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Coenzyme A thioester formation of 11- and 15-oxo-eicosatetraenoic acid

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ABSTRACT

Release of arachidonic acid (AA) by cytoplasmic phospholipase A2 (cPLA2), followed by metabolism through cyclooxygenase-2 (COX-2) and 15-hydroxyprostaglandin dehydrogenase (15-PGDH), results in the formation of the eicosanoids 11-oxo- and 15-oxo-eicosatetraenoic acid (oxo-ETE). Both 11-oxo- and 15-oxo-ETE have been identified in human biospecimens but their function and further metabolism is poorly described. The oxo-ETEs contain an α,β-unsaturated ketone and a free carboxyclic acid, and thus may form Michael adducts with a nucleophile or a thioester with the free thiol of Coenzyme A (CoA). To examine the potential for eicosanoid-CoA formation, which has not previously been a metabolic route examined for this class of lipids, we applied a semi-targeted neutral loss scanning approach following arachidonic acid treatment in cell culture and detected inducible long-chain acyl-CoAs including a predominant AA-CoA peak. Interestingly, a series of AA-inducible acyl-CoAs at lower abundance but higher mass, likely corresponding to eicosanoid metabolites, was detected. Using a targeted LC–MS/MS approach we detected the formation of CoA thioesters of both 11-oxo- and 15-oxo-ETE and monitored the kinetics of their formation. Subsequently, we demonstrated that these acyl-CoA species undergo up to four double bond reductions. We confirmed the generation of 15-oxo-ETE-CoA in human platelets via LC-high resolution MS. Acyl-CoA thioesters of eicosanoids may provide a route to generate reducing equivalents, substrates for fatty acid oxidation, and substrates for acyl-transferases through cPLA2-dependent eicosanoid metabolism outside of the signaling contexts traditionally ascribed to eicosanoid metabolites.

1. Introduction

Cyclooxygenases (COXs) are major enzymes responsible for the generation of bioactive lipids from the preferred precursor arachidonic acid (AA) [1]. The AA oxidation product 11-oxo-eicosatetraenoic acid (11-oxo-ETE) is generated by the sequential action of COXs, peroxidases, and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) on free AA [2]. The known intermediates of this pathway are the unstable 11-hydroperoxyeicosatetraenoic acid (11-HPETE) and the stable product 11-hydroxyeicosatetraenoic acid (11-HETE) [3]. 15-oxo-ETE can be generated by a COX/15-PGDH pathway from free AA or a 15-lipoxygenase (LOX)/15-PGDH or 12/15-LOX/15-PGDH pathway from free or esterified AA [4,5]. In multiple cancers and inflammatory conditions, COX-2 and 15-PGDH are counter-regulated, whereby COX-2 expression is increased and 15-PGDH levels are reduced [6–8]. Furthermore, the expanding involvement of eicosanoids as part of oxidized phospholipids in inflammation, and atherosclerosis, once again warrants interest in the metabolism of oxidized lipids beyond established roles as signaling mediators [9].

Eicosanoid signaling is responsible for a vast array of cellular responses through families of G- protein coupled receptors (GPCRs) [10]. Evidence for non-GPCR mediated signaling exists [3–8], but is still controversial for some mediators [11]. Electrophilic α,β-unsaturated ketone containing fatty acids derived from ω-6 and ω-3 polyunsaturated fatty acids have anti-proliferative, anti-inflammatory, and redox signaling properties [12–15]. Descriptions of the formation of polar lipids composed of glycerol and

Abbreviations: COX, cyclooxygenase; AA, arachidonic acid; HETE, hydroxyeicosatetraenoic acids; HPETE, hydroperoxyeicosatetraenoic acid; 15-PGDH, 15-prostaglandin dehydrogenase; oxo-ETE, oxo-eicosatetraenoic acid; PGE_2, prostaglandin E_2; EP, prostaglandin E receptor; FBS, fetal bovine serum; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2; LOX, lipoxygenase; ME, methyl ester; SPE, solid phase extraction.

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ethanolamine head groups with eicosanoid acyl-chains [16] was followed by identification of other polar lipids with common head groups but also with acyl-chains derived from eicosanoids [9,17]. Recent work has also demonstrated cPLA2-dependent bioenergetic metabolism is required for platelet function [18]. These lines of evidence suggest other, previously under-appreciated functions of eicosanoid metabolism outside of direct signaling processes. These studies also suggest, but did not examine, the existence of intermediates linking bioenergetic metabolism, especially β-oxidation, as well as the existence of eicosanoid substrates for acyl-transferases that can generate polar lipids with eicosanoid acyl-groups. Acyl-Coenzyme A thioesters are capable of performing both of these biochemical roles as ubiquitous, conserved, and localized substrates for both bioenergetics processes and synthetic pathways including those catalyzed by acyl-transferase enzymes. However, relatively little is known about the further metabolism of eicosanoids via acyl-CoA intermediates. Thus, this study was designed to determine the existence of these putative intermediates using oxo-eicosanoids for which no other function is currently described. We employed a COX-2/15-PGDH stably expressing human colon adenocarcinoma cell line (LoVo) that was used in previous studies of 11-oxo- and 15-oxo-ETE metabolism [2]. Specifically, we investigated the CoA thioester formation of 11-oxo- and 15-oxo-ETE using untargeted and targeted liquid chromatography-tandem mass spectrometry (LC–MS/MS) approaches, with confirmation in human platelets using LC-high resolution MS (LC-HRMS)

2. Materials and methods

2.1. Reagents

11-Oxoeicosatetranoic acid (11-oxo-ETE), 15-oxoeicosatetranoic acid (15-oxo-ETE), 11-oxo-eicosatetranoic acid methyl ester (11-oxo-ETE-ME), 15-oxo-eicosatetranoic acid methyl ester (15-oxo-ETE-ME) and the [$^{13}C_{20}$]-labeled 15-oxo-ETE internal standard were synthesized in-house as previously reported [2]. [$^{13}C_3$ $^{15}N_1$]-Arachidonoyl-CoA used as an internal standard was synthesized as previously reported [19]. Peroxide-free arachidonic acid (AA) was purchased from Cayman Chemical (Ann Arbor, MI). 5-sulfosalicilic acid (SSA), ammonium formate, glacial acetic acid, 2-mercaptoethanol (BME), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade chloroform as well as Optima LC–MS grade methanol, acetonitrile, water, isopropanol, ammonium acetate, and formic acid were purchased from Fisher Scientific (Pittsburgh, PA). 2-(2-pyridyl) ethyl functionalized silica gel (100 mg/mL) solid phase extraction (SPE) cartridges were obtained from Supelco Analytical (Bellefonte, PA). Streptomycin and penicillin were purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was from Gemini Bioproducts (West Sacramento, CA). Human colorectal adenocarcinoma cells (LoVo and HCA-7) were obtained from ATCC (Manassas, VA).

2.2. Acyl-CoA analysis

Human colorectal adenocarcinoma cells (LoVo) were grown to 80% confluence in F–12 K media with 2% FBS and 100,000 units/L penicillin and 100 mg/L streptomycin. Human colorectal adenocarcinoma cells (HCA-7) were grown to 80% confluence in DMEM/F12 media with 2% FBS and 100,000 units/L penicillin and 100 mg/L streptomycin. Cells were treated with stock solutions of eicosanoids in DMSO and final concentration was always kept under 0.1% of DMSO in the media. For platelet experiments, 1 mL of expired platelet concentrate from the Hospital of the University of Pennsylvania Blood Bank was washed and treated with vehicle control (0.25% DMSO), 10 μM 15-oxo-ETE, or 50 μM arachidonic acid in Tyrode's buffer containing 5 mM glucose as previously described [20]. After 1 h, the platelets were extracted as below, and analyzed by LC-HRMS as previously described [21].

Extraction and analysis were performed as previously described [19,22]. Briefly, cells were lifted with a cell scraper, and the resulting suspension was centrifuged at 500 x *g* at 4° C. For the time course experiments, cells were spiked with the [$^{13}C_3$ $^{15}N_1$]-arachidonoyl-CoA. The supernatant was removed, and the pellet was suspended in 750 μL of 3:1 acetonitrile:isopropanol (ACN:IPA). The suspension was then sonicated with a probe tip sonicator (Fisher) to disrupt the cellular membranes. 250 μL of 100 mM KH_2PO_4 (pH 6.7) was added, the suspension was vortexed, and then centrifuged at 16000 x *g* at 4° C. The resulting supernatant was transferred to a glass tube and acidified with 125 μL glacial acetic acid for solid phase extraction. 100 mg 2-(2-pyridyl)ethyl-functionalized silica gel solid phase extraction columns were equilibrated with 1 mL 9:3:4:4 ACN:IPA:H_2O:acetic acid. Supernatants were transferred to the column and filtrated under low vacuum. The columns were washed two times with 1 mL of the 9:3:4:4 mixture. The columns were eluted two times with 500 μL 4:1 methanol:250 mM ammonium formate into glass tubes. Filtrate was evaporated to dryness under nitrogen gas. The dry samples were dissolved in 50 μL of 70:30 water:acetonitrile with 5% SSA (w/v) and transferred into HPLC vials.

2.3. Liquid chromatography- mass spectrometry

Chromatographic separation was performed using a reversed phase Waters XBridge BEH130 C18 column (2.1 × 150 mm, 3.5 μm pore size) on an Agilent 1100 HPLC system using a three solvent system as previously described [23]: (A) 5 mM ammonium acetate in water, (B) 5 mM ammonium acetate in 95/5 acetonitrile/water (v/v), and (C) 80/20/0.1 (v/v/v) acetonitrile/water/formic acid, with a constant flow rate of 0.2 mL/min. Solvent C was used after analysis to wash the column. The gradient was as follows: 2% B at 0 min, 2% B at 1.5 min, 20% B at 5 min, 100% B at 5.5 min, 100% B at 13.5 min, 100% C at 14 min, 100% C at 19 min, 2% B at 20 min and 2% B at 25 min. The column effluent was diverted to waste before 8 min and after 18 min. For targeted acyl-CoA analysis, samples were maintained at 4 °C in a Leap CTC autosampler (CTC Analytics, Switzerland) during sample batch runs. 10 μL injections were used for LC–MS analysis. The LC was coupled to a API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) in the positive electrospray ionization (ESI) mode and analyzed using Analyst software as previously described [24]. The mass spectrometer operating conditions were as follows: ion spray voltage (5.0 kV), compressed air as curtain gas (15 psi) and nitrogen as nebulizing gas (8 psi), heater (15 psi), and collision-induced dissociation (CID) gas (5 psi). The ESI probe temperature was 450 °C, the declustering potential was 105 V, the entrance potential was 10 V, the collision energy was 45 eV, and the collision exit potential was 15 V. CoA thioesters were screened using a neutral loss of 507, then monitored using SRM (selected reaction monitoring mode) as previously published [19]. For LC-HRMS experiments, an Ultimate 3000 UHPLC coupled to a Thermo QExactive Plus operating with XCalibur (Thermo Fisher, San Jose, CA) as previously described was used [21]. Shift in retention times between the two systems is due to differences in the LC tubing length and injector cycle.