Exploring the structural properties of a novel metagenomic esterase for enhanced polylactic acid biodegradation

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Enzyme-catalyzed depolymerization of synthetic polyesters has advanced rapidly over the past decade, presenting promising opportunities for plastic recycling and addressing the growing plastic pollution crisis. In this study, we aimed to structurally characterize a novel metagenomic esterase, Mgy, and its distant ancestral variant, 118, both of which exhibit high catalytic activity toward DL-polylactic acid degradation. We employed size-exclusion chromatography (SEC), circular dichroism (CD) spectroscopy and X-ray crystallography for this characterization. SEC revealed a high level of soluble aggregates, prompting optimization of the buffer composition. AlphaFold modelling was used to examine surface properties, aiding in the engineering of more stable enzyme variants. We identified disordered regions at the N-terminus and additional cysteine residues, which may contribute to protein aggregation. To test this hypothesis, truncated versions of both enzymes, lacking the N-terminal region, were expressed, purified, and analyzed. SEC confirmed that all four enzyme variants (full-length and truncated) favor high ionic strength and reducing environments, minimizing soluble aggregates. The full-length proteins showed distinct oligomeric species distributions, predominantly in tetrameric form, while the truncated variants existed primarily as monomers. CD data indicated that the enzymes contain approximately 18% α-helices and 37% β-sheets, a pattern consistent across full-length and truncated forms. Preliminary X-ray diffraction data were collected for the full-length 118 at the XRD2 beamline, Elettra, Trieste up to 4 Å resolution. Ongoing efforts aim to optimize crystallization conditions for higher-resolution data. The 3D structure determination of this metagenomic esterase will provide critical insights into the structural properties underlying its plastic degradation activity, paving the way for engineering more robust enzymes for sustainable environmental applications.