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Investigating the Influence of Heat Stress to the Activity And Structural Changes of Lactase Enzyme

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1. Introduction

Biological pharmaceuticals offer a novel trend in the pharmaceutical industry regarding the research of protein based biological medicines in the past decade. Biologically active proteins and biopharmaceuticals carry a variety of modifications, some of which are essential to their function and specificity. Lactase (β-galactosidase) is an intestinal enzyme responsible for breaking down lactose, a disaccharide of milk, it deficiency causes lactose intolerance [1]. The treatment of this disease involves the administration of various lactase containing medication or dietary supplements. The enzyme proteins like lactase have different structures with different properties due to their diverse functions and variations [2]. The quality and stability of the drug is usually characterized by enzyme activity. A significant variety of degradation processes can be present in the background of activity loss, therefore producing a highly complex substance to analyze. Glycosylation patterns have a prominent role among post-translational modifications in proteins. These can affect different functions such as activity, but also protein stability or the half life time of proteins in plasma. The stability of the active ingredient can be influenced by varying external conditions such as pH, moisture, temperature and even the applied excipients during manufacturing and by the acidic circumstances of the gastric juice as well [3]. Because of these factors the products might lose their effectiveness before administration. The relationship between structure and activity changes are not completely characterized. In present work, the elaboration of an analytical tool combination adequate to structural changes due to stress conditions which are relevant to enzyme activity, and function in case of lactase enzyme was intended in solid phase. Besides the nanoLC-MS/MS, SDS-PAGE, DLS, DOSY-NMR and CD techniques were used to explore the possible changes in the primary sequence, glycolytic glycosylation pattern and in the higher structures.

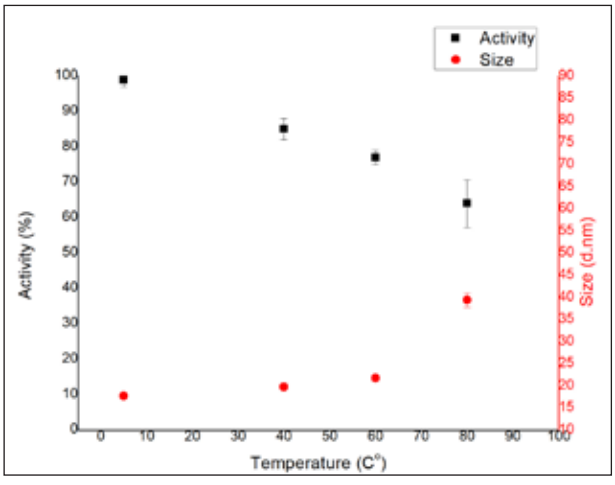

Figure 1 *Investigation of enzyme activity and size under heat exposure*

2. Materials and methods

The enzyme β-galactosidase from Aspergillus oryzae fungus was used in the solid state stability experiments. The exact parameters of the stability tests are shown in *Table 1.*

Table I *The parameters of the stability measurements*

Stress Conditions		Time
Temperature	40, 60, 80 °C	1 day, 3 days, 5 days, 7 days, 2 weeks

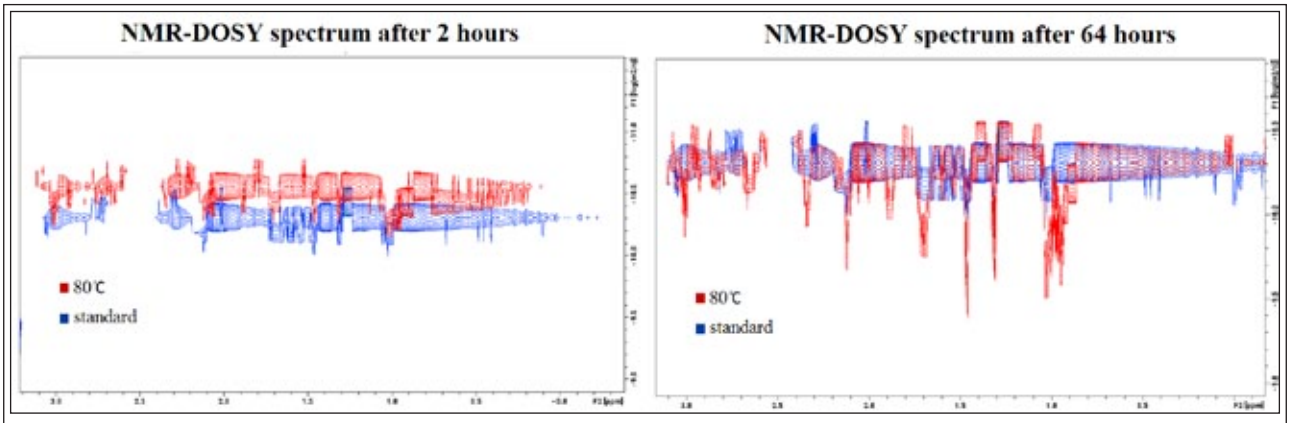

Figure 2 *The 2D DOSY ^{1}H NMR spectra of the same sample detected 2 h (left) and 64 h (right) after dissolving the solid samples in water. The DOSY profile belonging to the stressed sample (red) return to the standard's profile (blue) after 64 hours. Measurements were performed under the same conditions (288 K in water, 10% D_2O) on a Bruker Avance III 700 MHz spectrometer.*

Enzyme activity assay was used to determine the catalytic conditions of the enzyme. SDS gel electrophoresis, DOSY-NMR and dynamic light scattering methods were used to track changes in the size of the particles. Mass spectrometric measurements were applied to determine the exact mass, primary structure and glycosylation of the molecule.

3. Results

Increasing the temperature (40, 60, 80 ºC) results in various changes in the activity and structure of the enzyme: the size and mass of the molecule increase, the particles aggregate and consequently the catalytic property of the enzyme decreases. The degree of reduction and growth is illustrated in the *Figure 1.*

The change can be illustrated by the appearance of high molecular weight bands on the SDS-PAGE gel. According to the DLS and two-dimensional diffusion ordered spectroscopy (DOSY) ^{1}H NMR measurements, molecular size changes also show a similar tendency. At 40 and 60 ºC the change is significantly less than at 80 ºC. At high temperatures (80 ºC) enzyme activity does not return to baseline levels, but the long term NMR-DOSY studies suggest that aggregation in solution is reversible (***Figure*** **2**.). In contrast, UV exposure does not affect any of the tested properties. Quantitative mass spectrometric analysis of the glycans shows a significant difference for some sugar moieties due to high temperature. We can assume that structural changes in the higher order structural levels of the protein or within the glycosylation structures might be responsible for decrease of activity [4].

4. Conclusions

High temperatures (above 40 ºC) should be avoided during the manufacture of the lactase-containing preparation. Our investigations suggest that changes in the glycosylation structure and sugar components may affect the physico-chemical properties and aggregation tendency of lactase molecule. This may explain that the solubility properties of the samples stressed in solid phase are altered, which may cause a decrease in activity. This dataset may help us to develop better manufacturing, shipping and storage conditions for providing higher quality medications.

5. Acknowledgements

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