Prometheus Protocol PR-P-012

Ligand-Induced Stabilization of MHETase

The durability of polyethylene terephthalate (PET) debris is a long-term environmental burden, and the recycling efforts used at the present lack sustainability. The recently discovered bacterial enzymes PETase and MHETase of *Ideonella sakaiensis* which specifically degrade PET represent a promising solution. They allow *I. sakaiensis* to use PET as its major energy and carbon source for growth and MHETase likely acts synergistically with PETase to depolymerize PET. In this protocol, the effect on the thermal stability of the enzyme MHETase by different ligands is analyzed.

thermal unfolding | enzyme stability | ligand-induced thermal stabilization

---

A1. Target/Fluorescent Molecule

Mono(2-hydroxyethyl) terephthalate hydrolase (MHETase)
uniprot.org/uniprot/A0A0K8P8E7

A2. Molecule Class/Organism

Hydrolase
Ideonella sakaiensis

A3. Sequence/Formula

```
GGGSTPLPLP QQQPPQQEPP PPPVPLASRA ACEALKDGNG DMVWPNAATV VEVAAWRDAA PATASAAALP EHCEVSGAIA
KRTGIDGYPY EIKFRLRMPA EWNGRFFMEG GSGTNGSLSA ATGSIGGGQI ASALSRNFAT IATDGGHDNA VNDNPDALGT
VAFGLDPQAR LDMGYNSYDQ VTQAGKAAVA RFYGRAADKS YFIGCSEGGR EGMMLSQRFP SHYDGIVAGA PGYQLPKAGI
SGAWTTQSLA PAAVGLDAQG VPLINKSFSD ADLHLLSQAI LGTCDALDGL ADGIVDNYRA CQAAFDPATA ANPANGQALQ
CVGAKTADCL SPVQVTAIKR AMAGPVNSAG TPLYNRWAWD AGMSGLSGTT YNQGWRSWWL GSFNSSANNA QRVSGFSARS
WLVDFATPPE PMPMTQVAAR MMKFDFDIDP LKIWATSGQF TQSSMDWHGA TSTDLAAFRD RGGKMILYHG MSDAAFSALD
TADYYERLGA AMPGAAGFAR LFLVPGMNHC SGGPGTDRFD MLTPLVAWVE RGEAPDQISA WSGTPGYFGV AARTRPLCPY
PQIARYKGSG DINTEANFAC AAPP
```

A4. Purification Strategy/Source

MHETase was recombinantly expressed and purified as previously described[1]. Briefly, a DNA fragment encoding *I. sakaiensis* MHETase (amino acid residues 20–603) was cloned in a pColdII vector by FastCloning. For protein expression, *E. coli* Shuffle T7 express cells were transformed and protein expression was induced by 1 mM isopropyl β-D-1- thiogalactopyranoside (IPTG). The cell extract was purified on a gravity flow column with Ni-NTA sepharose, eluted with 200 mM imidazole, followed by desalting on a Superdex75 10/300 column (GE Healthcare, Solingen, Germany) with 20 mM TRIS pH 7.5, 150 mM NaCl.

[1] See Palm et al., Nature Communications 10: 1717 (2019) for more information.

A5. Stock Concentration/Stock Buffer

100 µg/mL
100 mM Tris-HCl, pH 7.5, 150 mM NaCl

A6. Molecular Weight/Extinction Coefficient

63 kDa

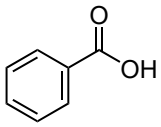
A7. Dilution Buffer

100 mM Tris-HCl, pH 7.5, 150 mM NaCl, with or without 20% DMSO

B1. Ligand/Non-Fluorescent Binding Partner

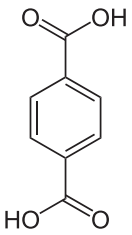
Benzoic acid (BA)

Benzoic acid is a compound comprising a benzene ring core carrying a carboxylic acid substituent. It has a role as e.g. an antimicrobial food preservative, a plant metabolite, a human xenobiotic metabolite, an algal metabolite and a drug allergen.



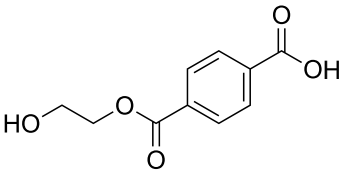
Terephthalic acid (TPA)

Terephthalic acid is one isomer of the three phthalic acids. It finds important use as a commodity chemical, principally as a starting compound for the manufacture of polyester (specifically PET), used in clothing and to make plastic bottles.



Monohydroxyethyl terephthalate (MHET)

Mono(2-hydroxyethyl) terephthalic acid ester is the monoester of terephthalic acid and ethylene glycol. It is the building block and one of the first enzymatic degradation products of poly(ethylene) terephthalate (PET).



B2. Molecule Class/Organism

Small molecule compounds

B3. Sequence/Formula

BA: C_6H_5COOH

TPA: $C_6H_4(COOH)_2$

MHET: $C_9H_9O_3COOH$

B4. Purification Strategy/Source/Batch-No.

BA: Merck
242381

TPA: Merck
185361

MHET: MHET was synthesized from BHET by partial hydrolysis with KOH. 8.7 mmol BHET was reacted with 8.4 mmol KOH in 18 mL $MgSO_4$-dried ethylene glycol at 110–130°C for 2.5 h. After cooling, 30 ml H_2O were added, and the mixture was extracted three times with 5ml $CHCl_3$. The aqueous phase was adjusted to pH 3 with 25 % HCl and filtered at 4°C. After two extraction steps with 30 ml hot H_2O and filtration at 4°C, the precipitate was dried at 60 °C (0.56 g (30%), Mp 185–190°C).

B5. Stock Concentration/Stock Buffer

BA: 21.7 mM
100 mM Tris-HCl, pH 7.5, 150 mM NaCl

TPA: Saturated solution
100 mM Tris pH 7.5, 150 mM NaCl, 43.5% DMSO

MHET: Saturated solution
100 mM Tris pH 7.5, 150 mM NaCl, 43.5% DMSO

B6. Molecular Weight/Extinction Coefficient

BA: 122.1 Da

TPA: 166.1 Da

MHET: 210.2 Da

D1. nanoDSF System/Capillaries

Prometheus NT.48 (NanoTemper Technologies GmbH)
High sensitivity Capillaries Prometheus NT.48 nanoDSF Grade (PR-C006, NanoTemper Technologies GmbH)

D2. nanoDSF Software

PR.ThermControl v2.1 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

D3. nanoDSF Experiment

1. Prepare 100 µg/mL solutions of MHETase in dilution buffer without and with ligands[2]:

1	100 µg/mL MHETase	100 mM Tris, pH 7.5, 150 mM NaCl	no ligand
2	100 µg/mL MHETase	100 mM Tris, pH 7.5, 150 mM NaCl	10 mM BA
3	100 µg/mL MHETase	100 mM Tris, pH 7.5, 150 mM NaCl, 20% DMSO	45% saturated TPA
4	100 µg/mL MHETase	100 mM Tris, pH 7.5, 150 mM NaCl, 20% DMSO	45% saturated MHET

2. Completely fill four capillaries from the four solutions, place them on positions 1 – 4 of the capillary tray and place the magnetic lid to fix the capillaries.
3. Start a new session of the *PR.ThermControl* software.
4. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Capillaries 1 – 4 selected
    b. 0.5°C/min
    c. 20°C – 95°C
    d. 60% excitation power
5. Start the measurement.

[2] A final concentration of 10 mM was only reached for benzoic acid. For terephthalic acid and monohydroxyethyl terephthalate, the saturated solutions were diluted 2.2-fold (i.e. to 45% of their original concentration).

D4. nanoDSF Results

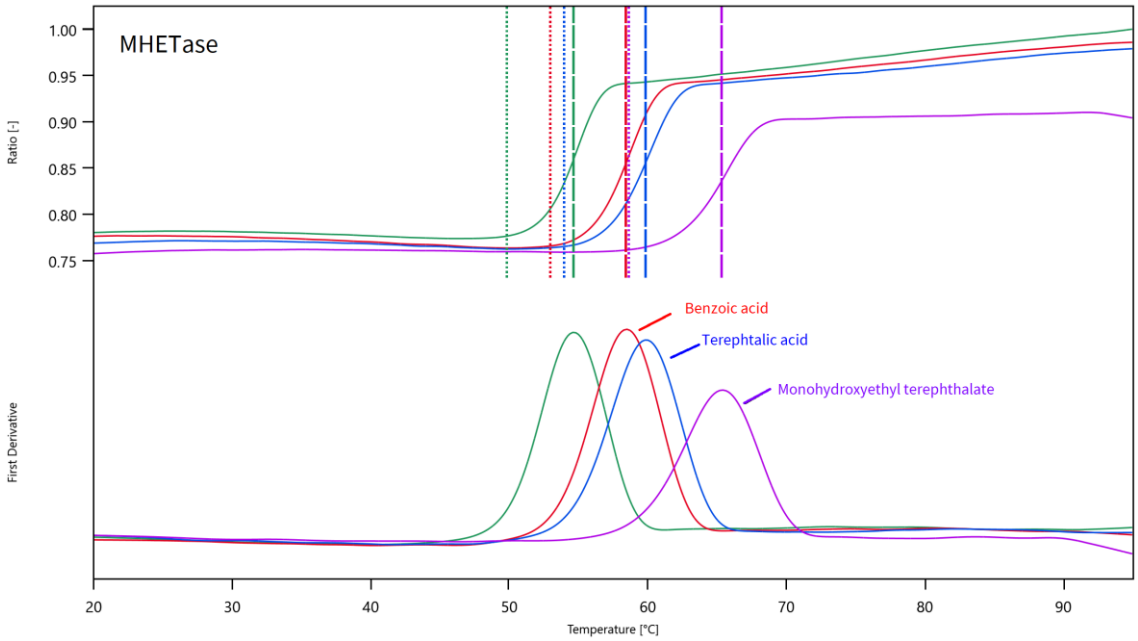
T_{on} = 49.8°C | T_m = 54.6°C (MHETase, green)

T_{on} = 53.0°C | T_m = 58.4°C (MHETase + BA, red)

T_{on} = 54.0°C | T_m = 59.9°C (MHETase + TPA, blue)

T_{on} = 58.6°C | T_m = 65.3°C (MHETase + MHET, purple)

Palm et al., Nature Communications 10: 1717 (2019)



D5. Reference Results/Supporting Results

N/A

E. Contributors

Gottfried Palm[3], Dominique Böttcher[4], Teresia Hallström[5]

[3] Department of Synthetic and Structural Biochemistry, Institute of Biochemistry, Greifswald University, Greifswald, Germany
[4] Department of Biotechnology & Enzyme Catalysis, Institute of Biochemistry, Greifswald University, Greifswald, Germany
[5] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com