Solution Stability of Adenine-based Cytokinins

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Introduction

Despite extensive research on cytokinins in plant development, signal transduction and metabolism, little information is available on the chemical stability of these compounds in solution or their autoclavability. For over 50 years it has been known that plants can adopt a range of morphogenetic states in response to relatively small changes in cytokinin concentration (when combined with auxins) [1]. Additionally, plant cell and tissue culture has been affected by variability and aberrant growth since its inception. Somaclonal variation [2] and epigenetics [3] have both been implicated for this variability in plant cell and tissue culture. It is however, unknown how the lack of knowledge on cytokinin stability could affect plant tissue culture variability with many different laboratory preparations and storage conditions. Therefore it is critical to understand the stability profiles of these compounds in solution for proper administration in plant tissue culture for research as well as commercial production.

This work describes the stability of three widely used adenine-based cytokinins: *trans*-zeatin, 2iP, and kinetin at storage temperatures of -20°C, 2-6°C, and 25°C in mild to strong alkaline solutions. We utilized HPLC and Mass Spectrometry to quantify and identify cytokinins and any degraded components. We also explored the stability of these cytokinins and additionally benzyladenine after one autoclave cycle.

Materials and Methods

The cytokinins used in each of these studies were dissolved in 0.01N – 0.5N KOH in triplicate. HPLC was performed isocratically for each of the compounds in 40-50% MeOH over a Kinetex™ 5µm C18 100 Å (Phenomenex) at 0.85 mL/min monitored at 270 nm. We isolated peaks from HPLC after accelerated degradation (40-75°C for 1 month) and analyzed them with MALDI-TOF MS (Perseptive Biosystems Voyager DE Pro) and ESI MS (Thermo Electron LTQ linear Ion trap) both in positive mode. We were able to quantify the cytokinin degradation based on the area of the parent compound's peak when it did not contain detectable amounts of degradant at known m/z. Samples that were autoclaved underwent 121°C at 1.1 bar for 30 min in a Sterilmatic STM-EL autoclave (Market Forge). The final concentration of the autoclaved samples was adjusted based on volume measurements of evaporation. Error bars are shown as one standard deviation, except in the case of data multiplied/divided with other data, in which the error bars were represented as root mean squares of the standard deviations.

Results and Discussion

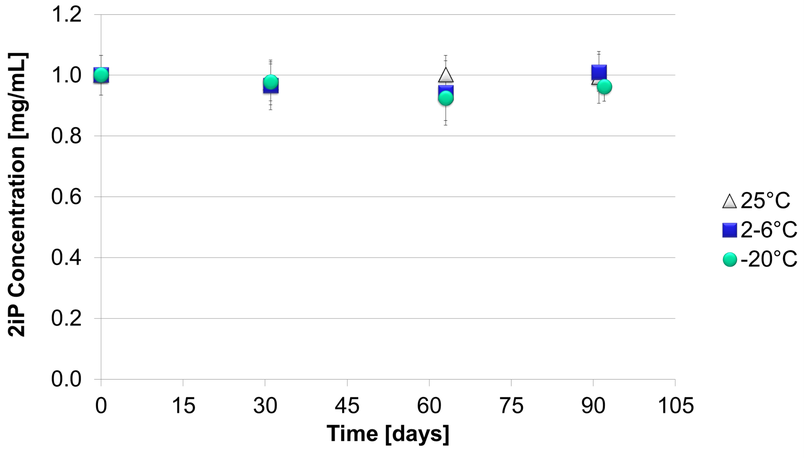

Figure 1. Stability profile of 2iP in 0.01N KOH

Results and Discussion (cont.)

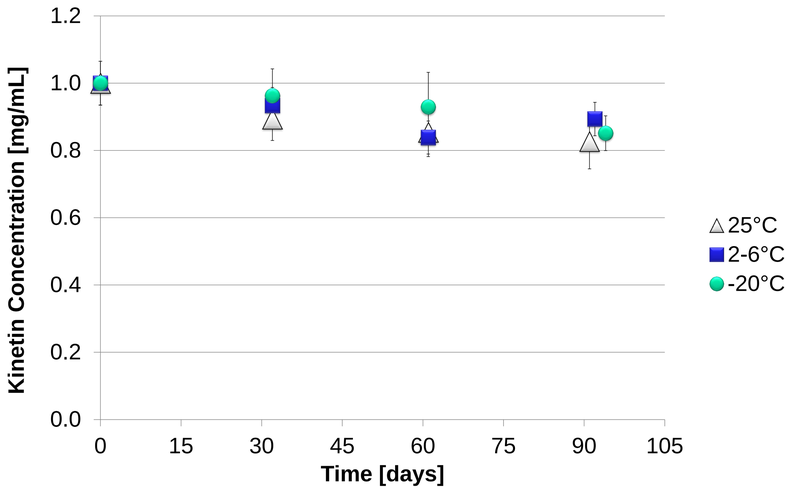

Figure 2. Stability profile of kinetin in 0.05N KOH

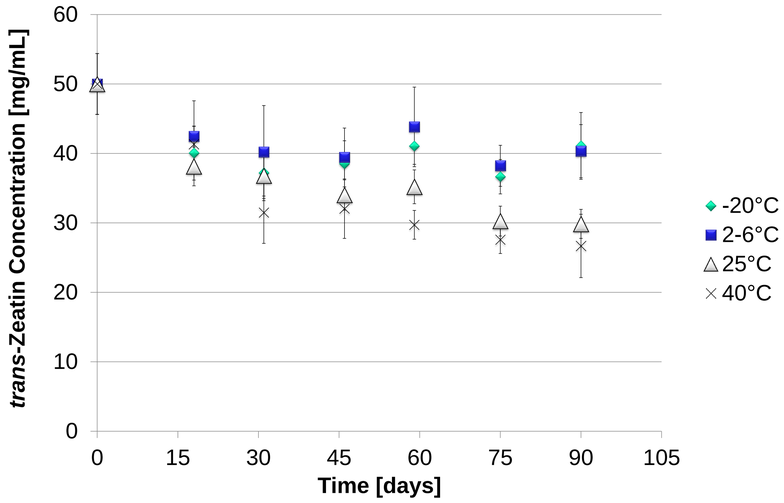

Figure 3. Stability profile of *trans*-zeatin in 0.5N KOH

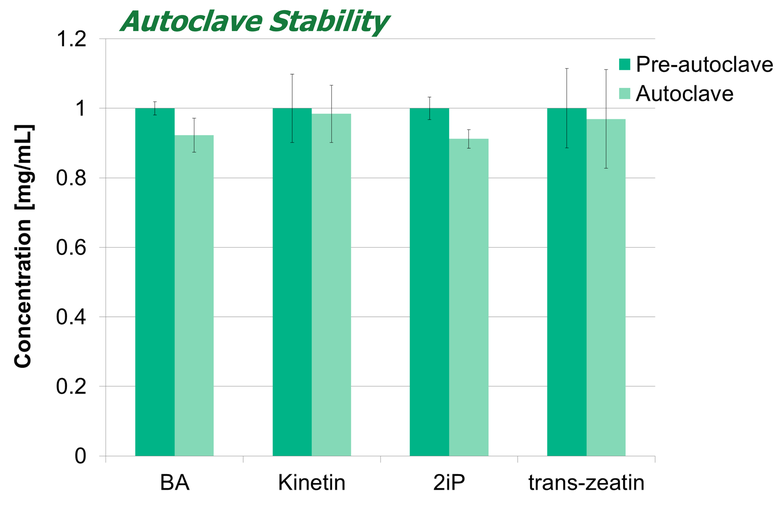

Figure 4. Stability of adenine-based cytokinins post autoclaving at 1.1 bar and 121°C for 30 min. Each cytokinin was dissolved in 0.01N KOH except for kinetin which requires 0.05N KOH for solubility. These concentrations were calculated to account for evaporation based on volume measurements.

- Figure 1 shows 2iP to retain greater than 90% of its mass at all temperatures studied over 90 days
- Figure 2 shows kinetin to retain greater than 80% of its mass at all temperatures studied over 90 days
- Figure 3 shows *trans*-zeatin to retain greater than 80% of its mass at relatively strong alkaline conditions (0.5N KOH) when the temperature was less than 2-6°C
- Figure 4 shows each of the four adenine-based cytokinins to lose non-statistically significant (t-test, 95% confidence) masses when autoclaved

Results and Discussion (cont.)

- *trans*-Zeatin lost 20% apparently due to degradation at all temperatures studied (Figure 3.) in the first 2 weeks.
- Figure 5 shows 0.5N KOH apparently affects the HPLC retention to C18 of a portion of the entire *trans*-zeatin population when compared to the same mass injected exposed to lower concentrations (0.01N KOH)
- We examined this further with milder alkaline conditions (Figure 6), however this 20% loss effect over 15 days is apparently independent of base concentration, but studies are on-going with more KOH concentrations/temperatures.

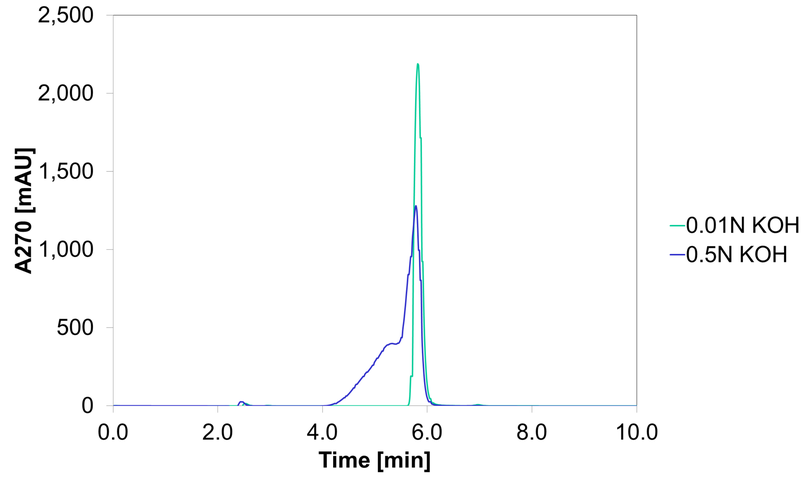

Figure 5. HPLC Chromatogram of trans-zeatin from 0.01N KOH and 0.5N KOH MALDI TOF MS did not detect any breakdown of *trans*-zeatin in this isolated peak from 3-6 min .

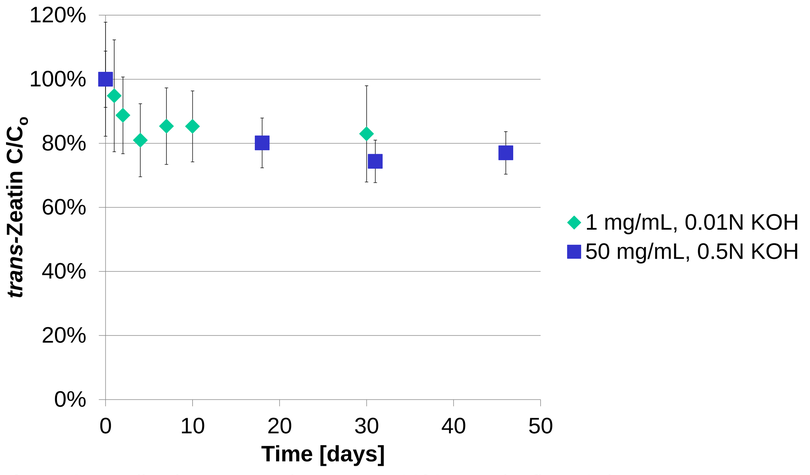

Figure 6. Decline in *trans*-zeatin mass at -20°C over the first 50 days

Summary

- The four adenine-based cytokinins studied here are stable to one autoclave cycle of 1.1 bar 121°C for 30 min with negligible loss apparently due to degradation
- 2iP is stable (>90%) for 3 months and kinetin is stable (80%) for 3 months at all temperatures studied.
- *trans*-Zeatin is stable (80%) for 3 months at -20°C and 2-6°C
- The effect of *trans*-zeatin losing 20% of its mass due to degradation over the temperatures studied here in the first 15 days is apparently base-concentration independent, but is still being investigated as base concentration does appear to affect the retention of *trans*-zeatin to C18

References

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[3] S.M. Kaeppler, R.L Phillips, In Vitro Cell Dev. Biol. Plant 29 (3), 125-130. 1993