NON-CANONICAL CYCLIC NUCLEOTIDE MONOPHOSPHATES IN *APHANIZOMENON FLOS-AQUAE*: NUCLEAR MAGNETIC RESONANCE AND MASS SPECTROMETRY

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INTRODUCTION

While the nutraceutical properties of the cyanobacteria, such as Aphanizomenon flos aquae (AFA) from Klamath Lake (Oregon, USA), are well known [1], there is an ongoing debate on their long-term effects on human health that remain unknown [2]. Some authors focus on the health-enhancing properties of blue-green algae supplements linked to the presence of bioactive compounds and anti-inflammatory and antioxidant substances [3], whereas others report the contamination of these supplements by microcystins and other toxins [4], thus arising health concerns about their use.
We recently reported that phytochemicals, such as mycosporine-like amino acids and low molecular weight glycosides, and a number of other metabolites can be detected directly on algae suspensions by NMR with the help of mass spectrometry (MS) [5].

We present a study that, combining multinuclear ^{1}H, ^{31}P and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy and high-resolution mass spectrometry, led to the detection of uncommon phosphorylated metabolites in AFA. We focused our attention on ^{31}P NMR signals at 20 ppm, a chemical shift that usually points to the presence of phosphonates. The molecules contributing to 20 ppm ^{31}P NMR signals revealed, instead, to be nucleoside 2',3'-cyclic monophosphates (cNMPs). These metabolites were fully characterized by multinuclear ^{1}H, ^{31}P and ^{13}C NMR spectroscopy and high-resolution mass spectrometry.

RESULTS

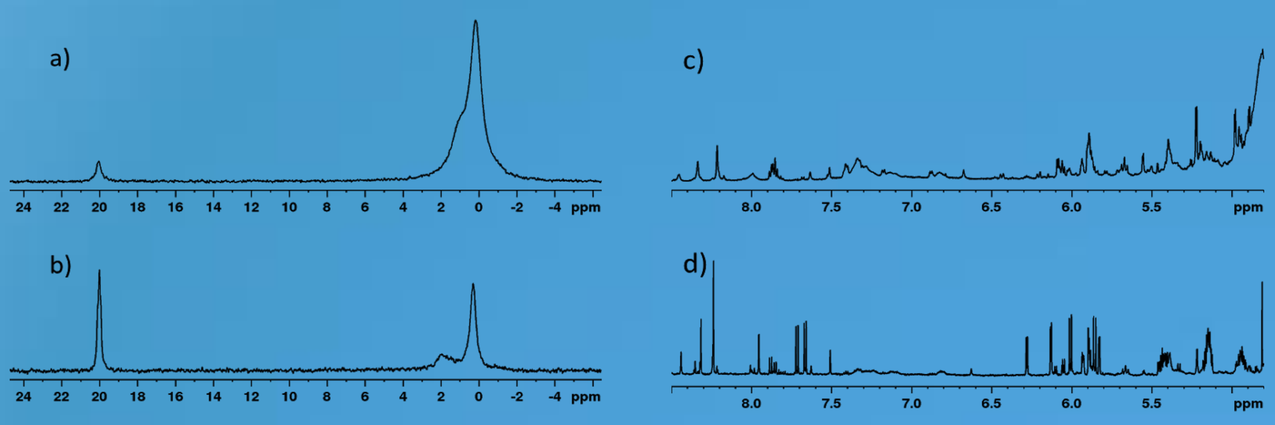

Figure 1: ^{31}P NMR spectra of (a) AFA-1 and (b) AFA-2 in D_2O and ^{1}H NMR spectra of (c) AFA-1 and (d) AFA-2 in D_2O in region 8.5-4.8 ppm.

The ^{31}P NMR spectrum of a D_2O suspension of dry powder of alga Klamath (AFA-1), Fig. 1a, confirmed us the presence of phosphonates (signal at 20 ppm). We isolated also a solid fraction of the AFA metabolome (AFA-2), derived from the depletion of ethanol- and methanol-soluble compounds, the ^{31}P and ^{1}H NMR spectra of which are reported in Fig. 1b and 1d. The same expansion of the ^{1}H NMR spectrum of AFA-1 is reported in Fig. 1c as a comparison, to show that, after enrichment, signals in this region are mainly due to the molecular species object of this study.
On this fraction H,P-HMBC (Fig. 2a and 2b), H,C--HSQC-TOCSY (Fig. 2c), was performed. The experimental data obtained, allowed us to assign the ^{31}P signals around 20 ppm to four cyclic nucleotides, cAMP, cGMP, cUMP and cCMP (Fig. 3), which therefore characterize the AFA metaboloma.
In order to confirm the NMR findings, AFA-2 samples were resuspended in H_2O and positive and negative ion high-resolution ESI-QO-MS and low-energy CID MS/MS spectra were acquired. The four cNMPs were detected both in positive (Fig. 4, Table 2) and in negative ionization mode, even though positive polarity was more efficient.

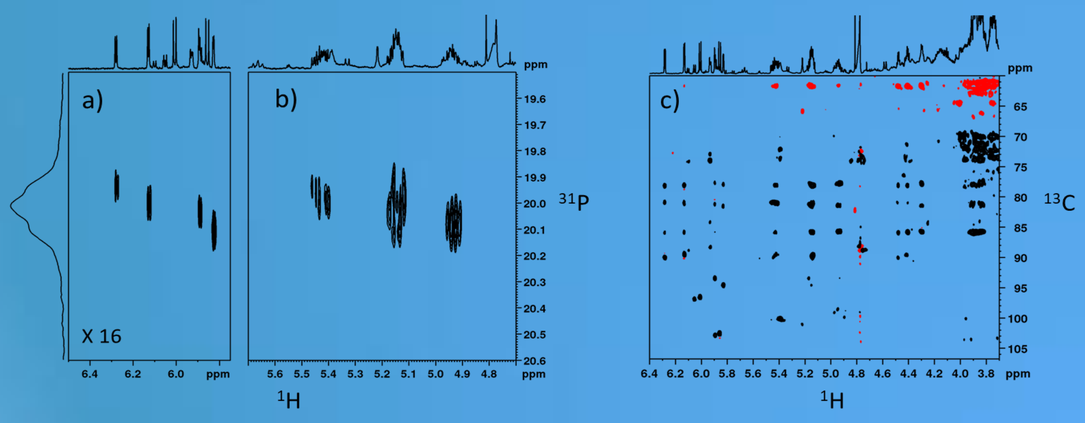

Figure 3: Relevant regions of the H,P-HMBC NMR spectrum of AFA-2 in D_2O plotted with different levels (a) 16 times lower than (b); (c) partial H,C-HSQC-TOCSY NMR spectrum of AFA-2 in D_2O.

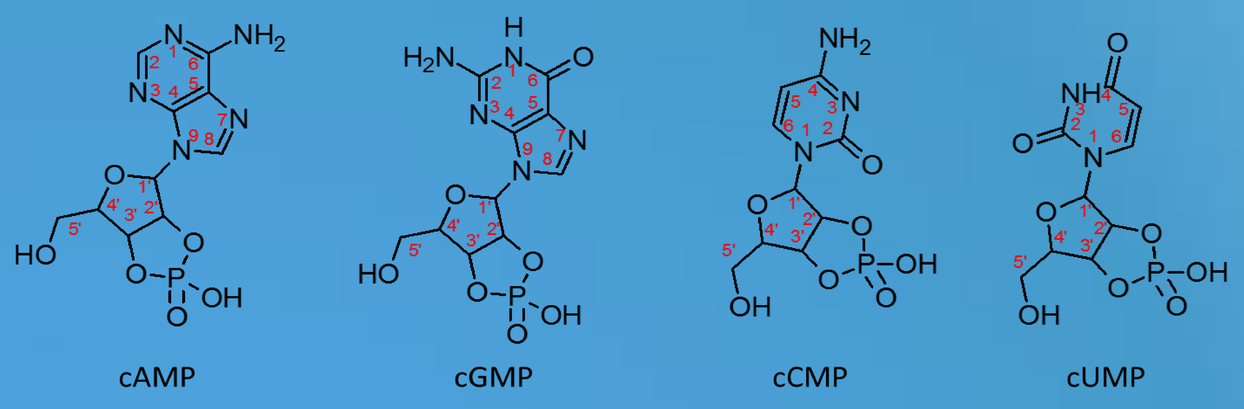

Figure 2: The four nucleoside 2',3'-cyclic monophosphates identified in this study.

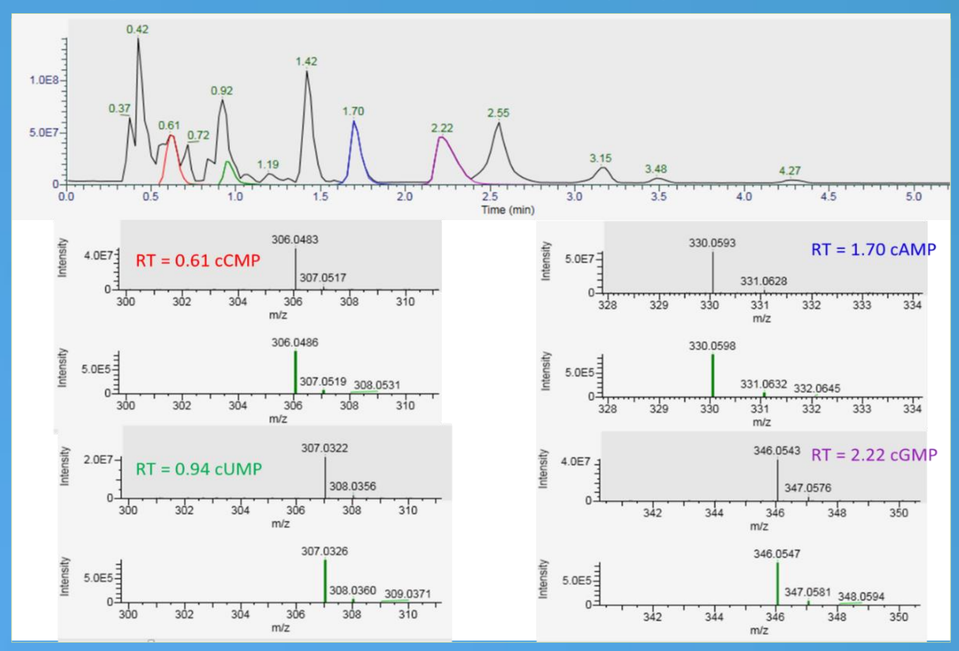

Figure 4: UHPLC chromatogram, high-resolution mass and isotopic clusters (black: experimental, green: calculated) of the [M+H]+ ions corresponding to the four cNMPs detected by ESI-QO MS.

compound	formula	detected mass	theoretical mass	delta (ppm)	Fit pattern	Combined score
cCMP	$C^{9}H^{13}N^{3}O^{7}P$	306.0483	306.04856	-0.93	100	96.97
cUMP	$C^{9}H^{12}N^{2}O^{8}P$	307.0322	307.03258	-1.24	100	95.89
cAMP	$C^{10}H^{13}N^{5}O^{6}P$	330.0593	330.05980	-1.42	100	98.18
cGMP	$C^{10}H^{13}N^{5}O^{7}P$	346.0543	346.05471	-1.21	100	97.50

Table1 High-resolution masses of $[M+H]^{+}$ ions of cNMPs.

CONCLUSIONS

In this study we focused our attention on AFA phosphorylated metabolites giving ^{31}P NMR signals at 20 ppm, a chemical shift that pointed to phosphonates. They instead revealed to be nucleoside 2',3'-cyclic monophosphates, that were characterized by multinuclear ^{1}H, ^{31}P and ^{13}C NMR spectroscopy and high-resolution mass spectrometry. Our data are fully consistent with the proposed structures and hence demonstrate, for the first time, the presence of cNMPs in AFA, and suggest AFA as potential source of cNMPs for future studies.

References

[1] D. Nuzzo, G. Presti, P. Picone, et al. Oxidative Medicine and Cellular Longevity. (2018) And references herein. [2] M. Gantar, Z. Svircev, Journal of Phycology 44 (2008) 260-268. [3] E. Christaki, P. Florou-Paneri, E. Bonos, International Journal of Food Sciences and Nutrition 62(8) (2011) 794-799. [4] A.S. Lyon-Colbert, S. Su, C. Cude, Toxin 10(7) (2018) E254. [5] V. Righi, F. Parenti, L. Schenetti, A. Mucci, Journal of Agricultural and Food Chemistry 64 (2016) 6708-6715.