Quantifying the Differences in Binding Affinity of Reduced Glutathione for Glutathione S-Transferase at pH 6.5 and 8.5 Using Isothermal Titration Calorimetry

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Abstract

The binding affinity between an enzyme and its substrate is often dependent on the pH of the local environment. Knowing the pH at which reduced glutathione (GSH) binds with the highest affinity to the enzyme glutathione S-transferase (GST) is useful for determining the optimal pH for purification of GST-fusion proteins during GST-affinity chromatography. In this study, GST of the species Schistosoma japonicum was purified, quantified, and utilized to study its binding interaction with GSH at pH 6.5 and 8.5 via isothermal titration calorimetry (ITC). After protein expression, extraction, and purification, the GST concentration was quantified using Qubit™ fluorometry. Thermodynamic properties and a dissociation constant (K_D) for each experiment were obtained utilizing the MicroCal PEAQ-ITC Analysis Software for the binding of GSH to GST at pH 6.5 and 8.5. Statistical analysis of the technical replicate data was performed to obtain an average and standard deviation of the K_D at each pH point. The results indicate a statistically significant difference (p<0.05) in binding affinity for the GSH-GST interaction, with the higher-affinity binding occurring at pH 8.5.

Methods

Expression

Recombinant GST was expressed in E. coli using the pGEX-4T-3 plasmid containing the gene for GST from the parasitic species Schistosoma japonicum. The cells were then pelleted and stored at -80°C for later use.

Purification

E. coli cell pellets were resuspended, lysed, and the GST was captured from the clarified cell lysate via GST-affinity chromatography. The GST was then dialyzed to remove GSH from the solution.

Quantification

The purified GST was quantified using Qubit™ fluorometry. BSA standards of known concentrations (0, 50, 100, 150, 200, & 250 µg/mL) were measured to verify the accuracy of the instrument’s measurements.

Results

The data in Table 1 suggest that the binding affinity of GSH for GST is significantly higher (~2-fold) at pH 8.5 than at pH 6.5, noting that a lower K_D corresponds to a higher binding affinity.

Figure 1 provides an example of a GSH-GST ITC run performed at pH 6.5. The baseline on the left side of the figure represents the change in the amount of power required to keep the sample cell at the same temperature as the reference cell throughout the titration. The binding of GSH with GST can be seen in the graph of the change in heat (expressed as binding enthalpy) vs. the molar ratio by the decrease in heat produced over time as more GSH is added to the system. Once GST becomes saturated with GSH, no more binding can occur between the two molecules, and only the heat of dilution can be seen.

Table 1: K_D and thermodynamic properties results (mean ± SD) of the statistical analysis of the technical replicates data of the GSH-GST ITC experiments performed at pH 6.5 (n=3) and 8.5 (n=4) at 25° C.

<table>
<thead>
<tr>
<th>pH</th>
<th>K_D (µM)</th>
<th>ΔH (kJ/mol)</th>
<th>ΔG (kJ/mol)</th>
<th>ΔT ΔS (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>63.3 ± 12.4</td>
<td>-11.4 ± 0.8</td>
<td>-24.0 ± 0.5</td>
<td>-12.6 ± 1.3</td>
</tr>
<tr>
<td>8.5</td>
<td>25.4 ± 3.8</td>
<td>-31.8 ± 2.3</td>
<td>-26.3 ± 0.4</td>
<td>5.49 ± 2.6</td>
</tr>
</tbody>
</table>

References

pGEX-4T-3 Vector Photo
https://www.lifescience-market.com/images/pGEX-4T-3-vector.png

GST-Affinity Chromatography Photo
https://www.ucdmc.ucdavis.edu/services/affinitychromatography.jpg

Qubit™ 3.0 Fluorometer Photo