Electronic Supplementary Material (ESI) for Catalysis Science & Technology

Supporting Information

Tuning lipase activity with perfluoro carboxylic acids as additives

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Contents

1. Chemicals and Additives

All chemicals were acquired from Sigma (Sternheim, Germany) unless otherwise indicated. *p*-nitrophenyl hexanote was obtained from TCI Europe (Eschborn, Germany). See Table S1 for details on the additives.

Table S1. Details of additives

ID #	*Name*	*MW (g mol^{-1})**	*Supplier*
1	Perfluoro-3,5,5'-trimethylhexanoic acid	464.08	Fluorochem (Derbyshire, UK)
2	Perfluorodecanedioic Acid	490.09	Sigma (Sternheim, Germany)
3	Perfluoropentanoic acid	264.05	Sigma (Sternheim, Germany)
4	Perfluoroheptanoic Acid	364.06	Sigma (Sternheim, Germany)
5	3-(Perfluorohexyl)propionic acid	392.12	ABCR (Karlsruhe, Germany)
6	Perfluorooctanoic Acid	414.07	Sigma (Sternheim, Germany)
7	Perfluoro-3,7-dimethyloctanoic acid	514.09	Fluorochem (Derbyshire, UK)
8	Perfluorononanoic acid	464.07	ABCR (Karlsruhe, Germany)
9	Perfluorodecanoic Acid	514.08	Sigma (Sternheim, Germany)
10	Perfluoroundecanoic acid	564.1	ABCR (Karlsruhe, Germany)
11	Perfluorododecanoic acid	614.1	Sigma (Sternheim, Germany)
12	Perfluorotridecanoic Acid	664.11	Sigma (Sternheim, Germany)
13	Perfluorotetradecanoic Acid	714.11	Sigma (Sternheim, Germany)
14	6-(Perfluoroethyl)hexanol	220.18	Fluorochem (Derbyshire, UK)
15	4,4,5,5,6,6,6-Heptafluoro-hexan-1-ol	228.11	Fluorochem (Derbyshire, UK)
16	1H,1H,2H,2H-Perfluorohexan-1-ol	264.09	Fluorochem (Derbyshire, UK)
17	1H,1H,2H,2H-Perfluorooctan-1-ol	364.11	Fluorochem (Derbyshire, UK)
18	1H,1H,2H,2H,3H,3H-Perfluoroun-decanol	478.14	ABCR (Karlsruhe, Germany)

2. Expression and purification of TTL

Expression

C-terminal His-Tagged TTL, which was kindly provided by Dr. Michael Hoesl (Technical University Berlin), is cloned in the vector pQE80L (Qiagen, Hilden, Germany) as described elsewhere.[1] The plasmid pQE80L-TTL-His-Tag (25 ng) was transformed into 50 µL of *E. coli* BL21-Gold (DE3) (Novagen, EMD Biosciences, Merck, Darmstadt, Germany) electro-competent cells using a cuvette and a Micropulser (BioRad, Munich, Germany) according to the manufacturer's instructions. Thereafter 1 mL of LB media was added to the cells and these were incubated at 37°C for one hour, followed by platting 100 µL of cells onto LB media plates with 100 µg mL^{-1} ampicillin or carbenicillin and incubation at the same temperature overnight. The next day, several clones were grown in liquid LB with the right antibiotic concentration at the same temperature and vigorous shaking until an OD_{600nm} of 0.6-0.8 was reached. The cells were then induced with 1 mM IPTG (Applichem, Darmstadt, Germany) at 30°C for 4 hr with vigorous shaking, followed by cell harvesting in a table-top micro centrifuge for 1 min at maximum speed. Ten µL of 1 OD_{600nm} of the non- and induced cultures were loaded on a 12% SDS-PAGE gel, to allow choosing the best expressing clone for large-scale protein expression. To this end, one litre LB with the right antibiotic was inoculated with the best-expressing clone, whose growth and induction was performed as described above.

Purification

The cells were harvested by low-speed centrifugation at 4°C and 3000 xg for 15 min. The pellet was suspended in phosphate buffer (50 mM NaH2PO4 / 300 mM NaCl / pH 8.0) containing 10 mM imidazole, followed by addition of 1 mg mL^{-1} of DNase, RNase as well as lysozyme (all from Applichem, Darmstadt, Germany) and sonication. The lysate was then separated into a soluble homogenate and insoluble cell debris by high-speed centrifugation (at 4°C and 30,000 xg for 30 min). The clear lysate was passed through 0.22 µm filters (Millipore, now Merck, Darmstadt, Germany) and loaded onto a 5 mL His-Trap FF column (GE Healthcare, Munich, Germany). Next, 10 column volumes (CV) of phosphate buffer containing 20 mM imidazole were added to wash out unbound proteins, followed by a 20-CV linear gradient of phosphate buffer with 300 mM imidazole to elute His-Tag bounded proteins. The eluted fractions were pooled together and concentrated using Vivaspin concentrators of 10,000 Molecular Weight Cut-Off (Sartorius, Aubagne, France) on 50 mM Tris-HCl pH 8.0 buffer. Some steps during the expression and purification process were documented using 10% SDS-PAGE (Fig. S1).

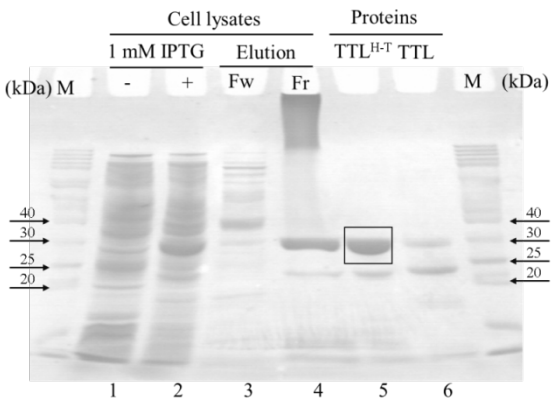

Fig. S1: TTL expression and purification. The cell lysates before (1) and after (2) induction are compared, but the one expressing TTL (2) was flowed-through (Fw) the column where TTL bound (3). After elution and pooling of TTL, the sample was exposed to 60°C for 30 min to discard non-desired bands although some precipitated (4), but these were removed by centrifugation and concentration (5). The TTL with His-Tag (TTL^{H-T}) showing purity close to 95% and theoretical mass of 30,130 Da (squared) was compared with a less-pure preparation of TTL without His-Tag (6). To each lane, 10 µL of sample were added, except for the 5 uL of the molecular weight marker (M), which was acquired from Fermentas (Thermo Scientific, Idstein, Germany). kDa: Kilo Daltons

3. Analytical characterization of TTL

Concentration

To estimate the concentration of TTL, Bradford´s method[2] was followed employing the BioRad Protein Assay, which contains an acidic solution of Coomassie® Brilliant Blue G-250 dye that can be detected at 595 nm upon binding proteins. For calibration, a reference of Bovine Serum Albumin (BSA #: R1281; formerly Fermentas, now Thermo Scientific, Bonn, Germany) was used, setting the concentration of TTL to 2.5 µg μL^{-1} (taking into account impurities as judged by Optical Density) with 50 mM Tris-HCl pH 8.0.

Mass Analysis

For the Liquid Chromatography-Electro Spray Ionisation-Mass Spectrometry (LC-ESI-MS), the samples were first completely desalted by HPLC using an Agilent 1100 system (Agilent, Boeblingen, Germany) and then applied to a monolithic 50/1 ProSwift RP-4H column (Dionex, Thermo Scientific, Idstein, Germany). Desalted proteins were eluted by the following gradient of buffer A (water/0.05% formic acid) and buffer B (acetonitrile/0.045% formic acid) at a column temperature of 40°C and a flow rate of 0.2 mL/min: Isocratic elution with 5% A for two minutes, followed by a linear gradient to 95% B within 8 minutes and holding 95% B for additional 4 minutes. Online mass spectrometric analysis was done with a Qstar Pulsar i mass spectrometer (ABSciex, Darmstadt, Germany) equipped with an ESI source. Parameters were as follows: DP1 75, FP 265, DP2 15, CAD 2, GS1 65, CUR 35. The voltage applied was 5500 V. Positive ions within the mass range of 500-2000 m/z were detected. For better performance, the “Enhance All” mode was activated, allowing the successful identification of TTL (Fig. S2).

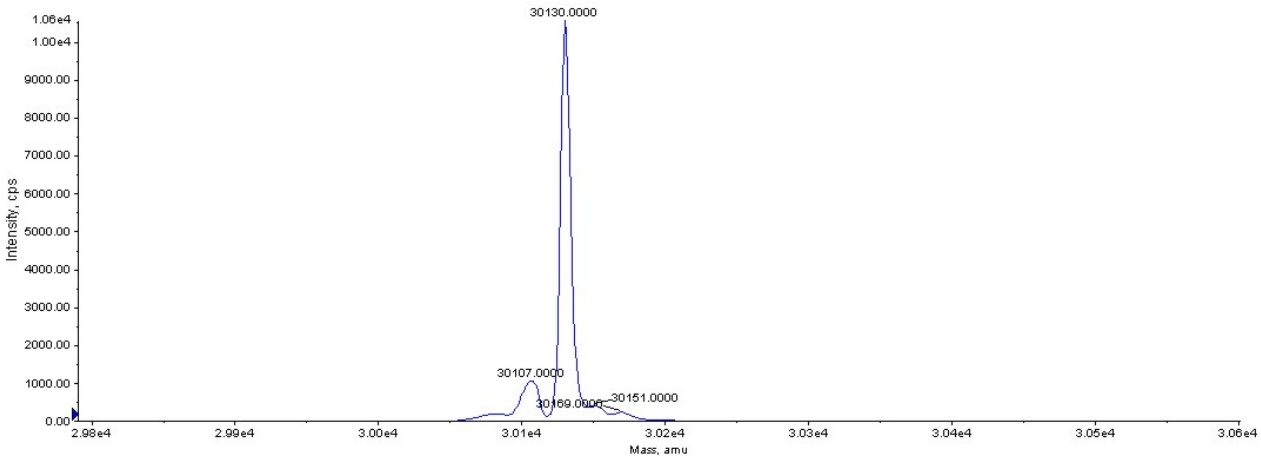

Fig. S1: Analysis of TTL by Liquid Chromatography-Electro Spray Ionisation-Mass Spectrometry (LC-ESI-MS). The expected theoretical mass for TTL of 30,130 Da, as determined by the program PROT PARAM[3], can be detected as the major peak in this time frame. The nature of the protein adducts of -23 and + 21 as well as +39 Da in respect to TTL is unknown, but most likely correspond to the deionisation and ionisation of one Na^+ ion as well as ionisation of one K^+ ion, respectively.

4. Spectrophotometric lipase assay

Lipase activity was measured according to the protocol of Winkler and Stuckmann[4] but adding Triton X-100 to avoid turbidity problems.[5] Briefly, a suspension of 0.5% Triton X-100, 1 mg mL^{-1} arabic gum (Across Organics, Geel, Belgium), and 1 mM substrate (*p*-nitrophenyl ester derivatives C2-C16) were homogenized in 50 mM Tris-HCl pH 8.0 buffer for 1 min at 13000 rpm using an Ultra-Turrax homogenizer (IKA Werke, Staufen, Germany). Then, 18 µL of TTL (0.25 - 2.5 µg) were added to 2 µL isopropanol without (control) and with 1 mM additive (final concentration 0.1 mM) and incubated for 60 min at 30°C. The reaction was started by adding 180 µL of buffered suspension to the 20 µl of TTL sample, followed by vigorous shaking at 1000 rpm in a standard Thermomixer (Eppendorf, Hamburg, Germany) and 75°C for 15 min. Finally, the samples were chilled on ice and an aliquot of 100 µL was placed onto a 96-well microplate to measure the absorbance of *p*-nitrophenol at 410 nm using the spectrophometer SpectraMax plus384 (Molecular Devices, Ismaning, Germany).

5. References

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