Annals of the University of Craiova
The Chemistry Series
Volume L, No. 2 (2024) 35-39
homepage: chimie.ucv.ro/anale/

Influence of zinc ions on the thermal stability of alpha-mannosidase

Cătălina Ionescu, Georgeta Ciobanu, Nina Pălăduți, Anca Moanță*

University of Craiova, Faculty of Sciences, Department of Chemistry, Calea București 107 I, Craiova, Romania

* E-mail: catalinagurgui@yahoo.co.uk

Received: 16.11.2024 / Accepted: 21.11.2024 / Published: 20.12.2024

Abstract:
The effect of zinc ions on the heating stability of alpha-mannosidase has been investigated at 2 different temperatures: 50 ℃ and 60 ℃. The enzyme has been heated in presence and absence of zinc ions for 5, 10, 20, 30 and 40 minutes, cooled in ice/water bath and assayed at room temperature, in presence or absence of zinc ions. Enzyme activity (EA) values showed a protective effect of zinc ions against enzyme activity loss due to heating. Zinc ions also increased EA values in non-heated enzyme.

Keywords: alpha-mannosidase, zinc ions, heating, residual enzyme activity

1. INTRODUCTION

Alpha-mannosidase (EC 3.2.1.24) is a lysosomal enzyme that catalyzes the hydrolytic degradation of glycoproteins [1-3], being found in mammalian, bacterial and plant sources [4]. Having one zinc ion in the active site, alpha-mannosidase is a metalloenzyme [5-7] and its activity is modulated by the presence of activators (like zinc ions) and inhibitors (like ethylenediaminetetraacetic acid, EDTA) [8]. Zinc ions restore enzyme activity in decayed preparations at acidic pH.

In this study, the effect of zinc ions on the activity of alpha-mannosidase, subjected to heating for various amounts of time at 2 different temperatures, has been investigated.

2. MATERIALS AND METHODS

2.1. Materials

The reagents were of analytical purity. Alpha-mannosidase and *p*-nitrophenyl-alpha-D-mannopyranoside were Sigma products.
Solutions:

- Commercial α-mannosidase *(Canavalia ensiformis,* Jack bean*)*, 5.1 mg protein/mL has been diluted to 26.5 μg/mL with acetic acid/ sodium acetate buffer solution, 200 mM (pH=4.5).
- *p*-nitrophenyl-α-D-mannopyranoside, 5 mM solution in acetic acid/ sodium acetate buffer solution, 200 mM (pH=4.5), was used as enzymatic substrate.
- Na_2CO_3 0.4M was used for stopping the reaction.

p-nitrophenol (pNP) 1mM - stock solution was used for the preparation of standards for the calibration curve.

2.2. Methods

Enzyme (26.5 μg/mL) has been incubated for 20 hours in buffer, in presence and absence of 1.25 mM $ZnSO_4$. 1.45 mL of enzyme solution was diluted with 12.18 mL of buffer, with and without addition of zinc ions. Each solution was split in 2 and heated at 2 temperatures: 50 and 60 °C. Therefore, 4 experiments were run: 50 °C in presence of zinc ions; 50 °C in absence of zinc ions; 60 °C in presence of zin ions; 60 °C in absence of zinc ions. At fixed time intervals (5, 10, 20, 30 and 40 minutes), aliquots of 0.47 mL were removed from each mixture and cooled in water/ice bath, then they were allowed to reach room temperature and they were assayed.

The assay method for enzyme activity (EA) has been described previously [8]. Briefly, the enzyme has been assayed at room temperature (23 ° C) for 60 minutes. Control samples have been prepared for each test, in which the enzyme has been added after Na_2CO_3 solution.

Residual enzyme activity has been calculated with eq (1).

$$\% \text{ Enzyme activity (residual)} = (EA_{test}/EA_{reference}) \times 100 \quad (1)$$

Where:

EA_{test}=Enzyme activity for heated enzyme

$EA_{reference}$=Enzyme activity for unheated enzyme.

2.3. Apparatus

The spectrophotometric measurements were performed with a Varian Cary 50 UV-Vis spectrophotometer using plastic cuvettes for spectrophotometer, having 1 cm pathlength. Reagents were weighted using an Adam PW124 Lab Balance.

3. RESULTS AND DISCUSSION

EA was higher in zinc containing samples (~ 0.030 U/mL), when compared with enzyme assayed in buffer only (~0.018 U/mL), at the beginning of the experiment (t=0 min). EA decreased with heating time in all experiments (**Figure 1**). After 40 minutes of heating: (a) at 50 °C, EA decreased at 0.023 U/mL in presence of Zn^{2+} and at 0.011 U/mL in absence of Zn^{2+}; (b) at 60 °C, EA decreased at 0.019 U/mL in presence of Zn^{2+} and at 0.008 U/mL in absence of Zn^{2+}.

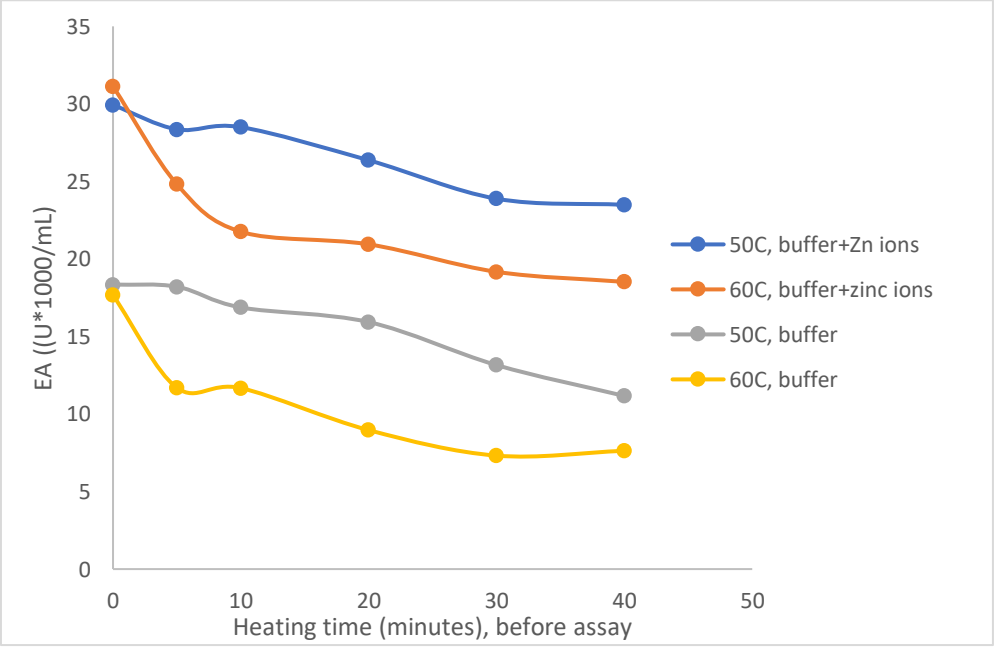

Figure 1. Decrease of enzyme activity (U*1000/mL) with heating time of the enzyme (heating has been realized before assay; the enzyme has been cooled in ice/water bath and assayed at room temperature, at 23 °C, for all tests).

Zinc ions activate the enzyme; therefore, EA was more elevated in all of the samples containing Zn^{2+}. In order to discuss the effect of zinc ions on enzyme thermal stability, enzyme residual activity has been calculated and compared in the experimental variants. Results are presented in Figure 2.

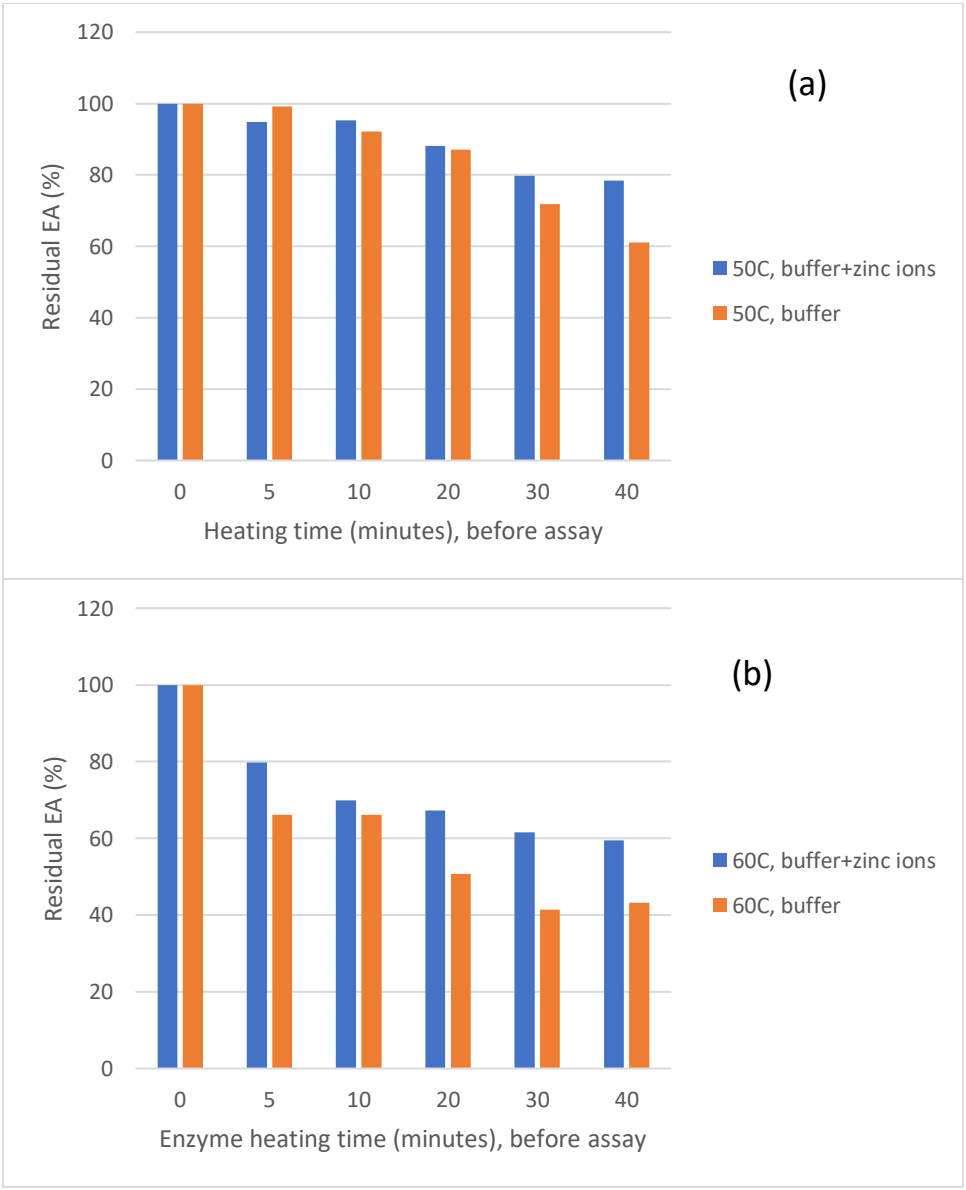

Figure 2. Decrease of residual enzyme activity (%) with heating time of the enzyme, with and without zinc ions at 50 °C (a) and 60 °C (b); heating has been realized before assay; the enzyme has been cooled in ice/water bath and assayed at room temperature, at 23 °C, for all tests.

From Figure 2, two effects may be observed:

a) temperature effect: when heated at 60 °C, the thermal degradation of the enzyme was more pronounced, compared with the heating at 50 ˚ C. This normal effect for enzymes is not correlated to the presence of zinc ions. After 40 minutes of heating:
    - in presence of zinc ions, at 50 °C, 78 % residual activity was present, versus 61 % residual activity at 60 °C;
    - in absence of zinc ions, at 50˚C, 59 % residual activity was present, versus 43 % residual activity at 60 °C.

b) zinc ions effect: the residual EA was more elevated when zinc ions were present in the mixture. After 40 minutes of heating:
    - at 50 °C, in presence of zinc ions, 78 % residual activity was present, versus 59% residual activity in absence of zinc ions;
    - at 60 °C, in presence of zinc ions, 61 % residual activity was present, versus 43 % residual activity in absence of zinc ions.

4. CONCLUSION

If the enzyme is not subjected to heating, zinc ions activate the enzyme, compared with the enzyme assayed in acetic acid/acetate buffer alone. If the enzyme is heated, zinc ions protect the enzyme against thermal inactivation, allowing it to retain higher residual activity, when compared to the enzyme heated in buffer only.

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