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Effect of Some Physiological Conditions on *Chromohalobacter* sp. İsolate's δ-Aminolevulinic Acide Dehydratase (ALAD)

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Abstract

Saline environments are frequently contaminated with heavy metals as a result of industrial or agricultural activities. Heavy-metal pollution represents an important environmental problem due to the toxic effects and their accumulation throughout the food chain leads to serious ecological and health problems. Lead (Pb) is one of the most widely used metals in industries and exposure to Pb continues to be a common problem almost in all over the world. δ-Aminolevulinic acide dehydratase (ALAD; E.C. 4.2.1.24) is a metalloprotein and plays a crucial role in heme synthesis. The inhibition of the enzyme δ-aminolevulinic acid dehydratase is recognized as a useful biomarker of Pb exposure and effect, both in humans and other animal species. Selective inhibition of ALAD with lead is an important biological marker of chemical contamination. In this study the effects of some heavy metals on ALADs activity of *Chromohalobacter* sp. isolates has been studied in order to determine whether their ALADs could be used as biosensor for lead and other heavy metals contamination. Spektrofotometric method was used to determine ALADs activity. According to the results, Pb negatively effected ALADs activity of *Chromohalobacter* sp. isolates. Some heavy metals inhibited its activity ratio of $Pb(NO_3)_2$ %15, $ZnSO_4$ %38, $MnCl_2$ %54, $CoCl_2$ %36, $FeSO_4$ %15, $Ni(NO_3)_2$ %52. Surprisingly, $MnSO_4$ increased its activity up to 8%. ALAD activity was observed at pH between 3 and 11, optimum pH was found to be 5 and it was observed that ALAD has an optimal temperature between 20 and 37 °C.
Key words: ALAD, *Chromohalobacter* sp., heavy metal, lead (Pb)

INTRODUCTION

Saline environments are constantly contaminated with heavy metals as a result of industrial or agricultural activities [1, 2, 3]. Heavy-metal pollution represents an important environmental problem due to the toxic effects and their accumulation throughout the food chain leads to serious ecological and health problems. Lead (Pb) is one of the most widely used metals in industries and almost in all over the world exposure to Pb continues to be a common problem. During the last decades, there was an increasing interest to investigate other sublethal endpoints, especially in relation to those biochemical responses that may be considered as early biomarkers of contamination [4]. Among them, the inhibition of ALAD is recognized as a useful biomarker of Pb exposure and effect, both in living organisms [5, 6, 7, 8, 9]. d-Aminolevulinate dehydratase (E.C.4.2.1.24, ALAD) is a cytosolic enzyme . 5-Aminolaevulinic acid dehydratase (porphobilinogen synthase; EC 4.2.1.24) catalyses the dimerization of two molecules of 5-aminolaevulinic acid to give porphobilinogen in a Knorr-type pyrrole synthesis

Recently halophilic bacteria have been utilized to treat saline and alkaline effluents containing heavy metals. Halophilic bacteria being tolerant to heavy metals could be employed as bioindicator organisms in polluted saline environments, while some halophilic bacteria have the ability to detoxify heavy metals. Halophilic microorganisms possess wide biotechnological applications and their abilities to degrade various environmental contaminants cannot be ignored [10, 11, 12, 13].

The objective of the present study was to determine whether ALAD of *Chromohalobacter* strain could be used as biosensor for lead and other heavy metals contamination.

MATERIALS AND METHODS

Extraction of the enzyme

Strain was cultivated in MGM broth for 7 days at 35°C. Crude enzymic extract was prepared from the cells as described by Ogunseitan [14]. Bacterial broth cultures were centrifuged at 3000×g for 10 min at 4°C in order to collect pelleted cells. The supernatant was discarded and the cell pellet was resuspended in 2 ml of cold lysis buffer solution [1 mM of dithiothreitol (DTT), 20 mM of Tris-Cl, 1 mM of phenylmethylsulfonyl fluorine (pH 7.4). The resuspended cells were lysed by sonication which uses a 3 mm microtip (Bandelin Sonopuls UW2070, Germany). Icebath was used to prevent protein denaturation during this process. Lysed cell suspensions were centrifuged at 18.400×g for 25 min at 4°C after the protein supernatant was transferred into a fresh tube. Total protein kit was used to determine the protein content of biological extracts (Sigma, TP0100, USA). Protein was stored at -20°C.

Determination of ALAD activity

The crude enzymic extracts, contain of 100µg protein, were added into microcentrifuge tube and the volume was adjusted to 200 µl with buffer A [50 mM potassium phosphate buffer, pH 7.2, containing 2 mM of dithiothreitol (DTT)]. Then, 200 µl of buffer B (50 mM potassium phosphate buffer, pH 7.2, containing 6 mM of δ-aminolevulinate and 1 mM of DTT) was added, and the reaction was initiated. The mixture was incubated at 42°C for 2 h. For ending the reaction, 1 ml of 4% trichloroacetic acid was used. The reaction mixtures were then centrifuged for 2 min at 12,000×g and 100 µl of each supernatants were transferred to the tubes containing 100 µl of Ehrlich's reagent [3.4 ml of acetic acid, 1.6 ml of 70% perchloric acid, 1 ml of 0.25 M mercury chloride, 0.1 g p-dimethylaminobenzaldehyde (DAB)], and waited for 5 min. Then they, developed pink color representing porphobilinogen formation, were measured spectrophotometrically (Jenway, 6305 UV/Vis) at A_{555} [14, 15] . One unit of enzyme activity was defined as the amount of enzyme that produced 1.0 µmol of porphobilinogen in 1 min. Specific activity was defined in terms of units per milligram of protein.

Effects of various metals on ALAD activity

Pb $(NO_3)_2$, $MnSO_4.H_2O$, $MgSO_4.7H_2O$, $Ni(NO_3)_2$, $ZnSO_4.7H_2O$, $CoCl_2.6H_2O$, $FeSO_4$ compounds were used during the preparation of metal media A (50mM K fosfat 0,0174g, 2mM DTT 0,00617g, 20 ml dH_2O). 100 µl bacterial protein were incubated for 1 h at 35°C in 50-µl metal solutions (500 µM of stock solution). After the incubation, the specific activity of ALAD in protein extracts was determined as described by Battistuzi et al. [15]. Control experiment without metals was conducted and all experiments were triplicated.

Dose effect of Pb(II) on ALAD activity

For this, 100 µl extract was incubated in the presence of different concentrations of Pb(II) (0, 50, 100, 250, 500, 750 µM) for 1 h at 35°C. After the incubation, ALAD activities were determined as described Battistuzi et al. [15], and all experiments were triplicated.

Determination of effect of pH temperature and salt consantration on ALAD activity

100µl crude enzymic extracts were added into centrifuge tube and incubated in the presence of different temperature, pH and salt consantratons. After the incubation, ALAD activities were determinated [15]. All experiments were triplicated

RESULTS

ALAD activity

In this study, when different heavy metals were applied to isolate *Chromohalobacter* sp., they inhibited the ALAD activity as follows: $MnCl_2$ (54%), $Ni(NO_3)_2$ (52%), $CoCl_2$ (36%), $ZnSO_4$ (36%), $FeSO_4$(15%) and $Pb(NO_3)_2$ (15%) respectively. Only $MnSO_4$ increased the ALAD activity (8%) of isolate *Chromohalobacter* sp. The results of the experiments conducted to test the effect of 250 ppm various metals on ALAD activity in protein extracts of *Chromohalobacter* sp. isolate are shown in Table 1.

Table 1. Effect of various metal ions on ALAD activity.

Metals (250 ppm)	Specific activity of ALAD (µmol PBG mg^{-1} $hour^{-1}$)	% Inhibition (-) or enhancement (+) of ALAD activity by metals
No metal	0.50	-
$Pb(NO_3)_2$	0.43	(-)15
$ZnSO_4$	0.31	(-)38
$MnCl_2$	0.23	(-)54
$CoCl_2$	0.32	(-)36
$FeSO_4$	0.43	(-)15
$MnSO_4$	0.54	(+)8
Ni(NO3)2	0.24	(-)52

It was determined that the increase of lead concentration (from 250 to 750ppm) inhibites the ALAD activity at *Chromohalobacter* sp. isolate (Table 2). The strongest ALAD inhibition measured was 36% at *Chromohalobacter* sp. when protein extracts were incubated with 650 ppm of Pb (Table 2).

Table 2. Effect of Pb concentration on ALAD activity.

Pb concentration (ppm)	Specific activity of ALAD (µmol PBG mg^{-1} min^{-1})	% Inhibition of ALAD activity by (Pb)
control	0,56	
250	0,43	(-)13
350	0,33	(-)23
450	0,29	(-)27
550	0,21	(-)35
650	0,20	(-)36
750	Not determinated	-

On the one hand, ALAD activity was observed at pH between 3 and 11, optimum pH was found to be 5 and it was observed that ALAD has an optimal temperature between 20 and 37 °C (Table 3-5)

Table 3. Effects of pH on ALAD enzyme activity.

pH	Specific activity of ALAD (µmol PBG mg^{-1} min^{-1})
3	0.22
5	0.26
7	0.25
9	0.24
11	0.13

Table 4. Effects of temperature on ALAD enzyme activity.

Temperature (˚C)	Specific activity of ALAD (µmol PBG mg^{-1} min^{-1})
4	0.41
20	0.51
30	0.49
37	0.51

Table 5. Effects of salt concentration on ALAD enzyme activity.

Salt concentration (%)	Specific activity of ALAD (μmol PBG mg^{-1} min^{-1})
15	0.20
20	0.28
25	0.47
30	0.35

DISCUSSION

When the ALAD activity of *Chromohalobacter* sp. was tested, only Mn increased the ALAD activity up to 8%, All other metals were decreased the ALAD activity (Table1). It was determined that the increase of lead concentration inhibites the ALAD activity at *Chromohalobacter* sp. (Table 2). Ogunseitan et al. [14] suggest that ALAD has been used extensively as a biomarker for lead contamination and further studies to improve the microbial biosensor approach to Pb contamination, including the characterization of the molecular diversity of ALAD in natural microbial communities. In our study we observed that increasing Pb concentration inhibited ALAD. The Zn^{2+} dependent enzymes include the ALADs from mammalian sources, which have pH optima of between pH 6.3 and 7.1. On the one hand, the yeast and *E.coli* enzymes can also be included in this class, requiring Zn^{2+} for activity but with more alkaline pH optima than their animal counterparts: 9.8 for the yeast. In addition to the enzymes obtained from *Streptomyces yokosukanensis* ATCC 25520 and *Escherichia coli* have higher alkali optimal pH. On the other hand, the optimal pH of the ALAD of the isolate, *H. argentinensis*, was found to be pH 7 [16]. In present study, although ALAD activity was observed at pH between 3 and 11, optimum pH was found to be 5 (Table 3) and it was observed that ALAD has an optimal temperature between 20 and 37 °C (Table 4).

Similarly Kutlu and Sümer [17] reported that ALAD from *Gammarus pulex* showed maximum activity at 37 °C. Korcan et al. [16] reported that ALADs from bacterial populations are sensitive indicators of bioavailability and physiological consequences of toxic compounds, particularly in cases where anthropogenic and geochemical factors donate to dynamic shifts in chemical speciation and concentration [14]. In this respect, the activity of δ-aminolevulinic acid dehydratase has been used extensively as a biomarker for lead exposure in prokaryotes and eukaryotes. ALAD of *Chromohalobacter sp*. has a very wide pH range, salinity tolerance, and temperature request. We conclude that it can be used as a biomarker for Pb contamination in the ambience of salty lead pollution caused by industrial processing.

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