



**SDI Review Form 1.6**

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| Journal Name:            | <a href="#">International Journal of Biochemistry Research &amp; Review</a>                |
| Manuscript Number:       | Ms_IJBCRR_31097                                                                            |
| Title of the Manuscript: | Temperature induced conformational entropy of $\alpha$ -amylase with and without additive. |
| Type of the Article      | Original Research Article                                                                  |

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This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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### PART 1: Review Comments

|                                     | Reviewer's comment                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
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| <b>Compulsory</b> REVISION comments | <p>The authors intended to analyze the temperature induced conformational entropy of human alpha amylase, working with saliva diluted in buffer. However, there are some experimental drawbacks that could lead to wrong results. Concerning the chosen buffer, Tris does not have buffering capacity at pH 6.9 (its pKa is 8.06 at 25 °C), and should be chosen a different buffer in order to repeat the experiments. Besides, Tris and is one of the worst buffers for analyzing temperature effects, since its pKa changes around -0.03 units of pH per degree Celsius, as can be checked at Applichem (<a href="https://www.applichem.com/en/products/laboratory-biochemicals/biological-buffer/">https://www.applichem.com/en/products/laboratory-biochemicals/biological-buffer/</a>). It means that, as temperature increases, the solution becomes more acid, introducing a hidden variable that also affects enzyme activity: pH.</p> <p>The authors also don't give details about the enzyme assay.</p> | <p>Thank you. It is true that pH changes with temperature. This is applicable to almost all buffer containing organic <b>components</b>. However, adjustment is made at each temperature with alkaline solution to maintain the same pH. Even in the preparation of most buffers, either the solution of the base or the acid is used to adjust the pH; this may not be stated in the method subsection, perhaps for the sake of brevity.</p> <p><b>Measurement of thermal inactivation of the enzyme:</b> The reaction mixture consisting of 1.31 exp (-8) M BAA, 25 mM Tris/acetate buffer (pH 7.0) and 0.002-0.02 mM CaCl<sub>2</sub> was incubated at 45-65 °C for the appropriate time intervals (<b>Tanaka &amp; Hoshino, 2003</b>).</p> <p>Figure 1: Kinetics of the thermal inactivation of BAA in the presence of calcium ion at 50 °C and pH 7.0:</p> <p>Calcium ion concentrations were: 0.002 mM; E, 0.005 mM; 0.01 mM; 0.015 mM;V, 0.02 mM. Protein concentration was 1.31exp (– 8) M in 25 mM Tris/HCl buffer. The remaining activity was the initial hydrolytic velocity against soluble amylopectin as a substrate. The reducing sugar was measured by dinitrosalicylic acid method (<b>Tanaka &amp; Hoshino, 2003</b>).</p> <p><b>Atsushi TANAKA and Eiichi HOSHINO</b><br/><i>Calcium-binding parameter of Bacillus</i></p> |



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|  |  | <p><i>amyloliquefaciens</i> <math>\alpha</math>-amylase determined by inactivation kinetics Biochem. J. (2002) 364, 635-639</p> <p>It is pointless citing other examples. But it is clear that be it with Tris/acetate or TrisHCl buffer, the pH cannot remain constant; perhaps it may be taken for granted that adjustment may have been made as expected from a scientist. However, it is better to state what has been done. On my part, as reported in the manuscript, I gelatinized the starch with distilled water (this cannot be verified, but the fact is that distilled water is cheaply available) for simple scientific reason; as noted by the reviewer, <i>as the mixture of raw starch and buffer solution is treated with heat, the solution becomes more acidic with the result that the starch may undergo acidolysis</i>. This may not give a true picture of the enzymes catalytic activity when it is assayed use of blank notwithstanding. Thus boiling distilled water was used to gelatinize the potato starch. The buffer was examined at each temperature. The pH was then adjusted by adding the base. Phosphate buffer may have been better but it can precipitate calcium from its salt.</p> |
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| <b><u>Minor</u></b> REVISION comments   | The quality of the text should be improved by a native English speaker.<br>The abbreviation of liter should be done using L (uppercase l), and the relative centrifugal force should be expressed as $xg$ , with $g$ in italics. | I appreciate the need for continuous improvement even if perfection seems to be utopian in nature; native English language speakers may have their manuscript edited let alone non English language speakers. Without discrimination lady speakers are preferred because they are generally better in spoken language.<br>$g$ has been changed to $g$ . |
| <b><u>Optional/General</u></b> comments | The experiments should be performed using a suitable buffer in order to obtain reliable results.                                                                                                                                 | It is good to repeat experiment but it may be better doing so with other homologues considering the reasons given.                                                                                                                                                                                                                                      |