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Effects of organic solvent and ionic liquids on the kinetic behavior of chromate reductase

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Abstract

Organic solvent and ionic liquids have received increasing attention as attractive solvents in medical and biotechnological usages. The present study has been carried out to study the comparative influence of 1-butyl-3-methylimidazolium, 1-methylimidazolium and some organic solvent on the kinetic parameters of the chromate reductase. Km and Vmax values for enzyme were calculated 1.39 mM and 0.26 μ mol.min-1.mg-1, respectively. As the concentration of ionic liquids increased, Km increased and kcat decreased. In addition, the associated tertiary structures changes of enzyme caused by ILs (100 mM) were studied by fluorescence method. The enzyme activity in the presence of 0.5 mM [MIm][Cl] and 0.4 mM [MIm][BF4] reduced to 35% of the initial reaction rate whereas this enzyme demonstrated 76% of its initial activity in the presence of organic solvent such as Tween 20, indicating that the chromate reductase is more sensitive to these ILs compared to Tween 20.

Keywords: Organic solvent, Ionic liquids, Chromate reductase,

1. Introduction

Environmental clean-up strategies for Cr(VI) removal involve physicochemical or biological detoxification [1]. The biological Cr(VI) detoxification which is more ecofriendly and an economically feasible technology. Some chromate resistant bacteria have been shown to reduce chromate to the trivalent form. Biological transformation of Cr(VI) to Cr(III) by enzymatic reduction may provide a less costly and more environmentally friendly approach to bioremediation [2]. Chromate reduction occurs both in anaerobic (chromate being used as final electron acceptor) and aerobic conditions.

In this work, removal of Cr (VI) from a culture medium and reducing to Cr (III) was reported. It is believed that the reduction in the *Bacillus licheniformis* strain ZT1 strain mediated by NADH-dependent enzymes, for this reason, here, we describe the purification and biochemical characterization of the chromate reductase activities.

2. Methods

Enzyme purification procedure

Proteins were precipitated by Amicon Ultra 0.5 mL filters to precipitate proteins and dialyzed in the preferred buffer. The dialysed sample was applied to Q-Sepharose column at a flow rate of 1ml/min, previously equilibrated with 50 mM Tris, pH 7. Proteins were eluted with a linear gradient (0–1M NaCl) in the identical buffer and flow rate. The active fractions were combined and concentrated in an ultrafiltration chamber (Amicon 8050) with a 30 kDa membrane cut-off and analyzed for proteins and enzyme activity. The concentrated sample containing 2M $(NH4)_2SO_4$ was loaded onto phenyl-Sepharose column previously equilibrated with Tris buffer containing 2M $(NH_4)_2SO_4$ buffer. After washing the column, adsorbed proteins were eluted with a linear 2–0 M $(NH4)_2SO_4$ gradients prepared in Tris–HCl buffer (pH 7). Following elution fractions revealing chromate reductase activity, were concentrated.

Enzyme assay and Protein determination

The chromate reduction activity was determined as described previously [3]. The assay mixtures reaction system (1.0 mL) was made up of varying Cr(VI) final concentrations (5–30 mM) in 700 µL of 100 mM potassium phosphate buffer (pH 7.0) added with 250 µL aliquots of CFE for chromate reduction and 50 µL of NADH. The system volume of 1.0 mL was kept constant for all experiments. The concentration of protein was analyzed with Bradford reagent, using Bovine Serum Albumin as the standard [4]. The method of Laemmli used for SDS–PAGE electrophoresis [5].

The effect of organic solvent (OS) and ionic liquids (IL) on enzyme characterization

Enzyme characterization was determined by enzyme incubation in the presence of Organic sovents (OS) and IL ([BMIm][Cl] and [EMIm][Br]). For enzyme stability studies, reductase incubated at 37 ˚C for 6 hours in the presence of OS and ILs and then at appropriate time intervals, 25 µl aliquots were removed from each solution mixture and were assayed for residual activity as described. Samples with no additive were used as controls.

3. Results and discussion

The strain was grown anaerobically and aerobically in the presence and absence of chromate in order to determine whether produced chromate reductase is inducible and respiratory enzyme or not. After purification, the obtained chromate reductase eluted between aldolase (150 KDa) and catalase (250 KDa). When calculated, the chromate reductase was found with a molecular mass near 196 kDa. The Km and Vmax values for enzyme were calculated 1.39 mM and 0.26 µ mol.min-1.mg-1, respectively.

As shown in Fig. 1 chromate reductase showed an optimal activity at pH 6.0, an observation that is consistent with previous report. The chromate reductase revealed the maximum activity at 70 °C .

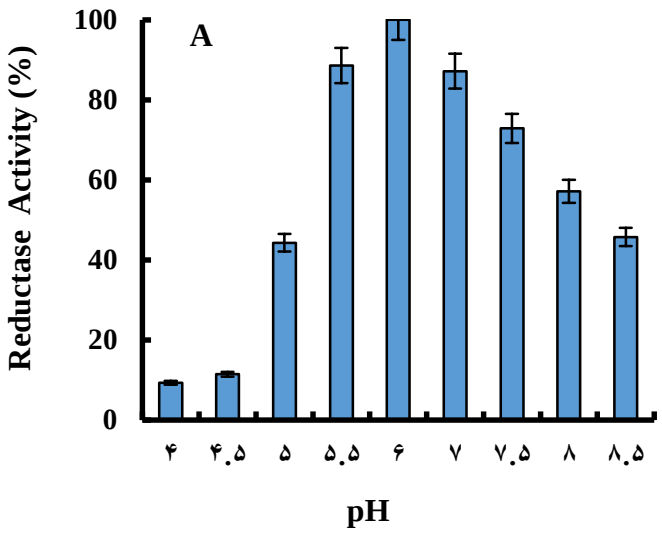

Fig. 1: pH optimum for activity of chromate reductase determined using mix buffer adjusted to the desired pH. The mix buffer contained 50 mM of succinic acid-NaOH (pH3.8 to 6),

imidazole-HCl (pH6.2 to 7.8), tris-(hydroxymethyl)- aminomethane (pH7.1 to 8.9), and glycine-NaOH (pH8.6 to 10.6).

In order to investigate correctly the effects of water-miscible organic solvents on the behavior of the enzyme, some organic solvents were selected for the investigation. The enzyme revealed 76% of its initial activity in the presence of Tween 20. Furthermore, N-propanol is less polar than water and it seems that disruption of hydrogen bonding, ionic, hydrophobic and vanderwaals interactions by a solvent less polar than water can lead to diminished substrate binding of catalytic turnover. The chromate reductase activity decreased in the presence of higher concentration of methanol. As results shown a clear decrease of the activity in the presence of [BMIm][Cl] for the studied enzyme was observed. The detected decay in enzyme activity could be due to either enzyme deactivation or enzyme inhibition.

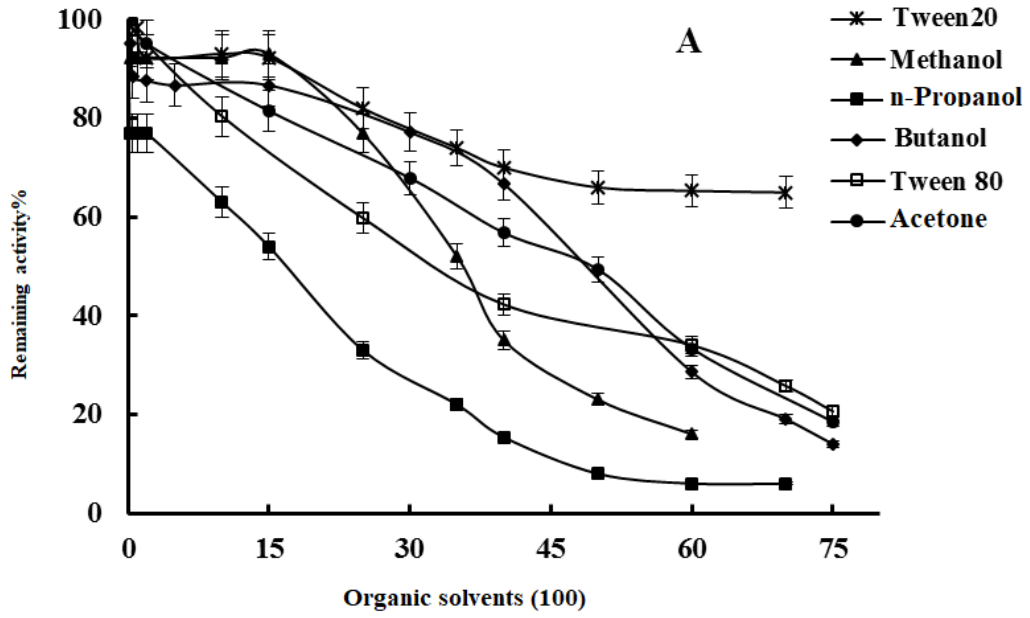

Fig. 2: Remaining activity of chromate reductase at different concentrations of organic solvents

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