Investigation of glycerol and Tween - 20 on bovine testicular hyaluronidase catalytic activity by capillary electrophoresis complemented with microscale thermophoresis fluorescence signal monitoring.

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Hyaluronidases are responsible for the degradation of hyaluronic acid (HA), an important polysaccharide abundant in the extracellular matrix, especially in the skin. Its quantity and size are regulated by bovine testicular hyaluronidase enzyme (BTH), which randomly cleaves the long chains to eventually produce tetrasaccharide (Tet). Capillary electrophoresis (CE) offline based assays and microscale thermophoresis (MST) were used to check the effect of glycerol and Tween-20 on the catalytic activity of BTH. CE provides information on the kinetic activity of BTH by quantifying the Tet product whereas MST allows, among others, the determination of the binding affinity between BTH and small molecules from µM to pM scale [1].

In this study, glycerol and Tween-20 were selected to investigate their effect on BTH storage conditions and to improve MST experimental results, respectively. Indeed, glycerol, a viscous, low volatile colorless and odorless liquid has been widely described to stabilize some enzymes when stored at -20°C [2]. Tween-20 or polysorbate 20, a viscous liquid, is a common surfactant used to avoid protein adsorption on the inner wall of glass capillaries used in MST experiments [3]. MST measurements require one of the investigated partners to be fluorescent either at native state or by adding exogenous dye. Fluorescence signals provide information on the distribution of the labelled protein solution inside the capillary, in the presence and absence of additives, revealing adsorption phenomena. Moreover, MST thermographs indicate the state of the protein in solution; soluble or aggregated, free or bounded [1].
In this study, different concentrations of glycerol (0.02%, 2%, 10% and 20 %) and Tween-20 (0.005, 0.01, 0.02 and 0.05%) were added to BTH solutions and their influences were monitored systematically by CE and MST.
CE results showed that labelling affect the catalytic activity of BTH as shown in Fig.1a and Fig2.a. Moreover, CE results showed a significant decrease of the catalytic activity of labelled and unlabelled BTH in the presence of increasing amount of glycerol in the reaction media. For instance, the catalytic activity of unlabelled BTH and labelled BTH was reduced by a factor of 2 in the presence of 20% of glycerol (Fig.1a). This observation is in total agreement with our previous study carried out on the effect of PEG 6000 on the catalytic activity of unlabelled BTH [4]. The results showed that the catalytic activity was strongly dependant on the crowding of the reaction media and its viscosity that resulted in reducing of the enzyme kinetic activity.

The MST results of labelled BTH stored in the absence of glycerol, fluorescence signals were similar regardless the percentages of glycerol added to the labelled BTH scanned solution at final concentration of 1nM as shown in Fig.1b. Additionally, corresponding MST thermographs were consistent at all tested percentages with an exception of solutions containing 20% of glycerol where MST traces were less reproducible (Fig.1b'). On the other hand, MST fluorescence signal results revealed that labelled BTH stored in the presence of 20% of glycerol (stock solution concentrations equal to 8.1µM) compared to aliquots stored in its absence preserves a good fluorescence signal as shown in Fig.1c and c'.

CE results presented in Fig. 2a showed that the catalytic activity of labelled BTH was reduced by 20 % in the presence of 0.05% of Tween-20. At the same final percentage of Tween-20, the catalytic activity of unlabelled BTH was unchanged compared to reactions carried out in its absence. Finally, the fluorescence signal of labelled BTH obtained with different percentages of

Tween-20 was fairly constant as shown in Fig.2b and the corresponding thermophoretic graphs were perfectly stackable in all tested conditions as shown in Fig.2c.

To conclude, glycerol influences the catalytic activity of labelled and unlabelled BTH but it has limited effect on thermophoretic movement of labelled BTH unless it is presented at very high quantity (> 20%). Tween -20 has a limited effect on catalytic activity on labelled and unlabelled BTH with no incidence on labelled BTH thermophoretic signals.

References

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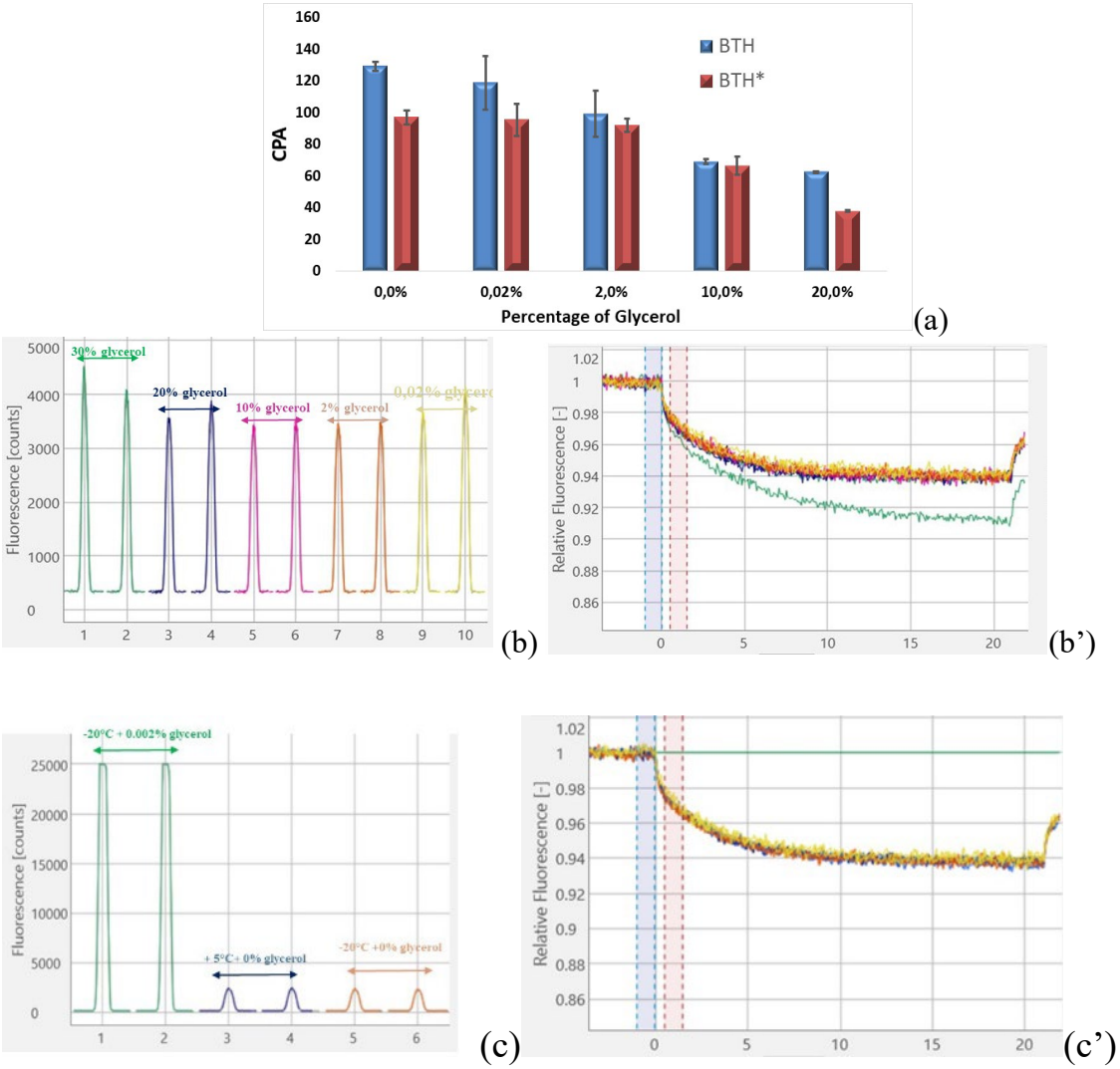

Figure 1 Histograms of corrected peak area (CPA), obtained by CE, of Tetrasaccharide, the final product of hyaluronic acid hydrolysis by labelled (red) and unlabeled bovine testicular hyaluronidase BTH (blue) in the presence of different percentages of glycerol (0.02, 2%, 10% and 20%) (a). MST fluorescence signal of labelled BTH at 1nM in the presence of different percentages of glycerol (b) and their corresponding thermographs (b'). Comparison of MST fluorescence signal of labelled BTH at 1nM at different storage conditions: –20°C with and without glycerol) and +5°C without glycerol (c) and the corresponding thermographs in (c').

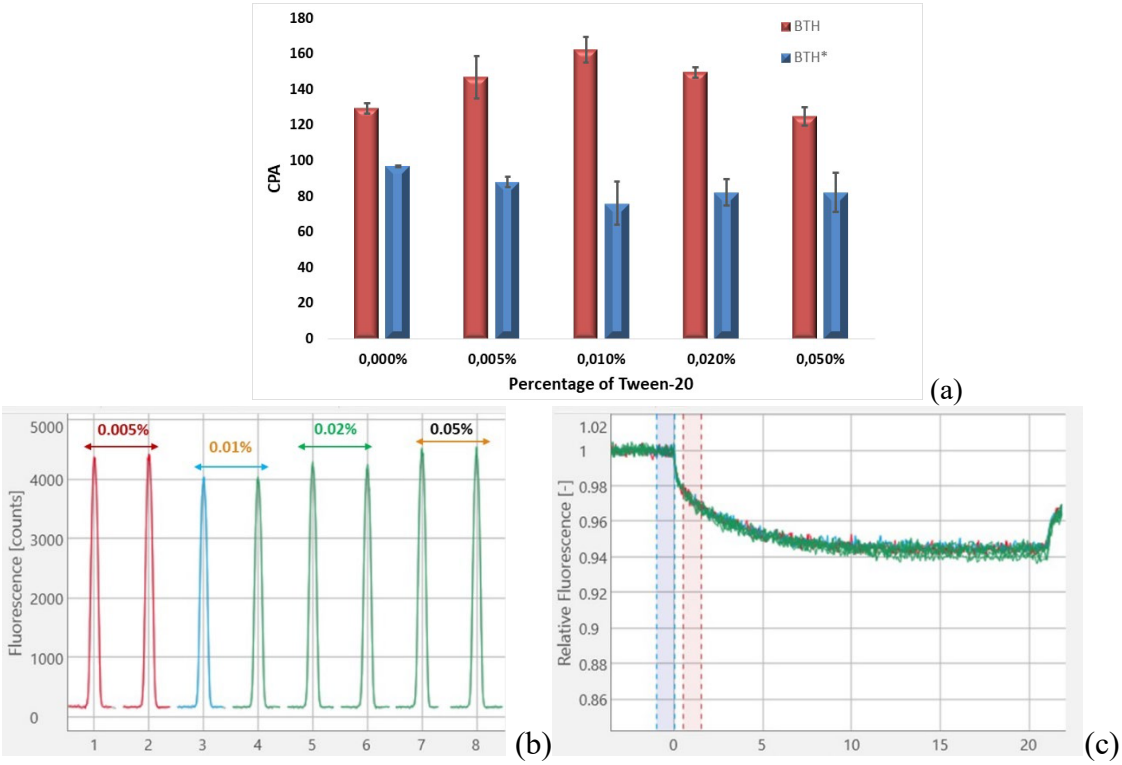

Figure 2 Histograms of corrected peak area (CPA), obtained by CE of Tetrasaccharide, the final product of hyaluronic acid hydrolysis by labelled (bleu) and unlabeled bovine testicular hyaluronidase BTH (red) in the presence of different percentages of Tween-20 (0.005%, 0.01%, 0.02% and 0.05%) (a). MST fluorescence signal results at 1 nM labelled BTH in the presence of different percentages of Tween -20 (b) and the corresponding thermophoretic graphs (c).