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Determination of 3′-phosphoadenosine-5′-phosphosulfate in cells and Golgi fractions using hydrophilic interaction liquid chromatography–mass spectrometry

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ABSTRACT

3′-Phosphoadenosine-5′-phosphosulfate (PAPS) is a key player in the sulfation of biomolecules, but methods for selective measurements are lacking. A liquid chromatography–mass spectrometry (LC–MS) approach for measuring PAPS was developed. A central feature of the method was employing hydrophilic interaction liquid chromatography (HILIC), which is highly suited for separating very polar/charged compounds, and is compatible with electrospray MS. Using simple instrumentation, the analysis time per sample was below 10 min and the method was characterized by easy sample preparation. The method was used to monitor decreasing levels of PAPS as function of sodium chlorate treatment (an inhibitor of PAPS synthesis) in whole-cell lysates as well as Golgi-fractions. The method allowed PAPS to be chromatographically separated from ADP and ATP, which can interfere with measurements if a less resolving LC–MS method is used.

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1. Introduction

In the secretory pathway of eukaryotic cells, the Golgi apparatus is a major site for post-translational modification of proteins. In animal cells, a wide variety of glycoproteins and proteoglycans (PGs) get their glycans synthesized and/or modified throughout the Golgi *cisternae*. The glycosaminoglycans (GAGs) attached to PGs are chains of repeating disaccharide units that become abundantly sulfated in the Golgi lumen of metazoan cells by a variety of sulfotransferases (STs). Other STs add sulfate to different classes of glycans of glycoproteins and glycolipids, or directly onto tyrosine residues. The substrate for these STs, 3′-phosphoadenosine-5′-phosphosulfate (PAPS, Fig. 1), is synthesized in the cytoplasm and nucleus in animal cells by a bi-functional PAPS synthase [1–4], and also in the cytoplasm and plastids of plant cells [5]. Utilization of PAPS by Golgi STs that modify PGs requires active uptake from the cytoplasm through PAPS transporters in Golgi membranes [6–8]. PAPS is the universal donor of sulfate to sulfotransferases, both in the cytoplasm and in the Golgi lumen. Since a number of xenobiotic molecules are sulfated in the cytoplasm, particularly in liver and kidney cells, competition with endogenous substrates can occur, and PAPS availability may become rate-limiting [9]. PAPS measurements in cells are appropriate for studying sulfation mechanisms and dynamics. However, common assays based on e.g. radioisotope incorporation [10,11] may e.g. have limited selectivity [12].

Liquid chromatography has previously been employed for measuring PAPS, for example with approaches incompatible with selective MS detection, e.g. ion pair chromatography or phosphate buffered mobile phases [12,13]. Our approach is to employ hydrophilic interaction liquid chromatography (HILIC) [14,15]. HILIC is a "multiple personality" hybrid of many separation principles, including e.g. partitioning, ion exchange and adsorption [16]. It is e.g. well suited for separating polar and ionized compounds (see e.g. [17–21]) with MS-compatible mobile phases. A great variety of HILIC stationary phases exists [22]. The ZIC-HILIC variant, which features a zwitter-ionic functionality, is well suited for compounds

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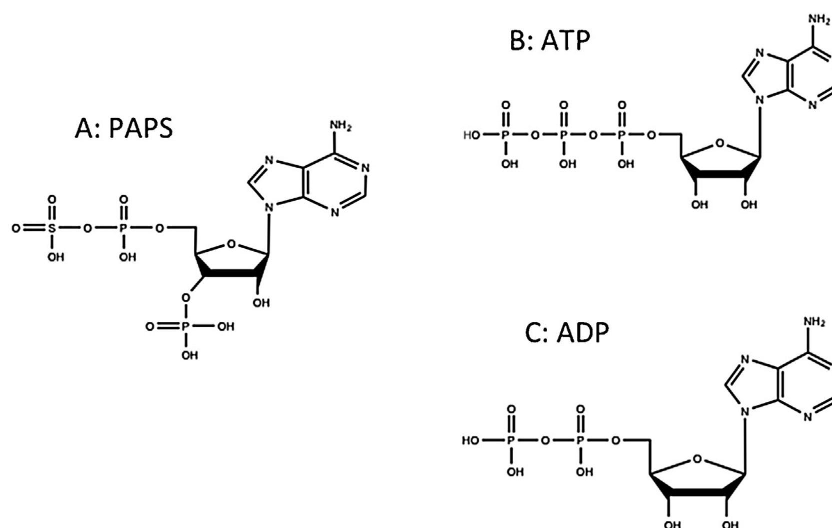

Fig. 1. Structure of A: PAPS (3′-Phosphoadenosine-5′-phosphosulfate), B: ATP (adenosine 5′-triphosphate disodium salt hydrate) and C: ADP (adenosine 5′-diphosphate).

similar to PAPS, especially when attached to organic polymer particles that are stable over a broad pH-range (ZIC-*p*HILIC) [17].

We have developed a ZIC-*p*HILIC-MS method for determining PAPS in whole cell lysates as well as Golgi fractions; the method is simple, has good precision and ensures interference-free mass spectrometric monitoring of PAPS.

2. Experimental

2.1. Reagents and solvents

Type 1 water (resistivity (MΩ cm @ 25 °C) >18.0) was from a Milli-Q ultrapure water purification system (Millipore, Bedford, MA, USA). HPLC grade ACN, acetic acid and methanol were from VWR (Radnor, PE, USA). Ammonium carbonate ($(NH_4)_2CO_3$), was from Fluka (Buchs, Switzerland). PAPS (adenosine 3′-phosphate 5′-phosphosulfate lithium salt hydrate), ATP (adenosine 5′-triphosphate disodium salt hydrate) and ADP (adenosine 5′-diphosphate) were from Sigma-Aldrich (St. Louis, MO, USA). See Fig. 1 for structures. Nitrogen (99.96%) was from AGA (Oslo, Norway). For synthesis procedure of the internal standard (^{13}C-PAPS ($^{13}C_{10}H_{15}N_5O_{13}P_2S$)) see **SM-1**. Sodium chloride for analysis was from Merk (Darmstadt, Germany).

2.2. Consumables and sample handling apparatus

Amicon® Ultra 0.5 mL centrifugal filters with a 10 K "cut-off" were from Merck Millipore (Billericia, MA, USA). Eppendorf® Safe-Lock microcentrifuge tubes (1.5 mL) and Microvials PP (0.3 mL) with snap rings were from VWR. Pipettes (Finnpipette®F2) 5–40, 20–200, 100–1000 μL and 1–5 mL were from Thermo Scientific. A NanoDrop™ 2000 UV–vis spectrophotometer was from Thermo Scientific. A Centrifuge 5415 R was from Eppendorf (Hamburg, Germany). A Speed-vac™ concentrator (SC110) was from Savant (Hicksville, NY, US). A sonicator (Branson 2510) was from Bransonic® (Bloomfield, USA).

2.3. Cell samples

See **SM-2** for details on cell cultering, fractionation and treatments.

2.4. Solutions

A stock solution of PAPS was made by dissolving the purchased 5 mg of PAPS standard in a mixture of ACN and type 1 water (65/35, v/v) to a final concentration of 1 mg/mL. The stock solution of the internal standard (IS) was prepared by diluting 1.0 mL of 1 mg/mL with a mixture of ACN and type 1 water (65/35, v/v) to 10.0 mL to a final concentration of 100 μg/mL. Stock solutions of ATP and ADP were prepared by dissolving 1 g of each in 10.0 mL of the mobile phase to a final concentration of 100 mg/mL. All stock solutions were divided in small aliquots and frozen (−80 °C). A 100 mM ammonium carbonate solution was prepared by dissolving 4.80 g of ammonium carbonate in 500 mL type 1 water. The solution was stored at 4 °C. A solution of 0.5 M NaCl was prepared by dissolving 14.6 g of NaCl in 500 mL type 1 water. The solution was stored at 4 °C.

2.5. Working solutions

Working solutions of PAPS/internal standard/ATP/ADP were prepared daily by diluting the stock solutions in the mobile phase or in the desired mixture of ACN and 100 mM ammonium carbonate solution.

2.6. Method evaluation solutions

Method evaluation solutions (also used for calibration) were prepared by spiking 50 μL of cell lysate samples (1×10^5 cells) with standard solutions to a concentration of 2, 10, 100 and 200 μg/mL (in addition to endogenous levels) and with internal standard to a concentration of 10 μg/mL.

2.7. Sample preparation

Cell lysis was carried out by adding 100 μL of an aqueous solution (type 1 water and 100 mM ammonium carbonate (50/50, v/v)) to the cell pellets (12×10^6 cells) and placing the mixture in an ultrasonic bath with cooling periods (10 s in the bath and 10 s on ice, six cycles) to disrupt cell membranes and release polar biological molecules (such as PAPS). Then the cells were centrifuged at full power (16100 rcf), 20 °C for 15 min, and the supernatant (cell lysate) was separated from the cell pellet carefully using a 20–200 μL pipette. The collected cell lysates were diluted with

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