Impacts of structural zinc and two conserved tryptophans on thermostability of thermoalkalophilic lipases

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Summary

Thermoalkalophilic bacteria produce thermostable lipases that stood out among other lipases owing to their large potential for industrial processes operated at elevated temperatures. Identification of the factors enhancing thermostability is critical to efforts to design of promising biocatalysts. Hitherto a few studies have elucidated that the structural zinc and the conserved tryptophan that is closely located to structural zinc have potentiated thermostability of thermoalkalophilic lipases.

In this study we investigate the impacts of the structural zinc and two tryptophans (W60, W211) that surround the zinc coordination site. For this project we chose the lipase from *Bacillus thermocatenulatus* (BTL2) as a representative of this family and delineated the interplay between zinc and the tryptophans in the context of thermostability by generating three mutants namely W60A, W211A and W60A/W211A.

First of all the purity of our different samples was tested by SDS-Page in order to obtain the final list of trustable samples to be analysed along all the experiments. DLS was used to find which the sizes of the different types of lipases are. It shows us that there is not a remarkable different between BTL2 and the single mutants, whereas producing the double mutant means loosening of structure. The other alteration of structure to be proved was the removing of the zinc. The behavior of our proteins in a wide range of temperatures in function of their concentration and their zinc condition was tested performing thermostability assays. These illustrated how higher concentrations and zinc containing forms show more activity when the temperature is increased than the other forms. Thermal denaturation and CD experiments demonstrated that the zinc is essential for structural stability of BTL2 while the presence of tryptophans (W60, W211) has contributed to stability with the W211 being the most dominant. Overall these results imply that the conserved tryptophans have a direct impact on the zinc mediated thermostability of BTL2. In the meanwhile, structural investigations were conducted paying attention in the position of the tryptophans and the zinc positions. In the figures acquired we can see how the tryptophans protect the zinc cation.
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1. **Abbreviations**

**pMCSG7**: bacterial expression vector with T7 promoter, adds N-terminal His tag and TEV protease site; amp resistance; ligation independent cloning (LIC).

**BTL2**: native lipase.

**W60A**: mutant lipase with an alanine substitution at the W60 tryptophan.

**W211A**: mutant lipase with an alanine substitution at the W211 tryptophan.

**W60/211A or W60-211A**: double mutant lipase with alanine substitutions at both W60 and W211 tryptophans.

**SDS-Page**: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis.

**APS**: Ammonium persulfate.

**TEMED**: Tetramethylethylenediamine.

**DLS**: Dynamic light scattering.

**$R_H$**: hydrodynamic radius.

**$C_P$**: standard deviation.

**TPEN**: N,N,N′,N′-Tetrakis(2-pyridylmethyl)ethylenediamine.

**CD**: circular dichroism.

**VMD**: visual molecular dynamics.
2. Introduction

Proteins are biological molecules that play important and versatile roles in all of the chemical processes of life. These biological species represent approximately 15 percent of the total cell mass. Proteins are built as the sum of a set of amino acids. Proteins are linear polymers derived from α-amino acid monomers, which are carboxylic acids with an amino group, a hydrogen atom and a further substituent (R) attached to the α-carbon. In the case of the proteins, the substituent R is limited to one of the 20 possible groups (or a derivative of one of these groups), each of these groups introduce a chemical diversity into the polymer, and thus a given protein shows biochemical properties depending on the amino acids forming it.

There are a huge variety of possible proteins depending on their roles. Enzymes are one of the types that can be found and act as a catalyst for essential chemical reactions in cells. They are capable of binding small molecules reversibly at some types of specific sites on their surfaces; this property is used by enzymes to catalyse chemical reactions. Moreover, the mechanisms of enzyme-catalysed reactions are usually directly related to those of organic chemistry.

There are three major groups needed for the human nutrition; lipids (fats) are one of these. In order to be able to digest them, it is necessary the action of one enzyme: the lipase. This kind of enzyme hydrolyzes lipids, the ester bonds in triglycerides, to form fatty acids and glycerol.

Bacterial thermoalkalophilic lipases grouped in the lipase family I.5 display only 30% homology with the lipases from gram positive bacteria. Despite their isolation from other bacterial lipases, thermoalkalophilic lipases have about 90% sequence identity with each other, which essentially makes any finding on one member valid for the whole family. Owing to high sequence conservation, these lipases share common biochemical features. Thermoalkalophilic lipases possess a unique zinc site and they also show increased thermostability compared with the mesophilic lipases, which attracts special attention to these lipases with regards to industrial processes that often operate at high temperatures. Previously studies concerning the conserved lid tryptophan has
demonstrated that the presence of the tryptophan W211 is critical to the thermostability of the thermoalkalophilic lipase originating from Bacillus thermocatenulatus (BTL2) \[^4\]. Although this conserved tryptophan W211 is closely located to the structural zinc, the interplay between the W211 and the structural zinc in the context of thermostability remains elusive.

In this study we aim to investigate this particular relationship and also along with W211 we located another tryptophan, W60 is also located very close to the structural zinc. Therefore we used three different mutants, namely W60A, W211A, W60A/W211A and the native BTL2 to delineate the interactions of the hydrophobic tryptophans with the structural zinc to achieve thermostability. To elucidate the particular impact of zinc, the zinc free forms of the lipases are obtained and used in the experiments. The insights revealed by this work are critical to the efforts to understand thermostability of thermophilic species and to the design of novel lipases with improved stabilities.
3. Experimental Methods

Here we describe the materials and methods that have been performed in the course of this project. Beforehand, the native and the mutant lipase genes were obtained as inserted in the expression plasmid of pMCSG7. This insertion was generated a fusion protein composed of an amino terminal polyhistidine tag and the mature lipases.

3.1 Expression and Purification

Escherichia coli SHuffle® cells (New England Biolabs) were transformed with the expression plasmids pMCSG7 containing the native and the mutant (W60A, W211A and W60A/W211A) lipases. These lipases were expressed in E. coli cells that were cultivated in Luria-Bertani media (low salt formulation) supplemented with 100 µg/ml ampicillin and with 1 mM of isopropyl-β-d-thiogalactopyranoside for induction. Expression was continued for 2 hours at 37°C and subsequent purification was carried out using HisTrap HP columns (GE Healthcare Life Sciences) by the availability of N-terminal polyhistidine tag. The purified lipases were stored in 10 mM sodium phosphate buffer pH 7.4 at 4°C. Protein concentration was determined using Bradford assay and purity of the recombinant lipase was tested by sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE).

3.2 SDS–Page

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is an analytical method used to determine the molecular weight of proteins. It works according to the electrophoretic mobility of the molecule. The mobility can be defined as a function of the chain length of the acrylamide polymer, molecular mass and other factors such as the charge of the molecule. Here we used this method to test the purity of the lipases.

Gel preparation

For the acrylamide and bis-acrylamide mixture we used a commercial solution and mixed it with %10 Ammonium persulfate (APS) and Tetramethylethylenediamine...
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(TEMED). The mixture is poured between two glasses placed 1 mm apart from each other. The gel is let about 1 hour for polymerization. The extra gels are kept at 4°C by wrapping with a wet tissue paper.

Sample preparation

The protein samples were diluted to 2 µg/µl, in water. 20 µl of the diluted protein samples were mixed with 5 µl of sample buffer containing bromophenol blue, SDS and glycerol. The mixtures were boiled at 95°C for 5 minutes and then directly loaded onto 12% polyacrylamide gel. During the heating procedure is important to cover our solution to prevent evaporation due to higher temperatures on the top of the machine. The samples can be kept at -20°C for later. The total amount of protein loaded onto each well is 50 µg. The gel was run at 100 V for the first 20 minutes and then at 120 V for an additional 2 hours.

Method performance

The equipment required for protein electrophoresis includes a tank, electrode chamber, power source, the gel and the samples prepared previously, a pipe and buffer (Figures 1 and 2). The gasket holding the gel is installed inside the tank which is filled with the running buffer. The tank lid is closed to complete the circuit and the power supply is adjusted according to the needs.

Figure 1. Schematic procedure
3.3 DLS

Dynamic light scattering (DLS) is a noninvasive method for measuring sizes of globular proteins. The sizes are typically in the submicron region.

The samples were prepared to contain the native and the mutant lipases at different concentrations in 10 µM of sodium phosphate at pH 7.4. The measurements were performed using Zetasizer NanoXS for three times. The hydrodynamic radius ($R_H$) and the standard deviation of the radius ($C_P$) were collected from monomodal data using the Zetasizer software.

3.4 Zinc removal

To determine the impact of structural zinc on the tested properties of the native and the mutant lipases, the zinc is chelated from the samples. Before zinc removal, all of the buffers (10 mM NaPO$_4$ at pH 7.4) were treated with Chelex (Sigma), a special resin that was developed to remove divalent cations such as zinc, for 1 hour at 37°C.
The samples were prepared to contain 10 mM NaPO$_4$ at pH 7.4 and 2 mM of N,N,N',N'-Tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) and 100 μM of protein. They were let with mild shaking at 4°C. The mixtures were concentrated by filters with pore size of 10 kDa. The filtration was carried out at 13,200 rpm for 10 minutes to remove the unbound TPEN and TPEN-Zn complexes. When this step is finished the samples of proteins were said to be free of zinc and kept at 4°C for further analysis.

### 3.5 Thermostability assay

This assay gives a picture of the protein behavior in different temperatures and how they also depend on their concentration. First of all the proteins were diluted, in this case at 10 nm and 10 μM. These two different concentrations of the lipase samples were incubated for 30 minutes at different temperatures, explicitly at 50 to 75°C with 5°C intervals. The control samples were kept in ice.

Enzyme assays were performed with 4-methylumberrylferyl (4-MU) caprylate as substrate in 10 mM phosphate buffer at pH 7.4 in a kinetic fashion. Gemini XS (Molecular Devices) was used to measure 4-MU fluorescence with excitation at 355 nm and emission at 460 nm. The assays were carried out at room temperature using 5 nm of lipase in the final reaction mixture. The percent activity values were obtained by setting the rate of the control sample to 100%. All of the assays were repeated at least three times to test for reproducibility.

### 3.6 CD Spectra

This method measures the absorbance of a solution when circularly polarized light impacts on it. Far-UV circular dichorism (CD) spectra of the samples at 0.5 mg/ml were collected with 1.0 mm path length, while near-UV CD spectra were collected from the samples at 1-3 mg/ml with 10.0 mm path length using J-815 spectropolarimeter (Jasco) in N$_2$ atmosphere equipped with thermostatically controlled cuvette at a scanning speed of 50 nm/min. The far-UV range is 190-250 nm and the near-UV range is 250-350 nm. The spectra scans were performed at 25°C and 3 scans were averaged to obtain final spectra which were corrected for the background. Thermal denaturation profiles were
determined before and after centrifugation (13,000 rpm for 30 minutes) by tracing ellipticity at 222 nm at a 6°C/min heating rate from 30°C to 90°C. $T_m$ values were calculated from the midpoint of the transition curves between folded and unfolded states of the lipases.

### 3.7 Structural investigation

The native thermoalkalophilic lipase structure (PDB ID: 1KU0) was investigated by visual molecular dynamics (VMD). The visuals were taken with special focus on the tryptophan residues W60A and W211A and the structural zinc site.
4. Experimental Results

As we said before the purpose of this project was to identify the impacts of the structural zinc and the tryptophans on the thermostability of BTL2. For this reason some mutants of the original enzymes were created and metal removal experiments were performed in order to eliminate zinc from the original molecule.

4.1 SDS

<table>
<thead>
<tr>
<th>Lane #</th>
<th>Sample Name</th>
<th>Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein Marker</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BTL 2 Pure</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>BTL 2 10 mM</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>BTL 2 ecoli 8 mg/ml</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>BTL 2 6 mg/ml</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>W211A 30 mg/ml</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>W211A 40 mg/ml</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>W211A 7 mg/ml</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>W60-211A 7 mg/ml</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>W60-211A 12 mg/ml</td>
<td></td>
</tr>
</tbody>
</table>
The first lane represents the protein marker that produced reference protein bands with known molecular weight (Figure 4). For this method the list of samples used is shown in Table 1.

Although 50 µg of protein was analyzed in SDS-PAGE, some of the samples (lanes 2, 4, 6 and 10) produced bands with lower intensity than those produced thick and intense protein bands (lanes 3, 5, 7, 8, 9). Thus those samples with lower protein concentration than predicted were discarded before characterizations.

Other kinds of results are obtained because of this method. The upper mark seen in W211A 40 mg/ml (sample number 6 in figure 1) might indicate the presence of oligomers such as dimers while other bands that do not correspond to any oligomeric form might indicate contamination in the sample, e.g. lane 5 (Figure 4).

Using SDS-Page as a method to test the purity of the samples gave us the best samples to use during all characterization process. But furthermore, this method is also useful to characterize the protein samples such as the contaminated or degraded protein samples can be distinguished via SDS-PAGE.

4.2 DLS

![DLS result for BTL2 0.5 mg/ml](image)

Figure 5. DLS result for BTL2 0.5 mg/ml
Figures 5, 6 and 7 show the size distributions by volume from the native (BTL2) and two of its mutants (W211A and W60-211A). BTL2 and W211A show a similar distribution, native gives a bigger amount of volume percentage using the same concentration amount. If the comparing is done between W60-211A and the last two mentioned.
proteins, the double mutant gives a larger distribution being less concentrated than the other two. This result might suggest a loosening of structure in the case of W60-211A.

DLS analysis was not preformed for W60A mutant. The reason of this decision is the insignificant outcomes in the size distributions made by the mutant W211A.

The significant difference obtained as a result of DLS between the double mutant and the other three polymers shows that the double mutant loosens the native structure.

### 4.3 Thermostability

![Thermostability result for BTL2 with and without Zn](image)

Figure 8. Thermostability result for BTL2 with and without Zn
Characterization of mutant lipases

Figure 9. Thermostability result for W60A with and without Zn

Figure 10. Thermostability result for W211A with and without Zn
Figure 11. Thermostability result for W60-211A with and without Zn

The figures 8, 9, 10, 11 show the thermostability results of the native and the three mutants (W60A, W211A and W60-211A). The first two columns (red, blue) represent the incubation conditions performed at the low concentration (1x) while the last two columns (orange, brown) show the incubation performed at high concentration (1000x).

Concentration comparison

Native and W60A mutant show a similar behavior, despite the fact that the mutant loses its activity before in higher concentrations (at 65°C instead of 70°C). Both proteins experience an increase in their activity at higher temperatures when thermal incubation was performed at high temperatures. However when the incubation was performed at low concentration, they failed to enhance the residual activity, essentially in the temperature range of 60-65 for the native and 55-60 for the W60A mutant. On the other hand the other mutants, W211A and the double mutant (W60A/W211A) displayed distinct thermostability profiles such that both of the mutants showed a much lower thermostability compared with the native and the W60A mutant. This result basically suggests that the W211 is located at a more critical location than the W60. Moreover both these mutant did not show any significant boost in thermostability upon an increase in the incubation concentration. Among these two mutants, the double mutant (W60A/W211A) is much less thermostable than W211A and also is much less responsive to the concentration increase during thermal incubation. These results implied that the
tryptophans located at 60th and 211th positions have a direct impact on thermostability of the native lipase with W211 being more significant. Also that the double mutant confirmed that the impact of the alanine substitutions at these positions is linearly related such that the double mutant showed a drastic decrease in the thermostability followed by W211A which is followed by W60A. Hence we suggest that both of the tryptophans act to potentiate thermostability also both of them are essential for the thermostability achieved by the native lipase.

Zinc comparison
When the zinc containing and the zinc-free form has been compared, a similar behavior in the activity of the native and the W60A mutant was also observed such that the zinc free forms showed lower thermostability compared with the zinc containing lipases. The activity of W211A is affected more rapidly at high temperatures than the native and the W60A. The samples without zinc lose all their activity at 60°C. The double mutant shows a different behavior in this aspect. Zinc presence and absence gave parallel results suggesting a minimal impact of zinc on the thermostability of the double mutant. In the analysis of thermostability, native shows to be more resistant to high temperatures than its mutants. Moreover, as the modification of its structure is high, thermostability of the native became worse with the lowest thermostability observed for the double mutant. Besides we see a different behavior between W60A and W211A, the results of the first one are more similar to the native. This fact means that the location of the changes in the structure plays an important role too. Ultimately, zinc free forms show a decrease in the activity in opposition to the zinc containing forms of the native and W60A and W211A mutants, but this is not shown for the double mutant. We can say that the role of the tryptophans play a more important role in the activity of the proteins than zinc.
4.4 CD – Far UV Spectra

Figure 12. Result of Far UV Spectra with Zn

Figure 13. Result of Far UV Spectra without Zn
Table 2. Resume of obtained results

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>BTL2</th>
<th>BTL2-Zn</th>
<th>W60A</th>
<th>W60A-Zn</th>
<th>W211A</th>
<th>W211A-Zn</th>
<th>W60-211A</th>
<th>W60-211A-Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>222</td>
<td>-36.84</td>
<td>-47.29</td>
<td>-12.02</td>
<td>-34.36</td>
<td>-51.16</td>
<td>-30.93</td>
<td>-17.74</td>
<td>-35.87</td>
</tr>
<tr>
<td>208</td>
<td>-33.75</td>
<td>-46.59</td>
<td>-12.09</td>
<td>-33.67</td>
<td>-49.98</td>
<td>-31.87</td>
<td>-16.35</td>
<td>-37.67</td>
</tr>
<tr>
<td>222/208</td>
<td>1.09</td>
<td>1.02</td>
<td>0.99</td>
<td>1.02</td>
<td>0.97</td>
<td>1.08</td>
<td>0.95</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Results of melting temperature

<table>
<thead>
<tr>
<th>Samples</th>
<th>T_m (Celsius)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTL2</td>
<td>73.01 ± 0.37</td>
</tr>
<tr>
<td>BTL2-Zn</td>
<td>68.98 ± 0.13</td>
</tr>
<tr>
<td>W60A</td>
<td>66.24 ± 0.02</td>
</tr>
<tr>
<td>W60A-Zn</td>
<td>63.16 ± 0.07</td>
</tr>
<tr>
<td>W211A</td>
<td>65.58 ± 0.05</td>
</tr>
<tr>
<td>W211A-Zn</td>
<td>58.59 ± 0.13</td>
</tr>
<tr>
<td>W60-211A</td>
<td>58.39 ± 0.01</td>
</tr>
<tr>
<td>W60-211A-Zn</td>
<td>57.09 ± 0.30</td>
</tr>
</tbody>
</table>

The graphics of the figures 12 and 13 show the far UV CD spectra of the samples in the zinc containing and the zinc free form.

In far-UV CD spectra, the ellipticity ratio $\theta_{222}/\theta_{208}$ can be used to evaluate the helical structure and association. Particularly, the n-$\pi^*$ transition (the band at 222 nm) is responsive to single stranded $\alpha$-helices, while the $\pi$-$\pi^*$ excitation (the band at 208 nm) is responsive to two-stranded helices that are interacting as oligomers [5]. Hence, a higher $\theta_{222}/\theta_{208}$ than 1.0 would suggest an increase in oligomeric ratio, while a lower ratio than 1.0 would present a monomeric form. In this sense, although zinc absence decreased the $\theta_{222}/\theta_{208}$ ratio of the native lipase, it did not become lower than 1.0. This result indicates that the zinc might have a role in the oligomerization. Indeed when the zinc is removed from the proteins all decrease in the ellipticity ratio of 222/208. The conclusion of this affect is that the intermolecular interactions are reduced in the mutants and also in the zinc free forms [6].

As it is showed in Table 3 all of the samples showed a decrease in their melting temperature when zinc is removed. While the decrease in the melting temperature is
significant in the native, W60A and W211A, it was not in the double mutant. This suggests that the structural zinc contributes to the thermal stability of the lipase molecules, with the double mutant being the least significant.

### 4.5 CD – Near UV Spectra

![CD - Near UV Spectra for W60A mutant](image1)

**Figure 14. Result of Near UV Spectra for W60A mutant**

![CD - Near UV Spectra for W211A mutant](image2)

**Figure 15. Result of Near UV Spectra for W211A mutant**
Characterization of mutant lipases

The graphics of the figures 14, 15 and 16 show the near-UV CD spectra of the mutant samples in the zinc containing and the zinc free form.

In near-UV CD spectra, proteins just give signal when they retain defined three-dimensional structures if not the results obtained when you perform this method are nearly zero. The important bands are located at 260 nm and 295 nm approximately. The signals located between 250 and 270 nm indicate the presence of phenylalanine. The ones located between 280 and 300 nm shows that these samples contain tryptophans in their structure. In the first graphic (figure 14) we can see the impact of the concentration and the meaning of zinc containing. When zinc is removed from our samples the signals given are stronger, meaning that zinc has an impact in these structures. Using higher concentrations, around 2.0 mg/ml, give more defined bands as a result.

As a result of the analysis of the structures we found out that zinc plays an important role in the oligomerization process, this metal cause a decrease in the intermolecular interactions as mutants do similarly. The proof of that are the ellipticity ratios values, which are decreased in the case of the mutants and the zinc free forms. The melting temperature is also affected for the presence of zinc; our proteins are more stable when zinc is in their structure. Additionally, the results show the presence of tryptophans in our structures.
5. Structural Figures

The figures 17 and 18 show the close and open lipase structures and the W60, W211 along with zinc respectively. The different between the native and the mutants is represented using the pink color to show that parts. Zinc is showed as a yellow ball. Although the tryptophan W211 is found in the lid domain that changes its conformation from the closed to open state, the tryptophans did not change their conformation with respect to the lid’s conformation, suggesting that regardless of the lipase’s conformation the tryptophans protect the structural zinc.

![Figure 17. Close conformation (PDB ID: 1KU0)](image)
In all of the metalloenzymes, aromatic residues surround the metal binding site and enhance binding specificity of metals\textsuperscript{[9, 10]}. Similarly, in this case W60 and W211 surround the zinc site (Figure 19); are suggested to be involved in binding specificity of
zinc cation. Thus presence of these aromatic residues around the zinc coordination site would potentiate the structural stability of the lipase, enhancing the compactness of the zinc domain.
6. Conclusion

The purpose to be achieved in the beginning of this project was to identify the impacts that can be caused for the modification of the original structure in the native lipase. For this reason some mutants of the original enzyme were created and metal removal experiments were performed in order to eliminate zinc from the original molecule.

Using SDS-Page as a method to test the purity of the samples gave us the best samples to use during all characterization process. But furthermore, this method is also useful to get more knowledge about the samples. As a result of this experiment we could also see that some of the samples were contaminated or others had the presence of oligomers.

The significant difference obtained as a result of DLS between the double mutant and the other three polymers shows that the creation of this double mutant means the loosening of structure.

In the analysis of thermostability, native shows to be more resistant to high temperatures than its mutants. Moreover, as much as its structure is modified the activity of the protein gets worst as can be seen in the results obtaining for the double mutant. Besides we see a different behavior between W60A and W211A, the results of the first one are more similar to the native. This fact means that the location of the changes in the structure plays an important role too. Ultimately, zinc free forms show a decrease in the activity in opposition of the containing forms for the native and W60A and W211A mutants, but this is not shown for the double mutant. We can say that the tryptophans play a more important role in the activity of the proteins than zinc.

As a result of the analysis of the structures we found out that zinc plays an important role in the oligomerization process, this metal cause a decrease in the intermolecular interactions as mutants do as well. The proof of that are the ellipticity ratios values, which are decreased in the case of the mutants and the zinc free forms. The melting temperature is also affected for the presence of zinc; our proteins are more stable when
zinc is in their structure. Additionally, the results show the presence of tryptophans in our structures.

To sum up, despite being an uncommon metal for lipases, thermoalkalophilic lipases possess a unique zinc site within their structures. In this study we identified that this unique zinc site play a major role in thermostability of native BTL2 such that in the absence of zinc the lipase showed decreased thermostability. More importantly we showed that the particular impact of structural zinc is dependent on the presence of two tryptophans that surround the zinc coordination site. Apart from expanding our knowledge on thermostability that occur naturally in thermophiles, these findings may also contribute to design of novel proteins with altered stabilities.
Bibliography


