES OF EDH PURIFIED FROM *M. SMEGMATIS* ATCC 20

Optimum pH. The enzyme showed a maximum activity around pH 9.7 in glycine/NaOH buffer and 6.5 in citrate/Na_2HPO_4 buffer in oxidative and reductive reactions, respectively. The oxidative reaction is very slow in TRIS/HCl or CAPS buffer.

Effect of metal ions. Mg(II), Mn(II), Ni(II), Ca(II), Zn(II), Co(II), Cd(II) and Cu(II) at 1 mM concentrations were not activators of this enzyme. At this concentration, Co(II) slightly inhibited (11% inhibition), Cd(II) significantly inhibited (52% inhibition) and Cu(II) completely inhibited (100% inhibition) the activity of *M. smegmatis* ATCC 20 EDH.

Substrate and coenzyme specificity. The ability of EDH to catalyze the oxidative reaction of nine polyols, between three and six carbon in size, was examined (Table 2). The enzyme oxidized to a high degree L-threitol, L-arabitol, glycerol, xylitol, erythritol and ribitol. According to Table 2, in the oxidative reaction, tri-, tetra- and pentipolyols, which contained –OH group in configuration D at C2, were substrates of enzyme. Moreover, the configuration –OH groups at C2-3 of polyols was either of D-erythro- or L-threo- type. The substrate specificity of the reductive reaction towards six ketoses and ten aldoses was examined (Table 2). In this case, the best substrates in a decreasing order were L-erythrulose, D-ribulose and dihydroxyacetone. The EDH was not specific to other tested ketoses and aldoses. The kinetic studies on velocity reaction catalyzed by the characterized enzyme demonstrated that it is not very specific for polyols substrates and K_m constants for these substrates were very similar (Table 2). It was observed in the case of ketoses that K_m constant for L-erythrulose was one range lower than these for dihydroxyacetone and D-ribulose (Table 2).

Table 2. Substrate specificity of *M. smegmatis* ATCC 20 erythritol dehydrogenase*

Substrate (Polyols)	Relative activity (%)	K_m (mM)	Substrate (Ketoses, Aldoses)	Relative activity (%)	K_m (mM)
Erythritol	100	3.3	L-Erythrulose	100	0.5
Ribitol	99	5.0	D-Ribulose	35	5.0
Glycerol	109	4.0	Dihydroxyacetone	29	4.0
L-Threitol	132	3.3	D-Fructose	0	-
Xylitol	107	2.9	L-Sorbose	0	-
L-Arabitol	119	4.5	D-Tagatose	0	-
D-Threitol	0	-	D-Arabinose	0	-
D-Mannitol	0	-	L-Arabinose	0	-
D-Sorbitol	0	-	D-Erythrose	0	-
			D-Galactose	0	-
			D,L-Glyceraldehyde	0	-
			D-Mannose	0	-
			D-Ramnose	0	-
			D-Ribose	0	-
			D-Xylose	0	-
			L-Xylose	0	-

* The possibility of using available polyols and estimating K_m values for this in the oxidative reaction was tested in glycine/NaOH buffer, pH 9.7 and NAD^+ as coenzyme. The substrate specificity and K_m values for ketoses and aldoses in the reductive reaction were examined in citrate/Na_2HPO_4 buffer, pH 6.5 and NADH as coenzyme

The EDH was specific to NAD^+/NADH as coenzymes. Phosphate forms of these pyridine nucleotides ($NADP^+$/NADPH) did not perform this function. The K_m constants for NAD^+ and NADH were 0.45 mM and 0.33 mM, respectively.

Effect of inhibitors. The effect of various inhibitors on the activity of *M. smegmatis* ATCC 20 EDH is shown in Table 3. For the estimation of -SH groups content, p-chloro mercuric benzoic sodium salt (pCMB); 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and N-ethylmaleimide (NEM) were used. The inhibitors: pCMB (making marcaptide linkage) and DTNB (oxidative compound) very strongly inhibited the mycobacterial EDH at concentration as small as 10^{-4}–10^{-5}M. NEM (alkylating compound) also inhibited the enzyme activity but at concentration 10^{-3}M. NBS, a specific reagent for modification of tryptophan in proteins, completely inhibited the activity of EDH at concentration 10^{-5}M. The activity of this enzyme was inhibited by chelating agents: EDTA and 1,10-phenantroline at concentration 10^{-3}M. When TRIS, also chelator, was added directly to the assay mixture, the half of the initial activity of EDH was observed at concentration 14 mM of inhibitor. The fact that the tested chelators significantly differed in their structures affected the activity of EDH from *M. smegmatis* ATCC 20 indicated that the enzyme is a metallprotein.

Table 3. Effect of various inhibitors on *M. smegmatis* ATCC 20 erythritol dehydrogenase*

Inhibitor	Concentration of the inhibitor (mM)	Inhibition (%)
pCMB	0.005	87
	0.01	100
DTNB	0.005	62
	0.01	83
NEM	0.1	17
	1.0	86
	5.0	100
NBS	0.005	4
	0.01	49
	0.05	100
EDTA	1.0	63
	5.0	100
1,10-phenantroline	1.0	28
	5.0	70

* The enzyme was preincubated with the inhibitors for 15 min. at RT. Then, enzyme activity was assayed in glycine/NaOH buffer, pH 9.7, by measuring increase of absorbance NADH using erythritol as substrate

Molecular weight. Based on the gel filtration on Sephadex G-200, the active EDH from *M. smegmatis* ATCC 20 had a molecular weight of about 160 kDa.

pI (isoelectric point). pI of the erythritol dehydrogenase from *M. smegmatis* ATCC 20 was determined by chromatofocusing and was shown to be 4.4–4.6. In this procedure the enzymatic activity of EDH was unstable.

Thermal and storage stability. The purified EDH lost 11% of its initial activity at 70°C and 50% at 80°C, respectively. When the purified EDH was stored in buffer A, the half life was six weeks at -15°C.

DISCUSSION

In this pilot study we described the procedure of purification and characterized the kinetic and physicochemical properties of erythritol dehydrogenase involved in the utilization of erythritol in *M. smegmatis* ATCC 20.

The substrate specificity of EDH reported here was different from yeast *Lipomyces starkeyi* CBS 1807 (*L. starkeyi)* and bacteria *Gluconobacter frateurii* CHM 43 (*G. frateurii*) erythritol dehydrogenases. For enzyme from *L. starkeyi*, D-threitol, (2R,3R)-2,3-butanediol, erythritol, glycerol and *meso*-2,3-butanediol were much better substrates than the other polyols in the oxidative reaction. Substrate specificity of the reductive reaction demonstrated that L-erythrulose and dihydroxyacetone were better substrates [11].

In thermotolerant G. *frateurii* two differentially dehydrogenases were described which are active with erythritol [9]. Quinoprotein membrane-bound erythritol dehydrogenase (QMEDH) showed broad substrate specificity toward C3-6 sugar alcohols such as glycerol, erythritol, ribitol, D-arabitol, D-sorbitol and D-mannitol. QMEDH well oxidized erythritol, D-arabitol, glycerol and D-sorbitol with relatively the same high rates while ribitol and D-mannitol were oxidized at the rate of 40–65% of those of erythritol. This enzyme also oxidized (2R,3R)-2,3-butanediol with that one third of erythritol. On the other hand, the cytosolic NAD^+-dependent erythritol dehydrogenase (CMEDH) of the same microorganism was regarded as a typical pentitol dehydrogenase of NAD^+-dependent. Glycerol, D-mannitol, and D-sorbitol were oxidized by the enzyme at 13, 68 and 18% of the relative rate to erythritol oxidation. It was interesting to see that L-arabitol, ribitol and xylitol were rapidly oxidized by the enzyme, while D-arabitol oxidation (126% of relative activity) was almost comparable to erythritol oxidation. Instead, the relative rate of L-erythrulose reduction was nearly ten times higher than that of erythritol oxidation in the cytoplasm. CMEDH in contradiction to QMEDH was independent of the oxidative fermentation of L-erythrulose production [9].

The preliminary study on a comparison of the substrate specificities of two enzymes: inductive erythritol dehydrogenase from *M. smegmatis* ATCC 20 grown on erythritol-enriched medium (13) and constitutive ribitol dehydrogenase (RDH) from *M. smegmatis (M. butyricum)* cultured on glucose-enriched medium (15) showed some similarities. The K_{ms} for erythritol (3.3 mM) and L-erythrulose (0.5 mM) for EDH from *M. smegmatis* ATCC 20 are comparable to the values for RDH from *M. smegmatis (M. butyricum)* (16), which have K_{ms} of 1.96 mM and 0.298 mM, respectively (Table 4).

Table 4. Comparison of selected properties of inductive erythritol dehydrogenase from *M. smegmatis* ATCC 20 and of constitutive ribitol dehydrogenase from *M. smegmatis (butyricum)*

Properties	Source	
	EDH	RDH [15, 16]
Molecular mass (kDa)	160	160
pH optimum	6.5 in reductive reaction 9.7 in oxidative reaction	6.5 in reductive reaction 9.7 in oxidative reaction
K_m (mM)	for erythritol 3.3 for L-erythrulose 0.5	for erythritol 1.96 for L-erythrulose 0.298
NAD^+/NADH requirement	Yes	Yes
pI	4.4-4.6	4.65-4.7
Highly sensibility for inhibitors specific for -SH group and chelators	Yes	Yes

M. smegmatis (M. butyricum) constitutive ribitol dehydrogenase exhibited a strict and unique catalytic preference for substrate bearing D-ribo configuration. The best substrates of this enzyme in oxidative reaction were ribitol, erythritol and glycerol. Xylitol (C3 epimer of ribitol) was the suitable substrate as well. D-sorbitol, D-mannitol, D-arabitol and D-galactitol were ineffective as substrates of this enzyme [15].

Moreover, a comparison of other properties of RDH from *M. smegmatis (M. butyricum)* (16) like optimum pH, specificity for coenzymes, high sensitivity towards chelating agents and inhibitors of thiols groups, molecular weight, isoelectric point with EDH from *M. smegmatis* ATCC 20 indicate that these mycobacterial dehydrogenases are similar (Table 4).

CONCLUSIONS

In this study we described the procedure of purification and some properties of inductive mycobacterial erythritol dehydrogenase, which was isolated from *M. smegmatis* ATCC 20. A large similarity between properties of this dehydrogenase and constitutive ribitol dehydrogenase from *M. smegmatis (butyricum)* was discovered.

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SUMMARY

In this pilot study we described the procedure of purification for erythritol dehydrogenase (EDH) involved in the utilization of erythritol in *M. smegmatis* ATCC 20. Some properties of this enzyme were studied. This enzyme purified from cytosolic fraction can be regarded as NAD^+-depedent polyol (C3-5) dehydrogenase, for which the configuration of –OH groups at C2-3 of polyols is either of D-erythro- or of L-threo-type. The K_m constants for erythritol and L-erythrulose are 3.3 mM and 0.5 mM, respectively. The enzyme has optimum pH around 9.7 in glycine/NaOH buffer and at 6.5 in citrate/Na_2HPO_4 buffer in oxidative and reductive reactions, respectively. The characterized mycobacterial EDH is specific to pyridine nucleotides NAD^+/NADH as coenzymes. The K_m constants for NAD^+ and NADH are 0.45 mM and 0.33 mM, respectively. Erythritol dehydrogenase from *M. smegmatis* ATCC 20 has pI 4.4–4.6 and molecular weight 160 kDa. Our results indicate that cysteine (amino acid with –SH group) and tryptophan are partially responsible for EDH activity. The inhibition activity of the characterized enzyme by tested chelators as EDTA, 1,10-phenantroline and TRIS indicate that the EDH is a metallprotein. A large similarity between the properties of inductive erythritol dehydrogenase from *M. smegmatis* ATCC 20 and constitutive ribitol dehydrogenase from *M. smegmatis (M. butyricum)* was discovered.

STRESZCZENIE

W pilotażowej pracy opisano procedurę oczyszczania dehydrogenazy erytrytolowej (EDH) zaangażowanej w przemianę erytrytolu w *M. smegmatis* ATCC 20. Badano wybrane właściwości enzymu. Enzym ten oczyszczony z frakcji cytozolowej wydaje się poliolową (C3-5) dehydrogenazą zależną od NAD^+, dla której konfiguracja grup –OH przy C2-3 poliolu może być typu D-erytro lub L-treo. Stałe Michaelisa dla erytrytolu i L-erytrulozy wynoszą odpowiednio: 3,3 mM i 0,5 mM. Enzym ma optimum pH około 9,7 w buforze glicyna/NaOH i 6,5 w buforze kwas cytrynowy/Na_2HPO_4 w utleniających i redukcyjnych reakcjach. Charakteryzowana mykobakteryjna dehydrogenaza erytrytolowa jest specyficzna do nukleotydów pirydynowych NAD^+/NADH jako koenzymów. Stałe Michaelita dla NAD^+ i NADH wynoszą odpowiednio: 0.45 mM i 0.33 mM. Dehydrogenaza erytrytolowa z *M. smegmatis* ATCC 20 ma pI 4,4–4,6 i jej masa cząsteczkowa wynosi 160 kDa. Nasze wyniki wskazują, że cysteina (aminokwas z gr. –SH) i tryptofan są częściowo odpowiedzialne za aktywność EDH. Hamowanie aktywności charakteryzowanego enzymu przez testowane chelatory, takie jak: EDTA, 1,10-fenantrolina i TRIS, wskazuje, że EDH jest metaloproteiną. Zaobserwowano duże podobieństwo właściwości pomiędzy indukcyjną dehydrogenazą erytrytolową z *M. smegmatis* ATCC 20 a konstytucyjną dehydrogenazą rybitolową z *M. smegmatis (M. butyricum)*.