

Temperature and pH Stability of Crude Rhodanese Enzyme Extracted from *Synodontis schall*

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Abstract- This research investigates the effect of temperature and pH on the stability of the enzyme extracted from the gills and liver of *Synodontis schall*. Rhodanese activity was assayed by measuring the amount of product formed in μ moles at 37°C and pH 8.2 per minute. The pH stability was done with pH 5.5 – 10.0, while 20 - 60°C was used to determine temperature stability. The results showed that gills rhodanese was stable within the range of pH 7.5 to 9.0, while the liver enzyme was stable within the pH range of 7.5 to 9.5. Both enzymes were stable at 30 and 40°C. At 50°C also the liver rhodanese was stable after incubating for 40mins, but lose 60% of its activity as the time of incubation increased. Rhodanese from the gills was not stable at higher temperatures as it loses more than 60% of its initial activity upon incubation for 10mins.

I. INTRODUCTION

Temperature and pH are two important factors that affect activity of rhodanese, an enzyme in the biotransformation of cyanide, because they denature the native structure of the enzyme protein. At extreme pH values, the charges on the ionizable groups in the side chains are very different from that under favourable conditions, hence the tertiary structure is usually disrupted (Palmer and Bonner, 2011). Most enzymes are denatured irreversibly in solutions with very high and very low hydrogen ion concentration as a result of attack on their tertiary structure (Whitaker, 1994). Generally, enzymes are active at small pH range, because they have several ionizable groups, thus changes in pH affect the enzyme conformation and essentially affect the maintenance of the catalytic capacity of the enzyme (Shuler and Kargi, 2002).

For most enzymes the turnover number increases with temperature until a temperature where the enzyme is no longer stable (Zubay *et al.*, 1995). This happens because the protein's tertiary structure held together by weak interactions is easily broken down at increased temperature (Bonner, 2018).

The temperature and pH dependence of rhodanese have been studied in bacteria (Itakorode *et al.*, 2019), fungi (Ezzi *et al.*, 2003), plants (Ehigie *et al.*, 2019) and animals (Eskandarzade *et al.*, 2012; Wodu *et al.*, 2021). However, the effects of these factors on *Synodontis schall* gills and liver rhodanese have not been fully reported, hence this investigation will elucidate the stability effects of temperature and pH on rhodanese.

II. MATERIALS AND METHODS

- Sample collection and Tissue Extract Preparation
Liver and gills of *Synodontis schall* used in this investigation were removed and stored at -4°C prior to extraction.

Ten grams (10g) of liver and five grams (5g) of gills were each homogenized in 30mls of sodium phosphate buffer (pH 8.2). The homogenate was centrifuged for at 4°C for 20 min at 4,000 rpm). The clear supernatant was used as the source of crude enzyme.

- Enzyme assay
Rhodanese assayed described by Agboola and Okonji (2004) was used with slight modifications. The 1.0ml reaction mixture contained 10mM Na₂S₂O₃, 10mM KCN, 0.25mM borate buffer, pH 8.2 and 10 μ l of enzyme solution. The reaction time was 1min at 37°C. The reaction was stopped with 0.5ml of formaldehyde (15%). Exactly 1.5ml of Sorbo reagent to develop the colour. Optical density was read at 460nm. The unit of enzyme activity was defined as the amount of thiocyanate formed in micromoles per minute at 37°C and pH 8.2.

- pH Stability
pH stability was done using 0.1 ml of enzyme solution incubated in 0.9 ml of buffer solutions with different pH (pH 5.5 – 10.0) for 10hrs at 4°C. Residual activity

of rhodanese was expressed a percentage of the pH optimum.

- Temperature Stability

The enzyme was assayed at temperatures range of 20 - 60°C to determine the effect of temperature on stability. The assay mixture was pre incubated at test temperatures for 10 minutes then the reaction was started by adding the enzyme already equilibrated at that temperature. Rhodanese was assayed was done as described above.

- Statistical Analysis

Statistical analysis was done with SPSS software system.

RESULTS AND DISCUSSION

Temperature and pH are two important factor that affect activity of enzymes because they denature the native structure of the enzyme protein. Figures 1 and 2 shows the plot of residual activity versus the pH after incubation at each pH studied for 10hrs. The results from the present work research showed that rhodanese extracted from the gills of *synodontis schall* is stable in the range of pH 7.5 to 9.0, while the liver enzyme was stable within the pH range of 7.5 to 9.5. The liver enzyme had a wider rang than the gills counterpart. Change in pH have a tremendous effect on rhodanese stability. The charges on the side chain of ionizable amino acids of rhodanese are critical to its structure and function (Westley, 1973). Ogata *et al.*, (1989) suggested that the control of sulphur transferring property of rhodanese *in vivo* is exerted by R-group phosphorylation at serine 124, which causes a change in conformational which is as a result of changes in the ionic interactions within the protein structure.

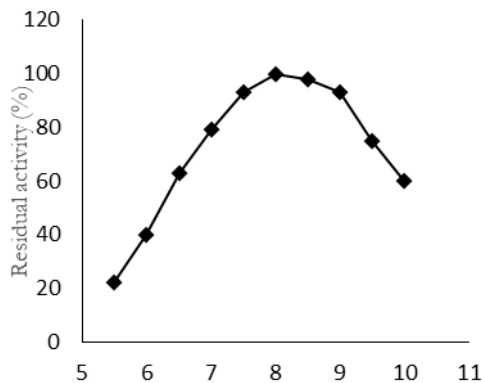


Fig 1: pH stability of *Synodontis schall* gills rhodanese

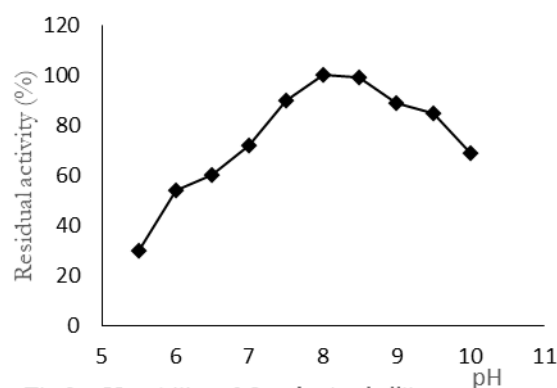


Fig 2: pH stability of *Synodontis schall* liver rhodanese

The stability of gills and liver rhodanese of *Synodontis schall* are shown in the plots of the residual activity of the enzyme versus the incubation time (figures 3 and 4). Both enzymes were stable at 30 and 40°C. Both enzymes at these temperatures retained upwards of 70% of their initial activity. *Synodontis schall* gills rhodanese was most stable at 30°C retaining approximately 80% of its initial activity when incubated at that temperature for 60mins. The liver enzyme on the other hand, was most stable at 40°C and retained upwards of 80% of its activity after incubating for 60mins. At 50°C also the liver rhodanese was stable after incubating for 40mins, but lose 60% of its activity as the time incubation increased. Rhodanese from the gills was not stable at higher temperatures as it loses more than 60% of its activity when incubated for 10mins. The liver enzyme was not stable at 60 and 70°C as there was total loss of activity after some minutes of incubation. *Synodontis schall* gills rhodanese was more stable at low temperatures than the liver enzyme.

The thermal stability study in this work revealed that the gills enzyme was more susceptible to thermal inactivation because it losses over 60% of its activity upon heating for 10mins at 50, 60 and 70°C. Tayefi-Nasrabadi and Rahmani, (2012) heat labile Rainbow Trout liver rhodanese that lost about 52% of relative activity when incubated for 5 min at 40°C. Gafar *et al.*, (2014) on the other hand, reported that tilapia liver rhodanese was stable when heated up to 60°C for about 30 mins. Liver rhodanese of *Synodontis schall* was stable at 50°C after 40mins of incubation.

Rhodanese has two domains of equal sizes with similar fold and are stabilized by hydrophobic interactions (Ploegman *et al.*, 1978). The enzyme rhodanese is sensitive to inactivation by heat (Horowitz and Bowman, 1987).

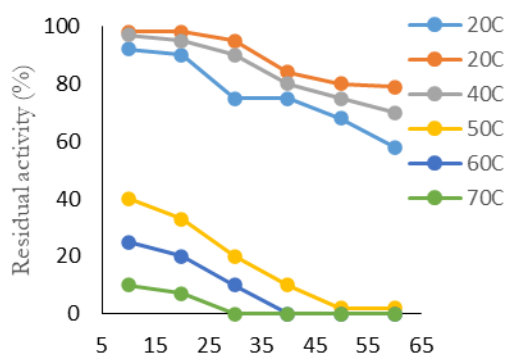


Fig. 3: Residual activity versus Time (min) incubation time (min) for *Synodontis schall* gills

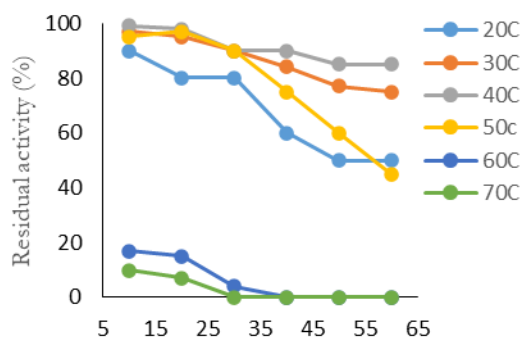


Fig. 4: Plot of residual activity versus incubation time (min) for *Synodontis schall* liver

III. CONCLUSION

In conclusion, the stability properties of *Synodontis schall* gills and liver rhodanese showed that the enzyme was stable in a range slightly alkaline. The temperature where the enzyme was stable were mainly near their respective optimum temperatures. The stability of *Synodontis schall* rhodanese is similar to rhodanese extracted from other sources.

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