

Effects of some alkali and alkaline earth metal ions on the initial velocities of *Congregibacter litoralis* KT71 β -lactamase hydrolysis of 4-nitrophenyl myristate.

ABSTRACT

The effects of some alkali metal ions (Na^+ and K^+) and alkaline earth metal ions (Mg^{2+} and Ba^{2+}) on the initial velocities of *Congregibacter litoralis* KT71 β -lactamase hydrolysis of 4-nitrophenyl myristate was investigated by varying the concentrations of the metal ions in the assay mixture which comprised of 100 μl of standard enzyme solution, 200 μl of varying concentration of metal ions, 500 μl of 50 mM sodium phosphate buffer pH 7.5 and 200 μl of 4-nitrophenyl myristate (substrate) which was added last to the assay mixture after an incubation time of 10 minutes at 44 °C. The enzyme activity was measured spectrophotometrically at a wavelength of 405 nm. The hydrolysis of 4-nitrophenyl myristate to yield 4-nitrophenol was monitored by reading the absorbance at 25 minutes. Results showed that the alkaline earth metal ions (Ba^{2+} and Mg^{2+}) had higher enzyme activation effect than the alkali metal ions (K^+ and Na^+). Also, all metal ions except Mg^{2+} showed enzyme stimulatory effect at low concentrations (<2 mM) but inhibitory at higher ion concentrations (2 mM - 3 mM). Mg^{2+} caused a proportionate decrease in enzyme activity from its peak (when metal ion concentration was lowest). Results from this research is of great significance to the industrialist especially where the search for novel lipases with unique characteristics suitable for the industries are inevitable.

KEYWORDS: *β -lactamase* , 4-nitrophenyl myristate, initial velocities, metal ions

INTRODUCTION

Several industrially useful biocatalysts are found in the marine ecosystem. The marine ecosystems are subject extreme temperatures ranging from 4 °C to 400 °C. This is also accompanied by high hydrostatic pressures [1]

Due to these extreme conditions, novel microbial cellular processes are conferred on these microorganisms present in the ecosystem. Consequently, they possess a unique enzyme system which includes increased salt tolerance and adaptivity to cold, both of which are key to industrial requirements. [2]

Marine sponges (phylum: *Porifera*) makes up one of the unique environmental niche, which inhabits several bacterial communities. Enzymes that are produced by microbe bound in sponges most likely have unique biochemical and physiological characteristics that makes it possible for them to adapt to the unique ecosystem. Metagenomic based approaches are now being used to access these novel enzymes [3]

One of the most important group of enzymes are the Lipolytic enzymes (Esterases (EC 3.1.1.1) and the Lipases (EC 3.1.1.3). These enzymes are involved in novel reactions in both aqueous and non aqueous media. There are very strong interests in researches involving lipases. This is because of their applications in the industries. Some of their applications include processes in biodiesel production, food flavoring, laundry, paper industries, cosmetic production, and in pharmaceutical industries. [4] With these potential applications, the marine microbial lipase still remains under exploited.

Generally, lipases differ from one another based on several criteria such as sizes, substrate specificities and stability profile. They also differ in their activities in the presence of various modulators. With these known features, and considering the importance of lipases in various industries, there is much interest in isolating novel enzymes from unique environmental niches.

Several metal ions are found in the environment and also in the body. They perform multiple functions in enzymes. These functions include the modification of protein structure and the enhancement of the protein structural stability in a conformation required for its function.

Many enzymes are dependent on alkali and alkaline earth metal ions for their activity. The two alkali metal ion in this study which are commonly found in living system are Na^+ and K^+ , while the alkaline earth metal ions in this research are Mg^{2+} and Ba^{2+} .

In this study, the interactions of some of alkali and alkaline earth metals with *Congregibacter litoralis* KT71 β -lactamase from the marine sponge *Haliclona Simulans* was investigated using 4-nitrophenyl myristate as substrate.

MATERIALS AND METHODS

Chemicals

Sodium chloride, Potassium chloride, Barium Chloride, Magnesium Chloride and 4-nitrophenyl myristate were all of analytical grade and purchased from reputable sources.

Methods

Enzyme purification

The protein coding sequence (Genbank Accession number: EAQ98391.2) was codon-optimised for expression in *E. coli* strain BL21 (DE3) *plysS* and synthesized by GeneArt (Invitrogen). Protein expression and purification was via Nickel affinity chromatography.

The purified β -lactamase from *Congregibacter litoralis* used in this work was provided by Dr Femi Olorunniji (School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University).

Enzyme Assay

The activity of purified *Congregibacter litoralis* KT71 β -lactamase was measured spectrophotometrically with the fatty acyl ester 4-nitrophenyl myristate as substrate at λ_{max} 405 nm (Selvin *et al* 2012). Reactions were performed in triplicate at 44°C and the amount of p-nitrophenol liberated at 25 minutes was determined.

RESULTS AND DISCUSSION

It has been shown that some enzymes have a catalytic preference for K^+ [5] Like K^+ channel proteins, they only allow K^+ but not Na^+ to pass through the plasma membrane.

Figure 1 shows the effect of sodium ion on the activity of *Congregibacter litoralis* KT71 β -lactamase hydrolysis of 4-nitrophenyl myristate. Results show that increasing the concentration of sodium ion from 0.5 mM increased the activity of the enzyme up to 1 mM. Further increase in concentration of sodium ion up to 3 mM resulted in a decrease in enzyme activity.

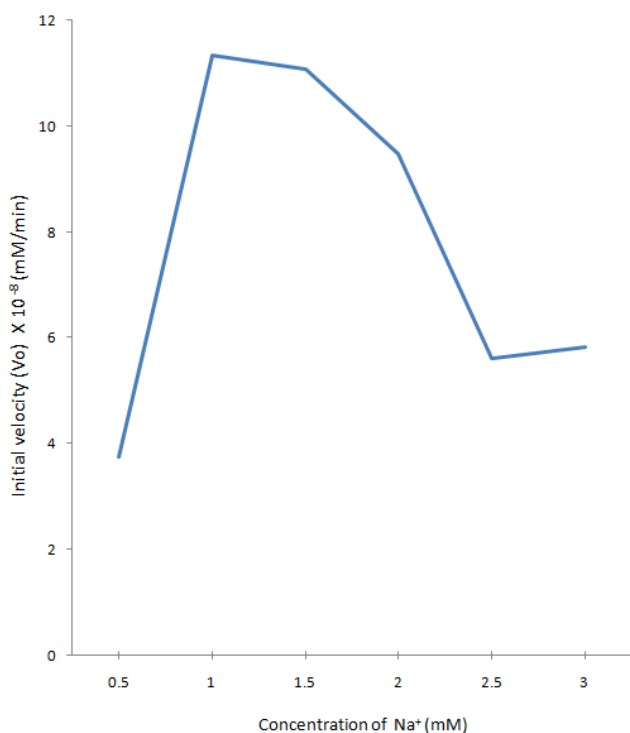


Figure 1: Effect of Sodium ion on the activity of *Congregibacter litoralis* KT71 β -lactamase activity in the hydrolysis of 4-nitrophenyl myristate

Figure 2 shows the effect of potassium ion on the activity of *Congregibacter litoralis* KT71 β -lactamase activity in the hydrolysis of 4-nitrophenyl myristate. Result show that increasing the concentration of potassium from 0.5 mM to 2 mM steadily increased the activity of the enzyme. Further increased in concentration of potassium ion up to 3 mM led to a decrease in enzyme activity.

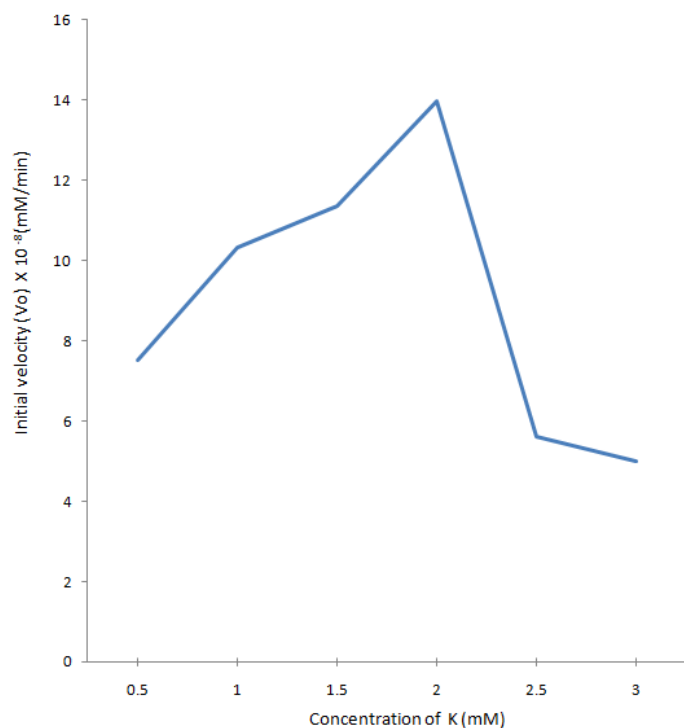


Figure 2: Effect of potassium ion on the activity of *Congregibacter litoralis* KT71 β -lactamase activity in the hydrolysis of 4-nitrophenyl myristate

A comparative study of the effects of the two alkali metal ions on the initial velocity of the enzyme (figure 3) showed that K^+ ions caused a steady increase in enzyme velocity which peaked at a velocity of 13.95×10^{-8} (mM/min) when the K^+ concentration was 2 mM unlike Na^+ which peaked at 11.35×10^{-8} (mM/min) at a Na^+ concentration of 1 mM. At high alkali metal ion concentration (3 mM), though a decrease in initial velocity was observed, K^+ had a higher initial velocity than Na^+ (as shown in figures 1 and 2).

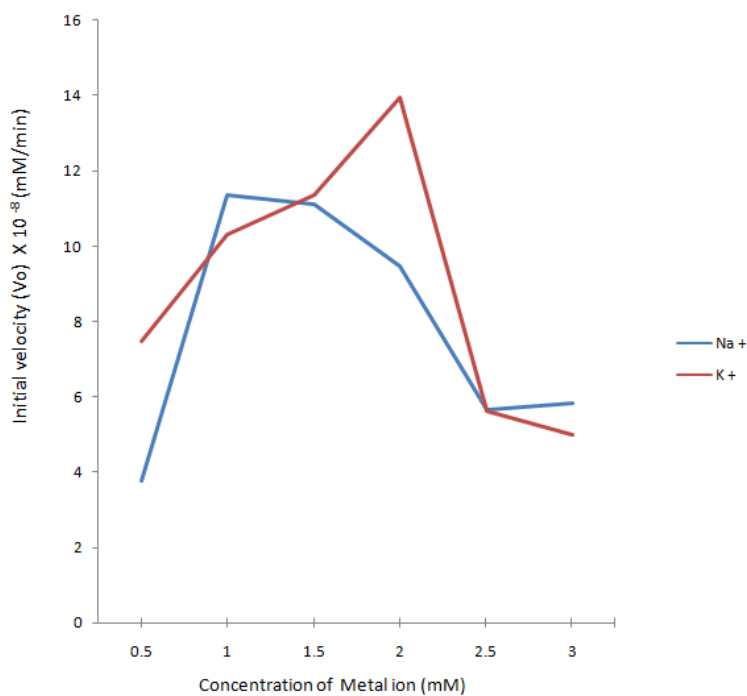


Figure 3: A comparative effect of sodium and potassium ion on the activity of *Congregibacter litoralis* KT71 β -lactamase activity in the hydrolysis of 4-nitrophenyl myristate.

This finding is similar to that observed by previous research [6] in a study on the ion specific effects of sodium and potassium on the catalytic activity of HIV-1 protease. In their study, it was observed that the initial velocity of peptide substrate hydrolysis increases with salt concentration more dramatically in potassium than in sodium chloride solutions. It can be inferred that the active site of the β -lactamase was more responsive to catalysis with substrate in the presence of K^+ than Na^+ .

Generally, for the alkali metal ion studied, low concentrations stimulated enzyme activity while high concentrations were inhibitory. It can be concluded that the alkali metal ions (Na^+ and K^+) have both stimulatory and inhibitory effect on *Congregibacter litoralis* KT71 β -lactamase depending on the concentrations of the metal ions.

Figure 4 shows the effect of barium ion on the activity of *Congregibacter litoralis* KT71 β -lactamase activity in the hydrolysis of 4-nitrophenyl myristate. Results show that increasing the concentration of barium ion from 0.5 mM to 1.5 mM increased the activity of the enzyme. Further increase in the concentration of Barium resulted in the decrease in activity of the enzyme up to 3 mM. This result is similar to that observed in using the alkali metal ions. Results from barium ion showed that it resulted in the highest activity of the β -lactamase compared to other metal ions in this study. This suggests a very high activating effect of barium ion.

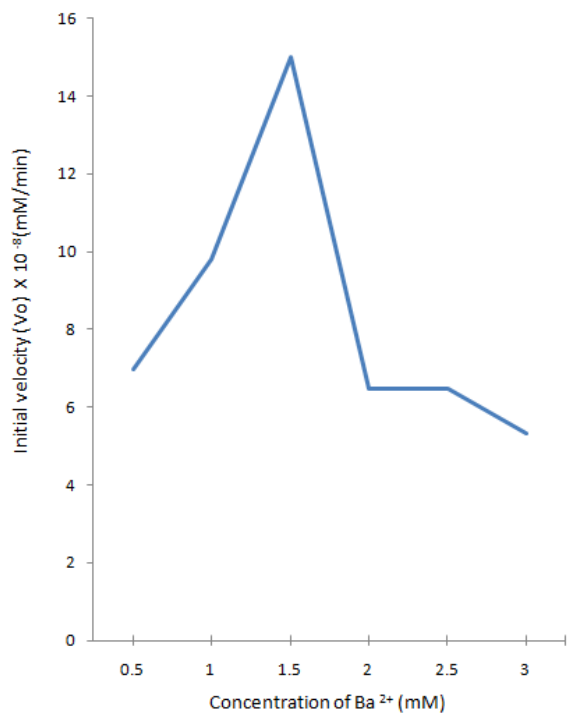


Figure 4: Effect of Barium ion on the activity of Congregibacter litoralisKT71 β -lactamase activity in the hydrolysis of 4-nitrophenyl myristate

Figure 5 shows the effect of Magnesium ion on the activity of Congregibacter litoralis KT71 β -lactamase activity in the hydrolysis of 4-nitrophenyl myristate. Results show that increasing the concentration of Magnesium ion from 0.5 mM to 3 mM resulted in a proportionate decrease in the activity of the enzyme. However at very low concentration of Mg^{2+} ion, the initial velocity was comparable to that of barium ion. Mg^{2+} has been shown to have inhibitory effect at high concentrations [7] in a research on the regulatory effect of divalent cations on rat liver alkaline phosphatase activity. In the research, Mg^{2+} acted both as an activator (optimal concentration) and inhibitor (at higher concentration).

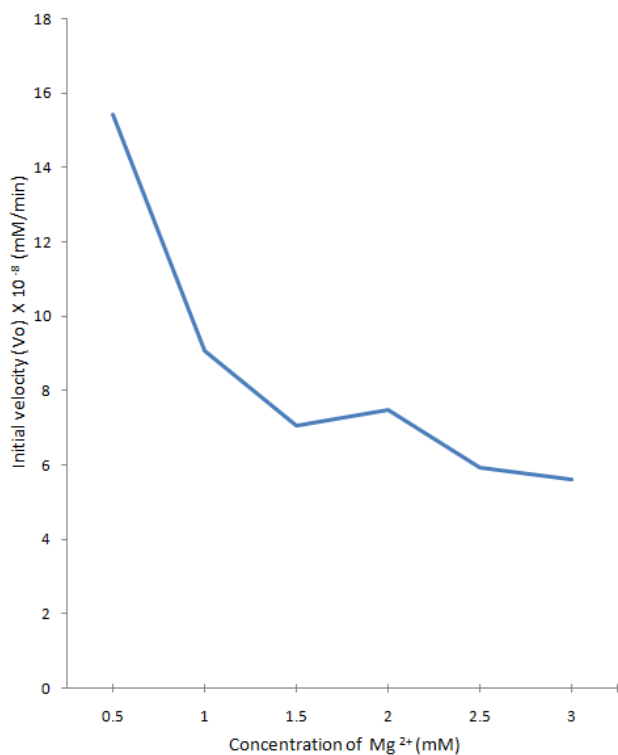


Figure 5: Effect of Magnesium ion on the activity of Congregibacter litoralis KT71 β -lactamase activity in the hydrolysis of 4-nitrophenyl myristate

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