The binding of phthalates with nuclease influences the DNA enzymatic degradation

Chao Qin
Nanjing Agricultural University - Weigang Campus: Nanjing Agricultural University
Hao Cheng
Nanjing Agricultural University
Bing Yang
Nanjing Agricultural University
Yanxing Xu
Nanjing Agricultural University
Xiaojie Hu
Nanjing Agricultural University
Yanzheng Gao
Nanjing Agricultural University
Wanting Ling (lingwanting@njau.edu.cn)
Nanjing Agricultural University https://orcid.org/0000-0002-2376-7760

Research Article

Keywords: exDNA, Enzymatic degradation, PAEs, Deoxyribonuclease I, Binding interaction, Molecular dynamics simulations

Posted Date: May 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1603487/v1

Abstract

Extracellular DNA (exDNA) degradation determines the persistence and the dissemination of antibiotic resistance genes which are the root cause for antibiotic resistance threatened human health. However, the effect of ubiquitous environmental endocrine-disrupting chemicals such as phthalates (PAEs) on this process is unclear. Here, we investigated the DNA degradation in the presence of five typical PAEs [dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), bis(2-ethylhexyl) phthalate (DEHP), benzyl butyl phthalate (BBP)] via gel electrophoresis experiments, spectroscopic analyses, atomic force microscopy, and molecular dynamics simulations. Results show that the PAEs inhibited DNA enzymatic degradation. PAEs were bound to amino groups in deoxyribonuclease I (DNase I) amino acid residues via van der Waals forces and hydrogen bonding, leading to the decrease of helix structure and structural deformation of DNase I, which in turn caused the decrease of its enzymatic activity. This binding interaction mainly contributed to the inhibition of DNA degradation. The maximum inhibition rates of PAEs on DNase I enzymatic activity were up to 83% (DMP), 64% (DEP), 60% (DBP), 77% (DEHP), 88% (BBP), respectively. This work provides interesting insights into how coexistent toxic organic contaminants affect the environmental fate of genetic macromolecules and further reveals their molecular ecological effects.

Introduction

Extracellular DNA (exDNA) are deoxyribonucleic acids exposed to the environment and not enclosed in living cells (Torti et al. 2015). After being released from biological cells, they are ubiquitous biopolymers that often occur in aquatic and soil environments (Vuillemin et al. 2017). The maximum content of exDNA is 44 $\mu g \cdot L^{-1}$ in water and 200 $\mu g \cdot g^{-1}$ in soils (Pietramellara et al. 2009). ExDNA is the dominant fraction of environmental DNA, carrying genes from a large number of species in ecosystems (Valentin et al. 2021).They are easily ingested and utilized by microorganisms, and complete gene exchange through horizontal gene transfer (HGT) (Zhang et al. 2018). This route is the main way for exDNA to influence the dissemination of antibiotic resistance genes (ARGs) as the root cause for antibiotic resistance threatening the health of human beings (Zou et al. 2022). Since exDNA are a genetic source of extracellular ARGs proliferation (Mao et al. 2014), the persistence of exDNA should be paid utmost attention to because its half-life can be as long as years (Pietramellara et al. 2009).

The exDNA degradation determines the persistence and dissemination of ARGs. Apart from hydrolysis and oxidation, the enzymatic reaction was considered the main exDNA degradation pathway (Ponnuswamy et al. 2017; Qin et al. 2019).This process can often be affected by the adsorption behavior of exDNA. For example, exDNA adsorption on soils can change its configuration and further protect it from enzymatic degradation (Stotzky 2000). For biochar, the resistance of exDNA to enzymatic degradation was not related to its configuration because it did not change after adsorption (Fang et al. 2021). In contrast, other investigations indicated that exDNA binding to soil colloids and minerals could lead to its conformation change but have a minor influence on its resistance to enzymatic degradation (Cai et al. 2006). The adsorption behavior of nucleases on adsorbents could also inactivate its enzyme

activity (Cai et al. 2007), thus inhibiting the degradation of exDNA. These previous studies on exDNA degradation mostly focus on its adsorption on the surface of materials such as soils, minerals, or biomass charcoals. However, there are few reports on the degradation behavior of exDNA in pure water systems apart from the interfacial behaviors.

In recent years, the influences of coexistence of toxic organic contaminants on exDNA enzymatic degradation are of increasing concern. The combination of aminoglycoside antibiotic neomycin B with exDNA could cause the configuration change of exDNA from B-DNA to A-DNA, thereby inhibiting the *in vitro* enzymatic degradation of exDNA (Woegerbauer et al. 2000). In addition, chlorpyrifos and chlorpyrifos-methyl binding with exDNA led to the non-covalent accumulation of bases, the expansion of grooves, the destruction of the hydration layer, and finally promoted the enzymatic degradation of exDNA (Yang et al. 2019). Similarly, our previous work found that hexachlorocyclohexanes could bind to exDNA and resulted in the increase of its helicity and base accumulation, and this more compact DNA molecular structure was beneficial to providing more degradation sites, thus enhancing the exDNA enzymatic degradation (Qin et al. 2019). In short, all studies have investigated the influence of toxic organic contaminants on exDNA enzymatic degradation from the perspective of changing DNA structure. However, no research reported their direct influence on the key target like DNA degrading enzymes.

In this study, phthalates (Jarošová et al. 2009) as ubiquitous environmental endocrine-disrupting chemicals were selected to investigate their influence and mechanism on exDNA degradation due to their high binding affinity with common pattern protein (human serum albumin) (Lv et al. 2021; Wu et al. 2016). Because DNase I is the most studied, the most abundant, and the most active nuclease (Fahmi et al. 2020), and it is often used to catalyze DNA (especially extracellular DNA) (Tetz et al. 2010; Whitchurch et al. 2002) degradation, this nuclease has served as the representative DNA-degrading enzyme in this work. The exDNA degradation induced by DNase I in the presence of PAEs was performed via agarose gel electrophoresis and spectroscopic experiments. Subsequently, the PAEs-DNase I binding interaction was examined by fluorescence quenching tests. Additionally, the binding sites, binding mechanisms and conformational change of DNase I were further detected by Fourier transformed infrared spectroscopy, molecular dynamics simulations and circular dichroism. This work provides new insights into the effects and mechanisms of toxic organic contaminants on exDNA environmental behaviors and develops more knowledge of their molecular ecological effects and mechanisms.

Experimental

The experimental and computational details are provided in Supporting Information (Supplemental methods). Sperm DNA from Salmon containing 41.2% guanine-cytosine pairs (Morawska et al. 2019) was selected as representative exDNA and acquired from Sigma-Aldrich (St. Louis, MO, USA). Representative PAEs (DMP, DEP, DBP, DEHP, BBP) were purchased from Wellington Laboratories (Guelph, ON, Canada), and their full names and structures are shown in Fig. S1. 1000 U DNase I (1 $U \cdot \mu L^{-1}$) including 10× reaction buffer (with $MgCl_2$) and 200 $mmol \cdot L^{-1}$ ethylenediaminetetraacetic acid (EDTA) solution was obtained from Jiangsu Kangwei Century Biotechnology Co., Ltd. (Taizhou, Jiangsu, China).

DNA was first dissolved in 10 mmol·L^{-1} tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl, pH 7.4, Solarbio, Beijing, China) to avoid its natural degradation. In particular, 1 mmol·L^{-1} PAEs solutions were prepared using ethanol as a solubilizer and further diluted by ultrapure water (18.25 MΩ·cm) to make the ethanol content < 5%. All these reagents were of analytical grade.

Results And Discussion

The exDNA degradation induced by DNase I in the presence of PAEs was first performed via agarose gel electrophoresis. As shown in Fig. 1a-e, the inhibitory effect of DNA enzymatic degradation exposed to a series of concentrations of PAEs was presented. In CK (without DNase I), all degraded DNA fragments appeared at the same position (> 2000 bp), suggesting that the undegraded DNA fragments were more than 2000 bp, which was consistent with our previous research (Qin et al. 2019). Furthermore, the brightness of DNA fragments (more than 2000 bp) was gradually strengthened (that was, the amount of undegraded DNA gradually increased), and the brightness of DNA fragments in the range of 100–2000 bp gradually became weakened (meaning that the degraded DNA amount gradually decreased) with the PAEs concentration increasing from 0 to 5.0 (DMP), 5.0 (DEP), 4.0 (DBP), 3.0 (DEHP), 3.0 (BBP) μmol·L^{-1}, respectively. This result indicated that PAEs at the concentration of this study could inhibit the enzymatic degradation process of DNA and enhanced the persistence of DNA. This result may arise because PAEs weakened the secondary bonds between DNA polymer molecules, that was van der Waals forces, and increased the distance and activity space between polymer molecular chains, making them less susceptible to degradation by the degrading enzyme (DNase I). The related morphologies of DNA before and after degradation influenced by DMP are shown in Fig. 2.

It was reported that DNA degradation led to the exposure of bases, causing the absorbance to increase at 260 nm (Yang et al. 2019). Therefore, the DNA absorbance increase (ΔA) at 260 nm was closely related to the DNA degradation degree. Thus, the ΔA values of DNA after degradation were also exactly recorded to confirm the dose-dependent inhibition effect of PAEs on DNA degradation (Fig. 1f-j). Interestingly, the ΔA values were gradually decreased in a nonlinear mode from 0.34 to 0.0084 with the DMP concentrations increasing from 0 to 5 μmol·L^{-1}. For DEP, DBP, DEHP, and BBP, the ΔA values showed the same nonlinear downward trends. Thus, we again confirmed that PAEs inhibited the enzymatic degradation of DNA in a dose-dependent manner and these spectroscopic results were consistent with the above agarose gel electrophoresis results.

To confirm whether PAEs binding with DNA molecules can occupy the enzymatic reaction sites of DNase I, we added more DNase I (0.002 ~ 0.014 U·μL^{-1}) into the condition that PAEs could inhibit DNA degradation. This was the best method to confirm whether PAEs had completely occupied the reaction sites of DNase I at the test concentrations (Fig. S2). The results illustrated that DNA degradation was restarted with the increasing addition of DNase I, indicating that PAEs did not occupy the enzymatic reaction sites of DNase I in the DNA molecular structure. This is probably because PAEs and DNase I bind more strongly to different sites on DNA–PAEs interacting primarily with the DNA bases, while DNase I

interacts with the sugar-phosphate backbone (Engavale et al. 2021). Since PAE binding to DNA did not induce any structural change of DNA (Cheng et al. 2022), we assumed that the inhibition by PAEs of DNA degradation was probably due to the interaction between PAEs and DNase I. This speculation was based on one previous study reporting that synthetic melittin binding with secretory phospholipase A2 the structure of which resembles DNase I inhibited its enzymatic activity (Saini et al. 1997).

To examine the influence of PAEs on DNase I activities, we detected the enzymatic activity change of DNase I after the addition of PAEs. The results are shown in Fig. 3a. With the DMP concentrations increased from 0 to 17.5 μmol·L^{-1}, the enzymatic activity of DNase I decreased accordingly from 1.00 U to 0.69, 0.55, 0.28, 0.20, 0.17, 0.17, 0.17 U, respectively. Similarly, the DNase I activity decreased from 1.00 U to 0.94, 0.82, 0.69, 0.62, 0.57, 0.47, 0.36 U after adding 0 ~ 15.4 μmol·L^{-1} DEP. The same phenomenon also appeared when DBP, DEHP, and BBP were added to the DNA degradation system because the enzymatic activity of DNase I decreased from 1.00 U to 0.40 U (DBP), 0.23 U (DEHP), 0.12 U (BBP) with the concentrations of these PAEs increased from 0 to 12.6 μmol·L^{-1} (DBP), 9.1 μmol·L^{-1} (DEHP), 11.2 μmol·L^{-1} (BBP), respectively. All these results suggested that PAEs inhibited the enzymatic activity of DNase I. The maximum enzymatic activity inhibition rates of PAEs on DNase I in our study were up to 83% (DMP), 64% (DEP), 60% (DBP), and 77% (DEHP), 88% (BBP), respectively (Fig. 3a). Nonetheless, the agreement between the PAEs' inhibition of DNA degradation and our enzyme activity change induced by PAEs suggested that the above speculation was quite plausible. Therefore, this was the first work that indicated PAEs could inhibit DNA enzymatic degradation by reducing the activity of nuclease.

Amino acid residues of DNase I can generate fluorescence at certain excitation and emission wavelengths, which are highly sensitive to organic matters and lead to their fluorescence quenching (Meng et al. 2021). Since PAEs-DNase I binding is the first step for the inhibition of DNase I enzymatic activity caused by PAEs, we next studied the possibility of PAEs-DNase I binding interaction by traditional fluorescence quenching tests. With the addition of various PAEs, the maximum fluorescence intensity of DNase I at the best excitation (295 nm) and emission (355 nm) wavelength was decreased appreciably (Fig. 3c), indicating that PAEs-DNase I binding interaction led to the alterations of the environment around amino acid residues (tryptophan) and finally induced its fluorescence quenching. To further identify the mechanism of how PAEs induced the DNase I fluorescence quenching, we plotted the Stern-Volmer curves (Fig. 3c). All fitted data (Table S1) showed an obvious linear relationship to the Stern-Volmer equation ($R^2 > 0.9$), and the quenching constant (K_{sv}) values of PAEs-DNase I binding were 2.3×10^3 (DMP), 1.5×10^3 (DEP), 1.8×10^3 (DBP), 1.6×10^3 (DEHP), 2.0×10^3 (BBP) L·mol^{-1}, respectively. The calculated quenching rate constant (K_q) values were 1.55×10^{11} (DMP), 1.03×10^{11} (DEP), 1.20×10^{11} (DBP), 1.07×10^{11} (DEHP), 1.34×10^{11} (BBP) L·mol^{-1}·s^{-1}. All these K_q values were larger than the highest diffusion collision K_q (2.0×10^{10} L·mol^{-1}·s^{-1}) (Lange et al. 1998), indicating the coexistence of static quenching (Mahmoudpour et al. 2020). Figure 3c shows the double-log plots of PAEs-DNase I and the corresponding fitted parameters are listed in Table S2. Log (K_A) values of DMP, DEP, DBP, DEHP, BBP were 7.59, 7.01, 8.00, 5.93, 7.71, respectively. All these K_A values were relatively close to the noncovalent

binding interaction between cyanidin-3-O-glucoside and whey protein isolate/β-lactoglobulin (Meng et al. 2021). The binding sites numbers were close to 1 (Table S2), suggesting that there was one binding site between PAEs and DNase I, which was similar to the binding interaction between small aromatic molecules and DNA (Qin et al., 2021). These results further confirmed the PAEs-DNase I binding interaction. Besides, dynamic quenching and static quenching both occurred in the binding interaction.

PAEs-DNase I binding sites were further investigated by the Fourier transform infrared spectroscopy (FTIR). The IR spectra of PAEs-DNase I complexes are illustrated in Fig. S3. The band located at 1632 cm^{-1} of the free DNase I spectrum was assigned to the amide I region that was often reported in the range of 1600 ~ 1700 cm^{-1}, which corresponded primarily to C(N) = O stretching (Souillac et al. 2002). The appearance of this band unambiguously indicated the β-sheet structure. Additionally, the peaks at 1554 and 1462 cm^{-1} were assigned to the stretching vibration of C-N and N-H in amide II. Besides, the band at 1400 cm^{-1} was attributed to C-N bending, and the band at 1297 cm^{-1} was attributed to N-H bending in amide III (Haris and Severcan 1999). Besides, the IR bands at 1162, 1081, and 930 cm^{-1} were presented as unsaturated bands in DNase I structure (Haris and Severcan 1999; Kang et al. 2010). After binding with PAEs, the band at 1297 cm^{-1} was moved to 1271 cm^{-1} (DMP), 1273 cm^{-1} (DEP), 1282 cm^{-1} (DBP), 1271 cm^{-1} (DEHP), 1281 cm^{-1} (BBP), respectively. This obvious IR band shift indicated the N-H bending change in amide III. By analyzing the molecular structure of DNase I, we believed that the main PAEs-DNase I binding sites were its amino groups.

Molecular dynamics simulations results revealed the dynamic process of PAEs-DNase I binding interaction (Fig. 4 and Table S3) (Kawamura and Ohta 2022). For DMP, its positions, and binding amino acids were changed from 0 ps (Arginine, Asparagine, Glutamate, Histidine, Proline), to 29798 ps (Alanine, Asparagine, Glutamine, Isoleucine), and 98122 ps (Asparagine, Threonine) in a relatively stable state (Fig. 4a). However, for DEP, DBP, DEHP, and BBP, the binding amino acids were various (Fig. S4). The appearance of the blue-green dots indicated by the yellow arrows suggested that the driving forces for PAEs-DNase I binding were van der Waals forces and hydrogen bonds. This result was consistent with the binding force analysis results of PAEs binding with human serum albumin (HSA) (Lv et al. 2021). Additionally, 1 ~ 3 hydrogen bonds were formed during the binding process. It was similar to PAEs-DNA binding interaction which formed 1 ~ 4 hydrogen bonds (Cheng et al. 2022). In addition, the Root-Mean-Square-Deviation (RMSD) values of PAEs-DNase I binding varied greatly from 5.00×10^{-7} to 6.65 nm (Table S4), and the average RMSD values were in the range of 2.34 ~ 3.05 nm, which were slightly smaller than the RMSD values of PAEs-DNA binding. Since the smaller RMSD values mean more stable complex formation, this result indicated that PAEs were more likely binding with DNase I than DNA, which could also be confirmed by the comparison of K_A values (Cheng et al. 2022). In addition, the relative stability could only be achieved when hydrogen bonds were formed (Fig. 4b). Therefore, PAEs-DNase I binding interaction was a dynamic and unstable process.

Because the secondary structure change of endonuclease can have a close relationship with its biological activity (Okabe et al. 1982), the residue ellipticity change at specific peaks can reflect the

helical structure deformation of DNase I (Qin et al. 2021; Okabe et al. 1982; 1993). The circular dichroism (CD) spectra of DNase I in the specific range from 185 to 250 nm in the presence of PAEs at various concentrations are shown in Fig. 3b. PAEs-DNase I binding caused the characteristic negative CD bands of DNase I near 215 nm to decrease, revealing that PAEs deformed the ordered DNase I structure by binding interaction. More importantly, the positive peaks at 199 nm were transformed to negative peaks at 197 nm (DMP), 199 nm (DEP), 201 nm (DBP), 200 nm (DEHP), and 197 nm (BBP), respectively. This characteristic peak change was probably due to the structural deformation of DNase I. However, the observed CD changes do not appear to be greatly influenced by the increasing PAEs concentrations. Then, we analyzed the CD change in the 250–300 nm range which could be more useful as it would indicate how phenylalanine (257 nm), tyrosine (278 nm), and tryptophan (295 nm) residues are affected by PAEs (Fig. 3b) (Sreerama et al. 1999). The characteristic peaks of DNase I at 257 nm, 278 nm, and 295 nm were changed by PAEs in intensity and position, indicating the binding of PAEs with phenylalanine, tyrosine, tryptophan residues changed the molecular structure of DNase I. The observed perturbations appeared to depend on the PAEs concentrations. Thus, we concluded that PAEs binding with DNase I induced structural deformation of DNase I which was the main mechanism for the inhibition of DNA degradation.In summary, we found the inhibition effect of coexistent PAEs on the degradation of extracellular DNA in a dose-dependent manner. This inhibition effect was mainly due to the binding interaction between PAEs and DNase I at amino groups via van der Waals forces and hydrogen bonds. Since PAEs-DNase I binding interaction could induce structural deformation of DNase I, PAEs decreased the activity of DNase I. To the best of our knowledge, this is the first report indicating the molecular ecological effects of PAEs pollution. We truly believe that coexistent PAEs can influence the persistence and the dissemination of specific genes such as ARGs.

Conclusion

In summary, a novel phenomenon that the binding of phthalates with nuclease inhibits the DNA enzymatic degradation has been found. PAEs can bind to amino groups in DNase I amino acid residues via van der Waals forces and hydrogen bonds. The binding interaction leads to the decrease of helix structure and structural deformation of DNase I. Moreover, The DNase I deformation finally results in the decrease of its enzymatic activity. The maximum inhibition rates of DNase I enzymatic activity are more than 60%. Thus, we conclude that PAEs-DNase I binding interaction mainly contributes to the inhibition of DNA degradation.

Declarations

Supplementary Information The online version contains supplementary material available at

Authors' contribution Chao Qin and Hao Cheng performed all experiments, analyzed the results, and wrote the manuscript. Bing Yang, Yanxing Xu, and Xiaojie Hu supplemented individual trials. Yanzheng Gao and Wanting Ling revised the manuscript and provided suggestions and support for all experiments.

Funding This work was funded by the National Natural Science Foundation of China (42107221, 42177016, 41977121), and the Guangdong Basic and Applied Basic Research Foundation (2020A1515110535). Numerical computations were performed on the Computing System in the National Engineering Laboratory of Soil Pollution Control and Remediation Technologies, Chinese Academy of Sciences.

Conflict of interest Authors declare they have no conflict of interest.

References

1. Cai P, Huang Q, Chen W, Zhang D, Wang K, Jiang D, Liang W (2007) Soil colloids-bound plasmid DNA: Effect on transformation of *E. coli* and resistance to DNase I degradation. Soil Biol Biochem 39:1007–1013. https://doi.org/10.1016/j.soilbio.2006.11.010
2. Cai P, Huang Q, Zhang X (2006) Interactions of DNA with clay minerals and soil colloidal particles and protection against degradation by DNase. Environ Sci Technol 40:2971–2976. https://doi.org/10.1021/es0522985
3. Cheng H, Qin C, Yang B, Hu X, Gatheru Waigi M, Vasilyeva GK, Gao Y (2022) Non-covalent binding interaction between phthalic acid esters and DNA. Environ Int 161:107095. https://doi.org/10.1016/j.envint.2022.107095
4. Engavale M, McCord J, Mapp B, Nzimulinda N, Bengtson E, Sutton RB, Keyel PA (2021) Dnase1 family in autoimmunity. Encyclopedia 1:527–541. https://doi.org/10.3390/encyclopedia1030044
5. Fahmi T, Wang X, Zhdanov DD, Islam I, Apostolov EO, Savenka AV, Basnakian AG (2020) DNase I induces other endonucleases in kidney tubular epithelial cells by its DNA-degrading activity. Int J Mol Sci 21:8665. https://doi.org/10.3390/ijms21228665
6. Fang J, Jin L, Meng Q, Wang D, Lin D (2021) Interactions of extracellular DNA with aromatized biochar and protection against degradation by DNase I. J Environ Sci 101:205–216. https://doi.org/10.3390/ijms21228665
7. Haris PI, Severcan F (1999) FTIR spectroscopic characterization of protein structure in aqueous and non-aqueous media. J Mol Catal B Enzym 7:207–221. https://doi.org/10.1016/S1381-1177(99)00030-2
8. Jarošová A, Harazim J, Krátká L, Kolenčíková D (2010) Screening of phthalic acid esters in raw materials, premixes and feed additives. Environ Chem Lett 8:387–391. https://doi.org/10.1007/s10311-009-0237-7
9. Kang F, Gao Y, Wang Q (2010) Inhibition of free DNA degradation by the deformation of DNA exposed to trace polycyclic aromatic hydrocarbon contaminants. Environ Sci Technol 44:8891–8896. https://doi.org/10.1021/es103215b
10. Kawamura Y, Ohta Y (2022) Annihilation dynamics of a dislocation pair in graphene: Density-functional tight-binding molecular dynamics simulations and first principles study. Comp Mater Sci 205:111224. https://doi.org/10.1016/j.commatsci.2022.111224

11. Lange DC, Kothari R, Patel RC, Patel SC (1998) Retinol and retinoic acid bind to a surface cleft in bovine β-lactoglobulin: a method of binding site determination using fluorescence resonance energy transfer. Biophys Chem 74:45–51. https://doi.org/10.1016/S0301-4622(98)00164-1
12. Lv X, Jiang Z, Zeng G, Zhao S, Li N, Chen F, Huang X, Yao J, Tuo X (2021) Comprehensive insights into the interactions of dicyclohexyl phthalate and its metabolite to human serum albumin. Food Chem Toxicol 155:112407. https://doi.org/10.1016/j.fct.2021.112407
13. Mahmoudpour M, Javaheri-Ghezeldizaj F, Yekta R, Torbati M, Mohammadzadeh-Aghdash H, Kashanian S, Ezzati Nazhad Dolatabadi J (2020) Thermodynamic analysis of albumin interaction with monosodium glutamate food additive: Insights from multi-spectroscopic and molecular docking approaches. J Mol Struct 1221:128785. https://doi.org/10.1016/j.molstruc.2020.128785
14. Mao D, Luo Y, Mathieu J, Wang Q, Feng L, Mu Q, Feng C, Alvarez PJJ (2014) Persistence of extracellular DNA in river sediment facilitates antibiotic resistance gene propagation. Environ Sci Technol 48:71–78. https://doi.org/10.1021/es404280v
15. Meng Y, Hao L, Tan Y, Yang Y, Liu L, Li C, Du P (2021) Noncovalent interaction of cyanidin-3-O-glucoside with whey protein isolate and β-lactoglobulin: Focus on fluorescence quenching and antioxidant properties. LWT 137:110386. https://doi.org/10.1016/j.lwt.2020.110386
16. Morawska K, Jedlińska K, Smarzewska S, Metelka R, Ciesielski W, Guziejewski D (2019) Analysis and DNA interaction of the profluralin herbicide. Environ Chem Lett 17:1359–1365. https://doi.org/10.1007/s10311-019-00865-1
17. Okabe N, Fujita E, Tomita K (1982) The effect of guanidine hydrochloride on the conformation of bovine pancreatic DNase I as measured with circular dichroism. BBA-Protein Struct M 700:165–170. https://doi.org/10.1016/0167-4838(82)90093-0
18. Okabe N, Takashma S, Watanabe K (1993) Conformational changes in deoxyribonuclease I in anionic and cationic surfactant solutions. Biol Pharm Bull 16:1294–1296. https://doi.org/10.1248/bpb.16.1294
19. Pietramellara G, Ascher J, Borgogni F, Ceccherini MT, Guerri G, Nannipieri P (2009) Extracellular DNA in soil and sediment: fate and ecological relevance. Biol Fert Soils 45:219–235. https://doi.org/10.1007/s00374-008-0345-8
20. Ponnuswamy N, Bastings MMC, Nathwani B, Ryu JH, Chou LYT, Vinther M, Li WA, Anastassacos FM, Mooney DJ, Shih WM (2017) Oligolysine-based coating protects DNA nanostructures from low-salt denaturation and nuclease degradation. Nat Commun 8:15654. https://doi.org/10.1038/ncomms15654
21. Qin C, Hu X, Yang B, Liu J, Gao Y (2021) Amino, nitro, chloro, hydroxyl and methyl substitutions may inhibit the binding of PAHs with DNA. Environ Pollut 268:115798. https://doi.org/10.1016/j.envpol.2020.115798
22. Qin C, Yang B, Zhang W, Ling W, Liu C, Liu J, Li X, Gao Y (2019) Organochlorinated pesticides expedite the enzymatic degradation of DNA. Commun Biol 2:81. https://doi.org/10.1038/s42003-019-0326-5

23. Saini SS, Peterson JW, Chopra AK (1997) Melittin binds to secretory phospholipase A_2 and inhibits its enzymatic activity. Biochem Bioph Res Co 238:436–442. https://doi.org/10.1006/bbrc.1997.7295
24. Souillac PO, Middaugh CR, Rytting JH (2002) Investigation of protein/carbohydrate interactions in the dried state. 2. Diffuse reflectance FTIR studies. Int J Pharmaceut 235:207–218. https://doi.org/10.1016/S0378-5173(01)00987-5
25. Sreerama N, Manning MC, Powers ME, Zhang JX, Goldenberg DP, Woody RW (1999) Tyrosine, phenylalanine, and disulfide contributions to the circular dichroism of proteins: circular dichroism spectra of wild-type and mutant bovine pancreatic trypsin inhibitor. Biochemistry 38:10814–10822. https://doi.org/10.1021/bi990516z
26. Stotzky G (2000) Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. J Environ Qual 29:691–705. https://doi.org/10.2134/jeq2000.00472425002900030003x
27. Tetz VV, Tetz GV (2010) Effect of extracellular DNA destruction by DNase I on characteristics of forming biofilms. DNA Cell Biol 29:399–405. https://doi.org/10.1089/dna.2009.1011
28. Torti A, Lever MA, Jørgensen BB (2015) Origin, dynamics, and implications of extracellular DNA pools in marine sediments. Mar Genom 24:185–196. https://doi.org/10.1016/j.margen.2015.08.007
29. Valentin RE, Kyle KE, Allen MC, Welbourne DJ, Lockwood JL (2021) The state, transport, and fate of aboveground terrestrial arthropod eDNA. Environ DNA 3:1081–1092. https://doi.org/10.1002/edn3.229
30. Vuillemin A, Horn F, Alawi M, Henny C, Wagner D, Crowe SA, Kallmeyer J (2017) Preservation and significance of extracellular DNA in ferruginous sediments from Lake Towuti, Indonesia. Front Microbiol 8:1440. https://doi.org/10.3389/fmicb.2017.01440
31. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS (2002) Extracellular DNA required for bacterial biofilm formation. Science 295:1487. https://doi.org/10.1126/science.295.5559.1487
32. Woegerbauer M, Burgmann H, Davies J, Graninger W (2000) DNase I induced DNA degradation is inhibited by neomycin. J Antibiot 53:276–285. https://doi.org/10.7164/antibiotics.53.276
33. Wu Z, Yi Z, Dong L, Zhang A (2016) Molecular simulation study of the specific combination between four kinds of phthalic acid esters and human serum albumin. Environ Toxicol Phar 41:259–265. https://doi.org/10.1016/j.etap.2015.12.017
34. Yang B, Qin C, Hu X, Xia K, Lu C, Gudda FO, Ma Z, Gao Y (2019) Enzymatic degradation of extracellular DNA exposed to chlorpyrifos and chlorpyrifos-methyl in an aqueous system. Environ Int 132:105087. https://doi.org/10.1016/j.envint.2019.105087
35. Zhang Y, Li A, Dai T, Li F, Xie H, Chen L, Wen D (2018) Cell-free DNA: A neglected source for antibiotic resistance genes spreading from WWTPs. Environ Sci Technol 52:248–257. https://doi.org/10.1021/acs.est.7b04283
36. Zou Y, Wu M, Liu J, Tu W, Xie F, Wang H (2022) Deciphering the extracellular and intracellular antibiotic resistance genes in multiple environments reveals the persistence of extracellular ones. J Hazard Mater 429:128275. https://doi.org/10.1016/j.jhazmat.2022.128275

Figures

Figure 1

Degraded DNA fragments and the absorbance increase (ΔA) of DNA induced by DNase I in the presence of PAEs (**a**, **f** DMP; **b**, **g** DEP; **c**, **h** DBP; **d**, **i** DEHP; **e**, **j** BBP). CK means without the addition of DNase I. DNase I was 0.002 $U \cdot \mu L^{-1}$, reaction time was 7 min, and the pH was 7.4.

Figure 2

AFM images of DNA (**a**, DNA; **b**, DNA + DMP; **c**, DNA + DNase I; **d**, DNA + DNase I + DMP). The concentrations of DMP were 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 $\mu mol \cdot L^{-1}$, respectively. DNase I was 0.002 $U \cdot \mu L^{-1}$, reaction time was 7 min, and the pH was 7.4. Blue circles represent DNA and its fragments after degradation.

Figure 3

The enzyme activity (**a**) and circular dichroism spectra (**b**) change of DNase I influenced by PAEs, and the PAEs-DNase I binding interaction was detected by fluorescence quenching tests (**c**). The DNase I concentration was 50 $mg \cdot L^{-1}$. The system was stirred at 37 °C and 180 rpm for 60 minutes to reach equilibrium.

Figure 4

The positions of DMP molecules, RMSD values, and the number of hydrogen bonds change with time during the PAEs-DNA molecular dynamics simulations. **a**, The DMP-DNase I molecules positions, binding forces, and binding amino acids at 0 ps, 29798 ps, 98122 ps, respectively. **b**, The RMSD values and the number of hydrogen bonds of PAEs binding with DNase I, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupportingInformation.docx