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Investigation of the Effect of Induced Macromolecular Crowding on Hyaluronidase Catalytic Activity and Interactions Using Capillary Electrophoresis and Microscale Thermophoresis

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Summary

The extracellular matrix (ECM) is dominated in-vivo by macromolecular crowding and resultant excluded volume effects [1]. It is composed of a large quantity of various macromolecules which fill the interstitial space within cells forming a hydrated gel [2]. ECM is a highly dynamic structure. It is constantly regenerated, remodeled and degraded to maintain tissue homeostasis, through the action of metalloenzymes such as collagenase, hyaluronidase (Hyal) and elastase [3]. The present investigation is part of a large multidisciplinary project, X-Crowd, aiming to scrutinize the kinetics of these enzymes in a realistic picture. For this purpose, crowded environments mimicking the ECM in-vitro are used. The crowding environment was simulated using dextran at two different molecular weights (40 and 476 kDa), respecting so the ratio between enzyme and crowder size.

We first intended to study the activity of Hyal, a glycosidase responsible for the degradation of hyaluronic acid (HA), a large polysaccharide responsible for skin hydration and cartilage lubrication. Capillary electrophoresis (CE), thanks to its miniaturized dimensions, was advantageously used to monitor the enzymatic reaction, after optimizing the injection step, taking into account the media viscosity and complexity. To better understand the effect of dextran on the catalytic activity of Hyal, a small substrate, decasaccharide (10-mers), was first used. Results were compared to those obtained with the high molecular weight natural substrate, HA. Moreover, the interaction between Hyal and the Dextran was characterized using microscale thermophoresis (MST), a biophysical miniaturized technique based on fluorescence detection. Hyal was thus labeled with ATTO-647 and studied in the presence of dextran at different buffer and pH conditions. CE combined to MST allowed to disentangle the impact of crowding on Hyal kinetics and confirmed the importance of considering it for the evaluation of compounds.

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