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Research article

Liquid chromatography/time-of-flight mass spectrometry for the analysis of plant samples: A method for simultaneous screening of common cofactors or nucleotides and application to an engineered plant line

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ABSTRACT

Intense efforts are currently devoted to improve plant metabolomic analyses so as to describe more accurately the whole picture of metabolic pathways. Analyses based on liquid chromatography/time-of-flight mass spectrometry (LC–TOF) are now widely distributed among plant science laboratories. However, the use of reliable, sensitive LC–TOF methods to identify and quantify micromolar or inframicromolar key metabolites is often impeded by the sensitivity of the technique to sample preparation or chromatographic conditions. Typically, the sample matrix has a substantial influence on ionization efficiency and therefore, on the detectability of such compounds. Here, we describe a new method to analyze simultaneously 23 nucleotides and cofactors from plant extracts, taking advantage of solid-phase extraction (SPE) prior to injection. The influence of common *m/z* fragments in several metabolites and adducts is considered. We applied this method to characterise metabolic intermediates of NAD biosynthesis in *Arabidopsis thaliana*, using a wild-type and an engineered transgenic plant line that produces bacterial quinolinate phosphoribosyl transferase (*nadc*). We show that sample pre-purification with SPE is strictly required not only for compound quantification and identification but also to allow ionization of matrix-sensitive compounds (*e.g.* nicotinamide) or alleviate fragmentation of others (*e.g.* NAD). When exogenous substrate quinolinate was infiltrated into *Arabidopsis* leaves to increase the natural content in downstream metabolites, a clear correlation between intermediates of NAD biosynthesis was seen, showing the accuracy of our method for quantification in biological samples. *Nadc* plants only showed very modest changes in NAD-related metabolites and furthermore, they were associated with slightly lower photosynthetic performance and ATP production.

1. Introduction

A growing number of studies take advantage of metabolomic methods to provide a general picture of plant metabolism. Several techniques (NMR, GC–MS, LC–MS) have been used to carry out non-targeted metabolomic analyses [1]. Multivariate statistics further allows the identification of metabolites that discriminate sample groups (for a recent application to LC–TOF data in plants, see [2]). However, all of these methods have advantages and disadvantages. Although widely used, NMR is insensitive to variations in trace constituents. GC–MS is not adapted to compounds with high molecular weight. Nowadays, LC–MS techniques are seen as essential, due to their inherent benefits in sensitivity and selectivity [3,4]. Specific applications of LC–MS methods to complex plant metabolites have been published in the past decade (*e.g.* naphtodianthrones, [5]; fungicide, [6]; metatonin, [7]; alkaloids, [8]; amino acids, [9]), including plant hormones [10,11]. Nevertheless, one remaining conundrum of plant metabolomics is targeted metabolomic analyses on large sample trials. In fact, satisfactory results may only be achieved under specific chromatographic conditions, possibly after cleanup treatments. In other words, while the separative step (LC) is crucial to enhance both sensitivity and selectivity, a further pre-purification step may be

Abbreviations: AdoHCys, S-adenosyl homocysteine; ADPG, ADP-glucose; Glut, glutathion; Me-THF, methyl-tetrahydrofolate; NaMN, nicotinate-D-ribonucleotide; NaAD, deamino-NAD$^+$; NMR, nuclear magnetic resonance; RuBP, ribulose-1,5-bisphosphate; SAM, S-adenosyl methionine; THF, tetrahydrofolate; TOF, time-of-flight mass spectrometry; UDPG, UDP-glucose; WUE, water-use efficiency.

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required, to avoid excessive ion overlapping and ion suppression of minor metabolites by major compounds (see, *e.g.*, [11] for cytokinins, [12] for phenolics).

The analysis of specific metabolites is of typical interest for physiological studies that use different genetic plant lines or varying environmental conditions so as to discriminate metabolic regulation points. Nevertheless, in the analysis of complex matrixes such as plant extracts, coeluting interferences could inhibit or enhance analyte ionization, decreasing or increasing signals and therefore, avoiding a correct quantification [13]. Accurate quantification of targeted metabolites thus requires maximal recovery of the compound of interest and chromatographic optimization. Here, we intend to develop a sensitive and reliable method for the analysis of nucleotides, cofactors and intermediates of NAD biosynthesis (quinolinate, nicotinate, etc.) or photosynthesis (ribulose-1,5-bisphosphate, RuBP) and photorespiratory metabolism (tetrahydrofolate, THF). Two steps of solid-phase extraction were performed before LC–TOF spectrometry, equipped with an electrospray interface in both positive and negative ion mode.

As many other compounds, intermediates of NAD synthesis and cofactors such as THF or S-adenosyl methionine (SAM) are present in rather low amount in plant leaves. In the present study, we tested the applicability of our method with *Arabidopsis* samples, taking advantage of *(i)* the transgenic plant line (*nadc*) producing quinolinate phosphoribosyl transferase (QPRT, EC 2.4.2.19) from *Escherichia coli* and *(ii)* the artificial stimulation of quinolinate metabolism with exogenous quinolinate. QPRT is believed to be a committed step of NAD synthesis (Fig. 1). QPRT catalyses the decarboxylating N-addition of phosphoribose to quinolinate, thereby producing nicotinate-D-ribonucleotide (NaMN). That is, QPRT is involved in *de novo* synthesis of NAD from aspartate. Although QPRT has been characterized in *E. coli*, the role played by QPRT catalysis in plants remains poorly documented. The plant genome contains a single, QPRT-encoding gene that is believed to

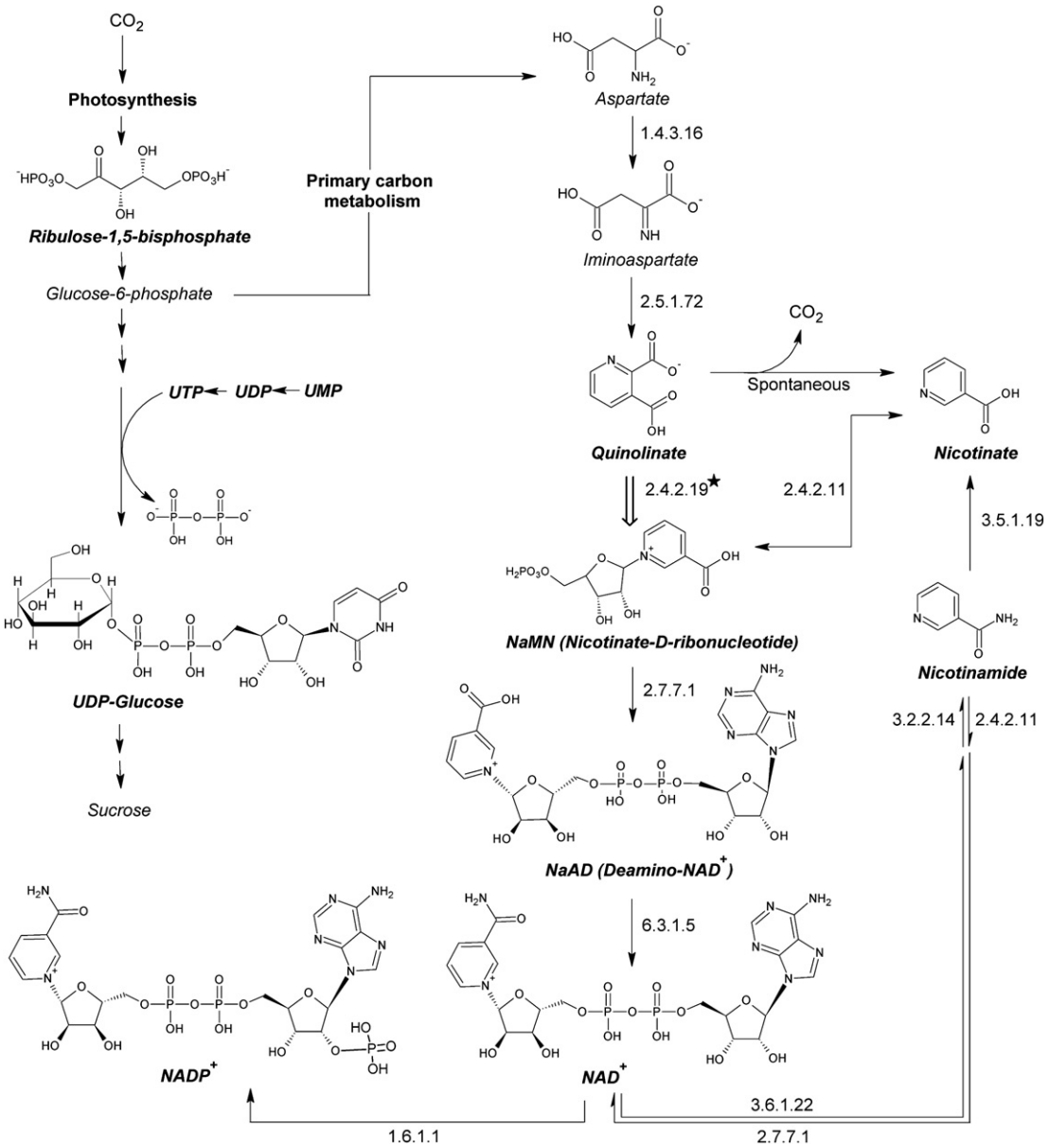

Fig. 1. The metabolic pathway investigated here, that includes nicotinamine dinucleotide (NAD) biosynthesis. The engineered *Arabidopsis* line examined in the present study (*nadc*) expresses the gene coding for bacterial quinolinate phosphoribosyl transferase (QPRT, EC 2.4.2.19), indicated with a star. Other enzymes are indicated by their EC number.