Short Communication

Effects of surfactants on the kinetics of Congregibacter litoralis KT71 β-lactamase

Uche-Enwere R. Chikwado and Martin O. Iniaghe.
Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

ABSTRACT: The effects of some surfactants such as sodium dodecylsulfate (SDS), Tween 80 and Triton X-100 on the kinetics of Congregibacter litoralis KT71 β-lactamase was determined by using 0.1% of each of the surfactant in the enzyme assay. The assay mixtures comprised of 100 µl of standard enzyme solution, 200 µl of 0.1% of surfactant, 500 µl of 50 mM sodium phosphate buffer pH 7.5 and 200 µl of 4-nitrophenyl dodecanoate (substrate) which was added last to the assay mixture after an incubation time of 10 minutes at 44 °C. The hydrolysis of 4-nitrophenyl dodecanoate to yield 4-nitrophenol was monitored by reading the absorbance at 25 minutes at a wavelength of 405 nm. The initial velocity and relative activity was determined and a concurrent assay was done using Thermomyces lanuginosus Lipase (TLL) which served as a reference enzyme. Results show that 0.1% of Non-ionic surfactants such as Triton X-100 and Tween 80 resulted in 25% residual activity in β-lactamase activity, while anionic surfactant like SDS resulted in a 3% residual activity of the β-lactamase. In the reference enzyme TLL a residual activity of 21%, 92%, 73% was observed for Triton X-100, Tween 80 and SDS respectively. It can be concluded that β-lactamase is highly susceptible to denaturation by surfactants especially SDS and Thermomyces lanuginosus Lipase appears relatively more stable in the presence of these surfactants.

KEYWORDS: β-lactamase, Congregibacter litoralis KT71, Surfactants.
INTRODUCTION

Lipases belong to a class of enzymes called hydrolases. They catalyze the hydrolysis of triglycerides to fatty acids and glycerol. They are produced by microbes of plant and animal origin, and by marine organisms. Since marine microorganisms can thrive in extreme conditions, lipases that are isolated from their origin possess characteristics of extremozymes, they retain their activity in extreme conditions and can therefore catalyze few chemical reactions which are impossible otherwise relative to the lipase produced from terrestrial microorganisms. (Patnala et al., 2016).

There is current interest in lipases because of their applications in the industries ranging from biodiesel production, to food flavoring, in laundry applications, paper industries, cosmetic production, and in pharmaceutical industries (Jaeger and Eggert, 2002). Despite these potential applications, marine microbial lipase remains under exploited.

Lipases differ from one another by their sizes, substrate specificities, stability profile, and activity in the presence of various modulators. Knowing the importance of lipases in various industries, there is much interest in isolating novel enzymes from unique environmental niches. β-Lactamases are bacterial enzymes that inactivate β-lactam antibiotics by opening the amide bond of the β-lactam ring (Christopher, 2013).

Surfactants are substances that create self-assembled molecular clusters called micelles in a solution (water or oil phase) and adsorb to the interface between a solution and a different phase (gases/solids). (Nakama, 2017). Surfactants decrease the interfacial tension between the two immiscible phases (oil and water). Based on the nature of polar head they are classified as cationic, anionic, non-ionic or zwitterionic. Surfactants have a special role in the cleaning process by lowering the surface tension of the solution. In general, surfactant activity mostly affects the interaction of the cleaning solution with the substrate surface. (Mahmood and Toofan, 2015)

In this study, the effect of the following surfactants: Triton X-100, Tween 80, and gelatinon on the kinetics of Congregibacter litoralis KT71 β-lactamase was investigated. Thermomyces Lanuginosus Lipase was used as a reference enzyme.

MATERIALS AND METHODS

Chemicals

Tween 80, Triton X-100, Sodium Dodecyl Sulfate and NaH₂PO₄, Na₂HPO₄ were all of analytical grade and purchased from reputable sources.

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 expressed in E. coli strain BL21(DE3)plysS and purified using Nickel affinity chromatography.

Effect of surfactants on the activity of the Congregibacter litoralis KT71 β-lactamase

Enzyme assay was done in the absence and presence of Triton –X, Tween 80 or SDS. Each of the assay mixtures comprised of 100μl of standard enzyme solution, 200μl of 0.1% of surfactant, 500 μl of
50 mM phosphate buffer pH 7.5 and 200 µl of 4-nitrophenyl dodecanoate (substrate) which was added last to the assay mixture after an incubation time of 10mins at 44 °C. The hydrolysis of 4-nitrophenyl dodecanoate to yield 4-nitrophenol was monitored by reading the absorbance at 25 minutes at a wavelength of 405 nm. The initial velocity and relative activity was determined with Thermomyces lanuginosus Lipase (TLL) which served as a reference enzyme. All assays were performed in triplicates. For the control, distilled water replaced the surfactants. The relative activities of the enzyme and their initial velocities were calculated and the mean was used for comparing of activities.

RESULTS AND DISCUSSION

Table 1 shows the effect of Triton-X, Tween 80 and SDS on the initial velocities and residual activities of Beta-lactamase. Results Show that SDS greatly reduced the activity of the enzyme when compared with control and other surfactants. Triton–X and Tween 80 had the least effect in reducing the enzyme activity when compared with the other surfactants. Similarly, the residual activity was greatly reduced in the presence of SDS than Triton –X and Tween 80 as shown.

Table 1. Effect of Triton-X, Tween 80 and SDS on the initial velocities and residual activities of β-lactamase.

<table>
<thead>
<tr>
<th>Surfactants (0.1%)</th>
<th>Initial Velocity (mmol/mim/ml) x10^{-5}</th>
<th>Residual activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton-X 100</td>
<td>0.49</td>
<td>25</td>
</tr>
<tr>
<td>Tween-80</td>
<td>0.37</td>
<td>25</td>
</tr>
<tr>
<td>SDS</td>
<td>0.04</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td>1.47</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Effect of Triton-X, Tween 80 and SDS on the initial velocities and residual activities of Thermomyces lanuginosus Lipase (TLL).

<table>
<thead>
<tr>
<th>Surfactants (0.1%)</th>
<th>Initial Velocity (mmol/mim/ml) x10^{-5}</th>
<th>Residual activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton-X 100</td>
<td>0.4</td>
<td>21</td>
</tr>
<tr>
<td>Tween-80</td>
<td>1.6</td>
<td>92</td>
</tr>
<tr>
<td>SDS</td>
<td>1.3</td>
<td>73</td>
</tr>
<tr>
<td>Control</td>
<td>1.8</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 2 also shows the effect of of Triton X-100, Tween 80 and SDS on the initial velocities and residual activities of Thermomyces lanuginosus Lipase (TLL). Results show that the residual activity of TLL using Tween 80 was the highest (92%) when compared with Triton-X and SDS. Unlike in Beta lactamase, the residual activity in the presence of SDS was significantly higher in TLL. The same trend was observed with the initial velocities.

In this study, the effects of surfactants on the activities of β-lactamase and *Thermomyces lanuginosus* Lipase was investigated. From the results obtained, all surfactants used in this research significantly reduced the initial velocities and residual activities of β-lactamase. On the contrary, the reference enzyme *Thermomyces lanuginosus* lipase had a different kinetics in the presence of these surfactants being able to resist the denaturing effect of these surfactants to a relatively high degree.

Sodium dodecyl sulfate (SDS) is a most effective detergent for solubilizing protein membranes, it is also an effective protein denaturant. Most enzymes that have been studied shows a complete loss in activity after treatment with SDS.

SDS which strongly reduced the activity of β-lactamase had a minimal effect on TLL. This may imply that 0.1% SDS does not cause appreciable changes in the secondary structure for TLL but caused an appreciable change in secondary structure for β-lactamase. This is similar to the reactions in interaction of sodium dodecyl sulfate with beta-galactosidase where 1% SDS did not change the secondary structure of the enzyme but 10% SDS did change the secondary structure of the enzyme. It was also shown that SDS can cause a conformational change involving enzyme inactivation and increase accessibility of the solvent to the protein core (Muga et al 1993).

Results from SDS interaction between the two enzymes shows that TLL has a stronger resistance to the denaturing effect of SDS than β-lactamase. In a similar manner, Tween 80 seem not to significantly change the conformation of TLL resulting in a residual activity of 92% compared with β-lactamase which had a residual activity of 25%. The effect Triton X-100 on both enzymes shows a marked decrease in the residual activity of the enzyme.

Generally, surfactants increase the lipid-water interface and thus enhance the rate of lipolysis. However, this does not hold true for all surfactants. The effect of surfactants is concentration dependent (Sangeetha et al, 2011). It was shown that high concentrations of Tween-80 (1%) inhibited lipase production by *B. pumilus* while at 0.5% concentration Tween-80 assisted maximum lipase production (Zhang et al., 2009).

SDS was also found to exhibit inhibitory effect on lipases while Triton X-100 and Tween enhanced reaction rates (Quyen et al., 2003; Lianghua and Liming, 2005). The reversal of this is also plausible; (Dutta and Ray, 2009) have observed that SDS exhibited stimulatory effect while Triton and Tween inhibited lipase activity. In conclusion, the effect these surfactants on enzyme activities are dependent on the properties of the enzyme.

From this study, the surfactants Triton X-100, Tween 80 and SDS exerted a denaturing effect on the secondary structure of the enzyme β-lactamase which consequently led to loss of activity. SDS had the greatest denaturing effect on β-lactamase relative to Tween 80 and Triton-X. The reference enzyme TLL possibly had a more resistant protein conformation hence was more resistant to the effects of these surfactants.
REFERENCES


