Non-targeted and targeted analysis of oxylipins in combination with charge-switch derivatization by ion mobility high-resolution mass spectrometry

Stefan Hellhake, Sven W. Meckelmann, Michael T. Empl, Kristina Rentmeister, Walter Wißdorf, Pablo Steinberg, Oliver J. Schmitz, Thorsten Benter, Nils Helge Schebb

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Materials and methods

Synthesis of epoxy PUFA

For synthesis of the desired epoxy PUFA, 0.64 mmol of PUFA (LA, ALA, AA, DHA or DPA) were dissolved in 10 mL chloroform and mixed with 100 mg (0.64 mmol) meta-chlorobenzoic acid (mCPBA) in a 50 mL round bottom flask sealed with a glass stopper and slowly stirred overnight at room temperature. The reduced mCPBA was separated by liquid/liquid extraction in a 100 mL separating funnel with 30 mL of a 50 mM sodium bicarbonate solution. The solution was extracted three times with chloroform. The combined chloroform phases were dried with sodium sulfate and evaporated using a rotary evaporator at 40 °C. The reaction process was monitored using thin-layer chromatography. The products were further purified by semi-preparative chromatography. Purity and concentration were determined by RP-LC-MS/MS.

Synthesis of dihydroxy PUFA

For the conversion of the PUFA to diols, acid hydrolysis was used. The isolated epoxy fatty acid regioisomers such as 17(18)-EpETE and 14(15)-EpETrE or different epoxide mixtures were dissolved in 10 mL acetonitrile and mixed with 10 mL 1 M H₂SO₄. The solution was stirred overnight at room temperature and subsequently neutralized using a 50 mM NaHCO₃ solution. The acetonitrile was removed using a rotary evaporator at 40 °C and the remaining aqueous phase was extracted three times with 10 mL chloroform. The combined chloroform phases were dried with sodium sulfate and the solvent was removed by rotary evaporation at 40 °C. The purity and concentration of the dihydroxy fatty acids was confirmed by RP-LC-MS/MS.

Synthesis of AMPP

Anhydrous pyridine (99.8 %), ethanol p.a. (EtOH), 1-chloro-2,4-dinitrobenzene, 4-[(N-Boc)aminomethyl]aniline, N-hydroxybenzotriazole, trifluoroacetic acid, N,N-diisopropylethylamine, ²H₅-pyridine, and all other chemicals were purchased from Sigma Aldrich (Steinheim, Germany).
4 mmol pyridine (0.344 mL) were mixed with 4.6 mL EtOH and 4 mmol 1-chloro-2,4
dinitrobenzene were added. The solution was refluxed at 98 °C for 16 h under a nitrogen
atmosphere. After cooling to room temperature, the EtOH was removed by rotary evaporation at
40 °C and the resulting crude N-2,4-dinitrophenyl pyridinium chloride was purified by
recrystallization in a small amount of hot EtOH, resulting in yellow-coloured pure N-2,4-
dinitrophenyl pyridinium chloride. N-2,4-dinitrophenyl pyridinium chloride was then dissolved in
14 mL EtOH/pyridine (3/1; v/v). After adding 6.72 mmol of 4-[(N-Boc)-amino-methyl] aniline, the
solution was refluxed at 98 °C for 3 h under a nitrogen atmosphere. The solution was allowed to
cool down to room temperature and 140 mL water were subsequently added to precipitate 2,4-
dinitroaniline. After filtration, the remaining solution was concentrated to a brown oily 4-[(N-Boc)-
amino-methyl] phenyl pyridinium chloride solution, using a rotary evaporator at 40 °C. 22.4 mL
trifluoroacetic acid (25 % in dichloromethane) were then added and the mixture was allowed to
react for 30 min at room temperature before drying by rotary evaporation at 40 °C. The residue
was triturated twice with toluene and dried again. Finally, the residue was dissolved in a minimal
amount of hot EtOH and allowed to cool down for about five minutes before treatment of the
solution with diethyl ether until it started to cloud up. The mixture was kept at -20 °C overnight,
yielding 578.3 mg of AMPP as a solid yellow-brown product. Synthesis was controlled by LC-MS
and NMR. Synthesis of labelled AMPP was carried out exactly as described above but using
4 mmol ²H₅-pyridine instead of pyridine.
Analysis of TPPU toxicity

**Fig. S1** Toxicity of ascending TPPU concentrations towards Caco-2 cells as determined using the SRB assay. Shown is the mean cell number (± SD) of three independent experiments after 24–72 h of incubation.

**Fig. S2** Structure of 10-hydroxy-8-octadecenoic acid derivatized with AMPP with suggested sites of fragmentation yielding fragment ions found by non-targeted feature analysis (Table 2)

**Table S1** (separate Excel File): List of detected features

**Separate py-File:** Python Skript