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Degradation of PET Nanoplastic Oligomers at the Novel PHL7 Target:Insights from Molecular Docking and Machine Learning

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Abstract

The versatility of Polyethylene terephthalate (PET) as a material with numerous applications in the food industry and its recalcitrance to chemical and microbial degradation has recently made it an environmental nuisance. In this study, we applied computational methods to ascertain the dependence of PET nanoplastic (NP) degradation on the chain length of the oligomer. The binding affinities of the NPs on the novel enzyme Polyester Hydrolase Leipzig 7 (PHL7) were used to relate their ease of degradation at the enzyme active site. The results revealed that the binding affinity of PET NPs at the enzyme target decreased from -5.2 kcal/mol to -0.8 kcal/mol, with an increase in PET chain length from 2.18 nm to 5.45 nm (2-5 PET chains). The binding affinities became positive at chain lengths 6.54 nm (6 PET chains) and above. These findings indicated that PET NP degradation at this enzyme's active site is most efficient as chain length decreases from 5-2 units and is not likely to occur at longer PET chains. A feedforward Artificial Neutral Network (ANN) analysis predicted that the energy of the PET NPs is a very important factor in its degradation.

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1. Introduction

Plastics are synthetic materials of long carbon chains that can be formed into different shapes when still molten and then transform into a slightly elastic solid form. Nanoplastics (NPs) are particles ranging from 1 to 1000 nm, unintentionally produced from the degradation and manufacturing of plastic objects [1]. Because they may pass through biological membranes, nanoplastics have a higher potential for danger than microplastics [2, 3]. NPs can be ingested by various creatures [4, 5], thereby raising concerns about possible bioaccumulation and biomagnification. There is increasing evidence that marine creatures consume NPs, some evidence of translocation outside the stomach, and much less evidence of transfer between trophic levels. Many studies have also demonstrated the exposure and toxicity of NPs to human health [6, 7].

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Plastic degradation is the change in the polymer's shape, colour, tensile strength, and molecular weight under the influence of chemicals, light, heat, or applied force. Though plastic degradation represents the failure of the polymer to perform a required service, the process can be useful as it concerns the recycling of polymer wastes to reduce the environmental pollution they can cause [3]. These days, the most popular methods for getting rid of plastic garbage in the environment include landfilling, burning, and mechanical and chemical recycling [8]. Landfilling is the main method of getting rid of plastic trash in most countries, especially impoverished ones because it is straightforward and inexpensive. However, accumulating plastic trash has consumed a substantial amount of space. While burning plastic garbage may assist reduce the need for landfill space and provide thermal energy, we also need to consider the environmental effects of secondary pollutants, including dioxins, carbon monoxide, and nitrogen oxides formed during the incinerating process. Even though mechanical recycling has grown to be the most popular method for recovering thermoplastic wastes, the bulk of recovered materials significantly degrade after a few processing cycles, limiting their economic value. The success of chemical recycling on the other hand depends on the accessibility of the processes and the effectiveness of the catalysts [9].

Currently, there are reports that natural enzymes can catalyze the hydrolysis of micro and nanoplastics as an alternative to chemical processes [10, 11]. The ester bond linkages in PET can be hydrolyzed by various esterases like PETase [12], cutinase [13], and lipase [14]. The extent of hydrolysis of PET by these enzymes is quite low and yields the monomeric units mono-2-hydroxyethylterephthalate (MHET) and bis(2-hydroxyethyl)terephthalate (BHET) [15]. These monomers can further be degraded into ethylene glycol and terephthalic acid, the initial reactants used for their formation. The high recalcitrant nature of PET waste is a major bottleneck in its hydrolysis by enzymes. Factors such as hydrophobicity, the crystallinity of PET, low accessibility, and structure usually limit enzyme function, thus making depolymerization very difficult [16]. Therefore, finding more effective and sustainable methods for breaking down PET plastic and other polymers could have significant benefits in terms of reducing pollution and increasing the recyclability of plastic waste.

In this study, we applied computational techniques [17] for the first time in studying the ease of hydrolysis of different PET nanoplastic chains by a microbial enzyme. Molecular docking of some PET nanoplastic oligomers was performed at the active site of the novel enzyme Polyester Hydrolase Leipzig 7 (PHL7). The binding affinities of the oligomers at the active site of the enzyme were used to estimate the chain lengths at which PET hydrolysis is most effective. Also, artificial neural network (ANN) was used to identify ligand-dependent factors responsible for the degradation process.

2. Computational Methods

2.1. Preparation of PET nanoplastic oligomers

Polyethylene terephthalate (PET) nanoplastic (NP) chains ranging from 1-10 polymer units were designed in Chemdraw and saved in MDL SDfile fileformat [18]. They were optimized using Open Babel in Python Prescription (version 0.8), which converted them to their most stable structures using Merk Molecular Force Field 94 (MMFF94). The optimized structures (Table 1) were used as small molecules in the study.

2.2. Identification and preparation of enzyme target

The 3D X-ray crystallographic structure of the novel enzyme Polyester Hydrolase Leipzig 7 (PHL7) with identity 7NEI [19] was retrieved from the protein data bank (PDB). The Chain A of the enzyme was used as target to study the effect of chain length on PET NP hydrolysis. Removal of the interfering crystallographic water molecules and minimization of the protein was done using UCSF Chimera 1.14 [20, 21, 22].

2.3. Molecular docking studies

Site-directed docking of the PET NP chains was performed on the active site of the enzyme with Autodock Vina in PyRx software version 0.8 [23]. The amino acids at the active site were selected and toggled on the enzyme surfaces in the Pyrx software. The specific site on the receptor was set using the grid box with dimensions:center x: 22.249, center y: – 1.869, center$z$: – 22.211, and size x: 23.795, size y: 14.112, size$z$: 15.463. At the end of the molecular docking, the binding poses of the enzyme-ligand complex were generated, and their scoring results were also created. Theinteractions between the enzyme-NP complex were visualized using BioviaDiscovery Studio 4.5 [24].

2.4. Artificial Neural Network (ANN) Analysis

Computational models with numerous processing layers may learn data representations at various abstraction levels [25, 26]. The current study employed a feedforward artificial neural network (ANN) made up of many perceptron layers (with threshold activation).

Backpropagation is a supervised learning method that the ANN uses during training. A minimum of three layers of nodes make up this ANN: the input layer, the hidden layer, and the output layer. Each node, except the input nodes, is a neuron that employs a nonlinear activation function. The network information for the ANN is summarized in Table 2.

The input layers include the determined variables from the PET NPs oligomers, including energy, molecular mass (MM), and chain length (CL). The ANNs used 70 % of the input data to train the model, while 30 % was used for testing the model. The ANN had two hidden layers based on a hyperbolic tangent activation function. The dependent variable or binary classifications are contained in the output layer. The ANN will examine the output layer's dependent and the input layer's independent

Table 1: Optimized NP chains used for the study

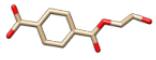
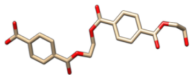
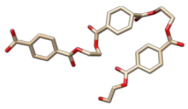
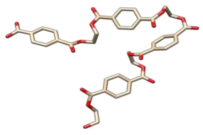
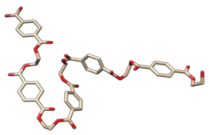
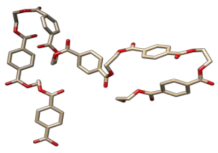
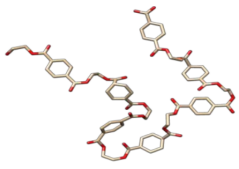
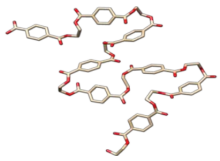
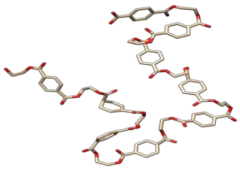
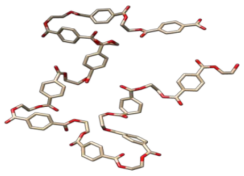
NP Chain length	Structure	Chemical formula	Molecular mass	Minimized energy (Ha)
NP1		$C_{10}H_{10}O_5$	210.18	76.01
NP2		$C_{20}H_{18}O_9$	402.35	195.80
NP3		$C_{30}\ H_{26}\ O_{13}$	594.52	418.82
NP4		$C_{40}\ H_{34}\ O_{17}$	786.69	577.84
NP5		$C_{50}\ H_{42}\ O_{21}$	978.86	633.86
NP6		$C_{60}\ H_{50}\ O_{25}$	1171.02	995.98
NP7		$C_{70}\ H_{58}\ O_{29}$	1363.19	1427.46
NP8		$C_{80}\ H_{66}\ O_{33}$	1555.36	1159.54
NP9		$C_{90}\ H_{74}\ O_{37}$	1747.53	2118.52
NP10		$C_{100}\ H_{82}\ O_{41}$	1939.70	2415.53

Table 2: Network Information for the ANNs

Input layer	Covariates	1	Energy
		2	Molecular mass (MM)
		3	Chain length (CL)
	Number of Units[a]		3
	Rescaling Method for Covariates		Standardized
Hidden Layer(s)	Number of Hidden Layers		2
	Number of Units in Hidden Layer 1[a]		1
	Number of Units in Hidden Layer 2[a]		1
	Activation Function		Hyperbolic tangent
Output Layer	Dependent Variables	1	Binding affinity
	Number of Units		1
	Rescaling Method for Scale Dependents		Adjusted normalized
	Activation Function		Hyperbolic tangent
	Error Function		Relative error Sum of Squares

[a] Excluding the bias unit

variables during training to see how they relate to one another. The hidden layer's nodes include mathematical functions that define the relationships. Once the connections have been established, the testing data will be used to validate them. Error functions, such as the relative error (RE) and the sum of squares error (SSE) shown in equations (1) and (2), would be used to verify and evaluate how well an ANN model predicts the output.

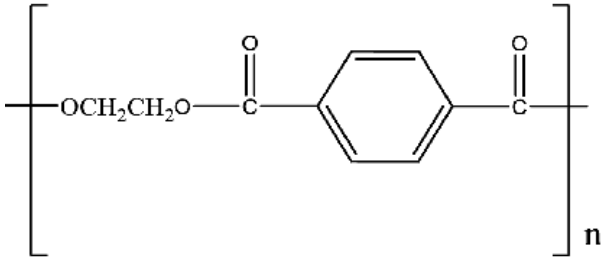


Figure 1: Structure of PET

$$RE = \left|\frac{\mathrm{BA}_A - \mathrm{BA}_P}{\mathrm{BA}_P}\right| \times 100 \quad (1)$$

$$SSE = \sum (\mathrm{BA}_P - \mathrm{BA}_A)^2, \quad (2)$$

where $(BA)_P$ is the estimated value of the binding affinity in kcal/mol by ANN model, $(BA)_A$ is the experimental value of the binding affinity in kcal/mol.

Table 3: Chain lengths and binding affinity of NPs chains at the PHL7 active site

NP oligomers	Chain length (nm)	Binding affinity (kcal/mol)
NP1	1.09	- 5.4
NP2	2.18	- 5.2
NP3	3.27	- 5.1
NP4	4.36	- 3.8
NP5	5.45	- 0.8
NP6	6.54	23.2
NP7	7.63	38.5
NP8	8.72	51.4
NP9	9.81	137.8
NP10	10.90	139.8

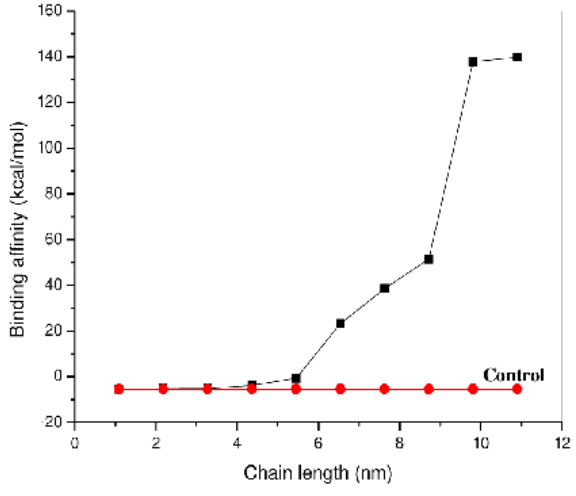


Figure 2: Plot of PET chain length against binding affinity at PHL7 active site

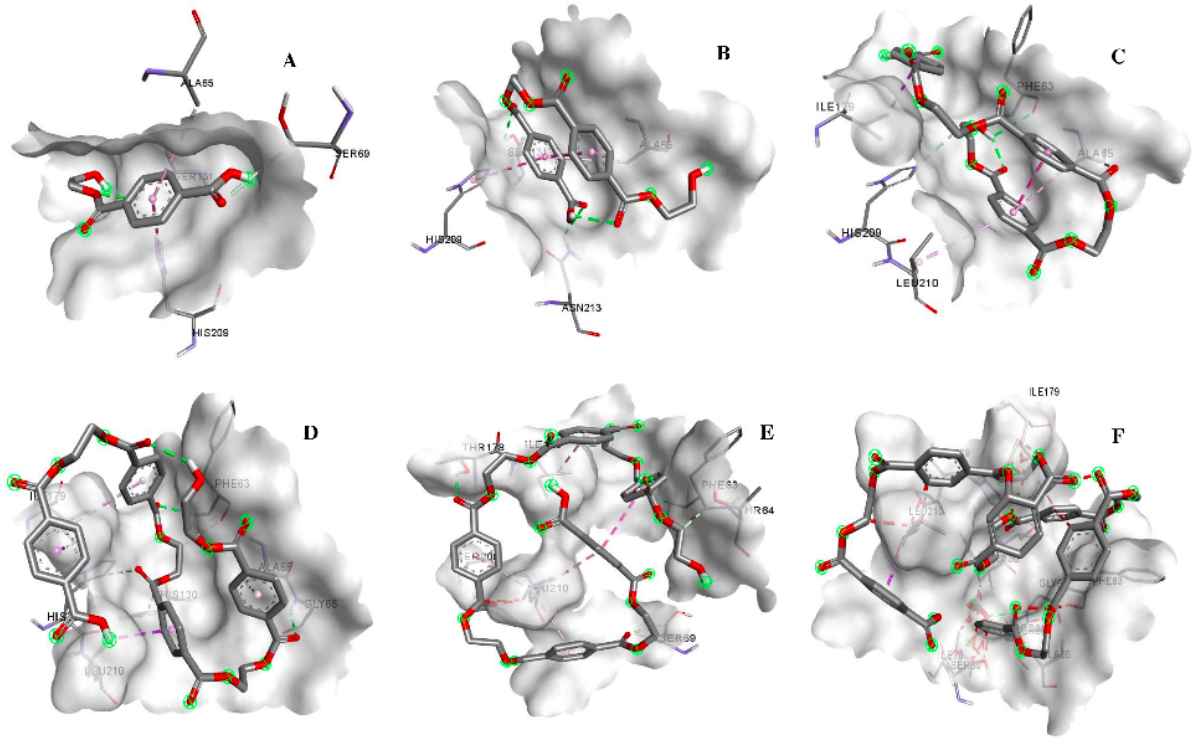


Figure 3: Fitting of NP oligomers at the enzyme binding pocket (A) NP1 (B) NP2 (C) NP3 (D) NP4 (E) NP5 (F) NP6

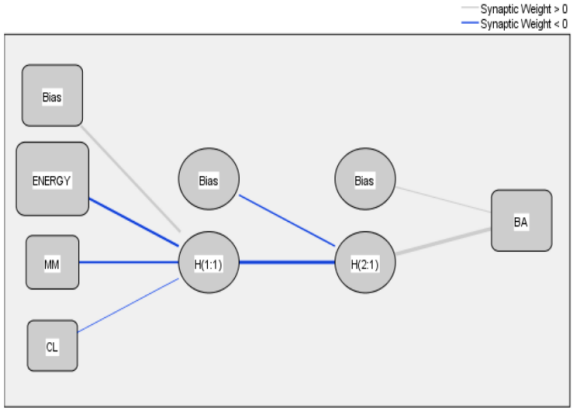


Figure 4: ANNs for predicting PET NPs degradation based on its properties-Energy, Molecular mass (MM), and Chain length (CL)

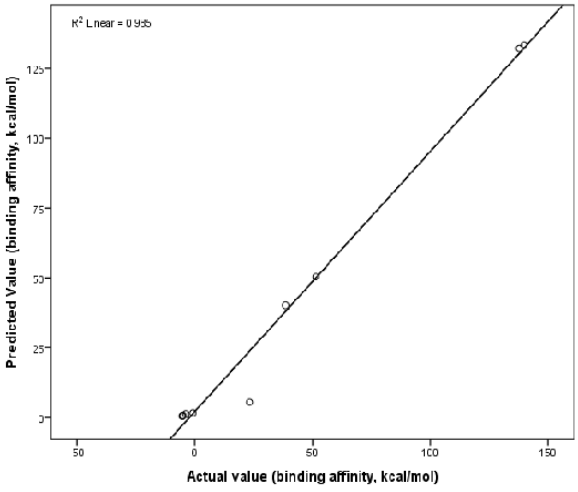


Figure 5: Linear correlation of predicted binding affinity and actual values from molecular docking

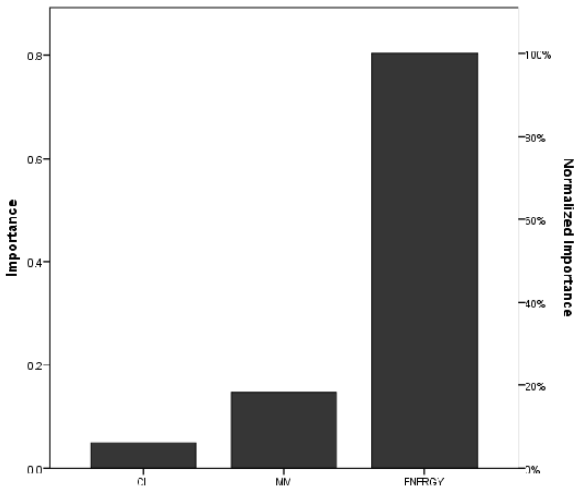


Figure 6: The most important property for NPs degradation by PHL7 from the ANN prediction

3. Results and discussion

Polyethylene terephthalate is a semi-crystalline polymer produced from the reaction of terephthalic acid and ethylene glycol. Each monomer unit (Figure 1) has a physical length of about 1.09 nm and a molecular weight of ≈ 200 [27].

It is a thermoplastic material with excellent chemical resistance, melt mobility, and spinnability. The bacterial strain *Ideonellasakaiensis* 201-F6 was recently found to exhibit a rare ability to grow on PET as a major carbon and energy source [28]. A novel enzyme, Polyester Hydrolase Leipzig 7 (PHL7), isolated from a compost metagenome, can completely hydrolyze amorphous PET films within hours has been freshly reported by Sonnendecker and coworkers [19]. We have shown in this study the binding affinities of modeled PET NP oligomers at the PHL7 active site, and the results are given in Table 3.

The PET monomer, which served as control in this study, had a binding affinity of - 5.4 kcal/mol at the enzyme target and was closely followed by NP2 and NP3 with binding affinities of -5.2 kcal/mol and - 5.1 kcal/mol respectively. These results suggested that the degradation of PET at this enzyme target would be most efficient at 2.18 nm and 3.27 nm chain lengths. The drop in the binding affinity from -3.8 kcal/mol for NP4 to – 0.8 kcal/mol for NP5 indicated a reduction in the hydrolyzing ability of the enzyme as the chain length increased from 4.36 nm to 5.45 nm.

Positive binding affinity values, which increased steadily, were obtained with NP6-NP10 oligomer units. This implied that the hydrolysis of PET within the range of 6.54 nm chain length and above was not feasible with this enzyme. Therefore, PET polymer materials should be within 5.45 nm and below for efficient binding and hydrolysis at the PHL7 active site. A plot of the binding affinity of PET NP oligomers as a function of NP chain length is shown in Figure 2. As chain length increased, three notable degradation characteristics of PET at the enzyme target were observed. There is a reactive stage at NP2-NP5 where hydrolysis was feasible, an intermediate nonreactive stage at NP6-NP8 where enzyme action is highly limited, and a recalcitrant stage at NP8 and above where total recalcitrance of the NP was manifest.

The 3D views of enzyme-NP interactions showed that the PET trimer (NP3) is the maximum chain unit that can fit perfectly in the enzyme binding cavity (Figure 3). This fitting becomes increasingly difficult from NP5 and above. The recalcitrant nature of PET polymer and the difficulty in its hydrolysis could therefore be reduced by subdividing it to less than 5.45 nm chain lengths which have good fitting in the enzyme pocket.

The ANN for the degradation of PET NPs oligomers by PHL7 based on the intrinsic properties of the oligomers is shown in Figure 4. The network involved two hidden layers, which processed the input variables. The output results from this network were compared with the actual binding affinity values from the molecular docking by linear regression (Figure 5). The results showed that the ANNs could predict the binding affinity with high accuracy with R^2 of 0.985. The ANN was further checked using error models, which gave small errors for SSE (0.045) and RE (0.028). The results indicated that the energy of the oligomers was the most important property for its degradation, with 100 % normalized importance (Figure 6). The energy of the oligomers increases with an increase in their sizes. The high energy of the longer oligomers reduced their binding affinity at the active site, which makes their degradation difficult at the enzyme target. Efficient degradation would therefore occur at a lower energy of the NPs, which is obtainable at chain lengths between NP2-NP4.

4. Conclusion

The molecular docking of PET NP oligomers ranging from 1.09 nm - 10.90 nm at the PHL7 active site was performed and their binding affinities were used to determine the effect of chain length on the degradation of PET. Binding affinities of the NPs decreased from -5.2 kcal/mol to -0.8 kcal/mol, as PET chain length increased from 2.18 nm to 5.45 nm (2-5 PET chains). At chain lengths of 6.54 nm (6 PET chains) and above, the binding of the PET NPs on the enzyme was non-spontaneous, as was seen in the resulting positive binding affinity values obtained with these chains. Artificial Neural Network analysis revealed that structural energy is the major determinant factor in the PET degradation process.

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