Structural Assessment of Polymer-Enzyme Complex Nanoparticle Stability

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Statement of Purpose

Hybrid nanoparticles in which a polymer is used to stabilize the secondary structure of enzyme provide a means to preserve its activity in non-native environments. This approach is illustrated here with horseradish peroxidase (HRP), an important heme enzyme used in medical diagnostic, biosensing, and biotechnological applications. Polymer chaperones in these polymer-enzyme complex (PEC) nanoparticles can enhance the utility of enzymes in unfavorable environments. Structural analysis of the PECs is a crucial link in the machine-learning driven iterative optimization cycle of polymer synthesis and testing. Here, we discuss the utility of small-angle X-ray scattering (SAXS) and quartz crystal microbalance with dissipation (QCMD) for evaluating PECs.

Materials and Methods

Six polymers were synthesized by automated photoinduced electron/energy transfer-reversible addition-fragmentation chain-transfer (PET-RAFT) polymerization directly in 96-well plates.[1] Multiple molar ratios of enzyme:polymer (1:1, 1:5, 1:10, and 1:50) were characterized. HRP was mixed with the polymer and heated to 65 °C for 1 hr to form PECs. Enzyme assay and circular dichroism measurements were performed along with SAXS and QCMD to understand polymer-protein interactions. SAXS data were obtained at NSLS-II beamline 16-ID.

Results and Discussion

SAXS data were analyzed to determine the radius of gyration (R_g), Porod exponent and pair distance distribution functions (P(r)) (**Figure 1**). R_g, which corresponds to the size of the PEC nanoparticles, is sensitive to the polydispersity of the solution and does not change significantly in the presence of the polymer GEP1. Notably, the maximal dimension does not change as significantly upon heating to denaturation in the case of the PEC as it does with HRP alone. The effect of denaturation induced by heating seems to depend on the molar ratio of the polymer to enzyme. The Porod exponent, which is related to roughness, decreased from about 4 to 3 upon complexation indicating polymer binding to the enzyme's surface. These were confirmed by modeling the structures of the HRP, the polymer and the PEC were modeled using DAMMIF/DAMMIN and MONSA (ATSAS software). The changes observed in the structure could be correlated to the measured enzymatic activity.

Figure 2 shows the evolution of the PEC when the polymer is deposited onto the enzyme immobilized on gold-coated QCM sensors. The plots show the changes in frequency (*f*) and dissipation (*D*) with time as HRP is first deposited and is followed by the adsorption of the polymer. Large Δf and ΔD show that the polymer forms a complex with HRP. Such changes were not observed with negative controls, Pluronics and poly(ethylene glycol). Comparison of the data from free particles in solution with QCM data from immobilized enzymes, shows that the conformation of the complexes in solution and surface-bound HRP could be different. This way, we were able to explore the various states of complex formation under different conditions with different polymers.

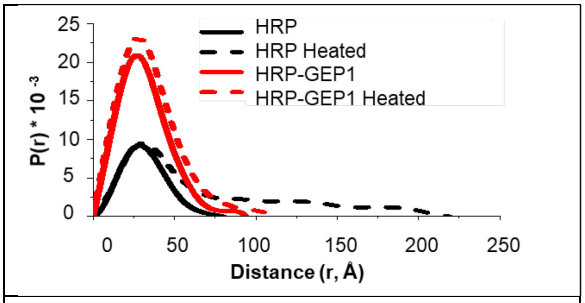

Figure 1. P(r) plots for PEC vs. HRP before and after heating, illustrating the increased enzymatic stability due to polymer additives.

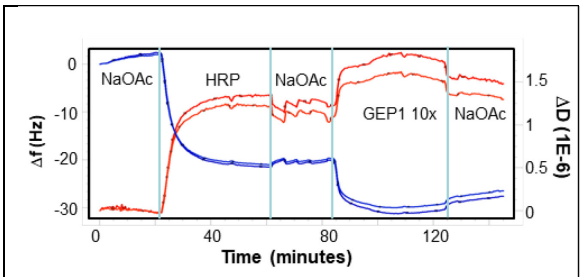

Figure 2. QCMD data showing the interaction between the immobilized HRP and the polymer. 3rd and 5th harmonics are plotted (blue -Δf; red-ΔD).

Conclusion

SAXS and QCMD data show that stabilization of the enzyme activity by inhibiting the unraveling of the secondary structure as seen in size, surface roughness, pair distribution function and percent helicity.

Acknowledgment

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References

[1] Tamasi, M, et al. *Adv Intell Syst* 2020, 2(2): 1900126.