The Effects of Crowding on Enzyme Function: The Case of a Viral Protease

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Biochemical processes in cells, including enzyme-catalyzed reactions, occur in crowded conditions with various background macromolecules occupying up to 40% of the cytoplasm's volume. Consequently, viral enzymes in the host cell also encounter crowded conditions. We focus on one such enzyme, named the NS3/4A protease, encoded by the hepatitis C virus. This protease is crucial for viral replication and is used as a therapeutic target.

To gain an understanding of how the crowded environment affects the activity of this protease, we performed experimental and computational studies. The crowded milieu was mimicked by synthetic polymers: polyethylene glycol (PEG) and branched polysucrose (Ficoll). Using a spectroscopic assay with a fluorogenic peptide substrate, we found that PEG slowed down and Ficoll enhanced the reaction catalysed by NS3/4A. However, Ficoll increased the inhibition constant (Ki) of the telaprevir by about 40%, while the same concentration of PEG did not affect the inhibitory activity of this drug.

To explain the reasons for these observations, we performed extensive atomistic molecular dynamics simulations of NS3/4A in the presence of PEG and Ficoll crowders as well as substrates. As expected, the diffusion of the protease, crowders, and substrates was reduced in the simulations with the crowding agents, although to a different extent. The crowders also promoted the folding of the NS3/4A disordered fragments to a helical conformation potentially helping in membrane anchoring of the protease that is necessary in the host cell. Importantly, we found that the crowders not only physically restricted the space but formed up to several nanosecond long interactions with the enzyme and substrates. Both crowders enhanced the presence of substrates near the active site, with Ficoll crowders increasing substrate binding more than PEGs. Overall, we have demonstrated that the crowder type can modulate the effect of crowding not only on the enzymatic reaction but also on the kinetics of its inhibition and provided atomistic details of the interactions between the substrates, crowders and the enzyme. The results bring us closer to answering the question of how cellular crowding modulates the behaviour observed during *in vitro* biophysical experiments and simulations which is key for inhibitor design.

References

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