Sucrose Metabolism in Mandevilla velutina Cell Cultures Analyzed by ^{1}H-NMR Spectroscopy

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Abstract: *Experiments were performed to compare the suitability of using peak-surface or peak-height ratio in ^{1}H-NMR spectra for analyzing sucrose (Suc) metabolism in Mandevilla velutina (line A) cell suspension cultures. The ^{1}H-NMR spectrum data analysis of the time-course experiment monitoring Suc content in the culture medium revealed a hyperbolic profile for the metabolization of that sugar in M. velutina cell cultures. By measuring peak area or peak height, similar results were obtained. However, the error found for sucrose quantitative analysis was significant as measured by integral area (SD = 20.86) relative to peak height ratio (SD = 0.63). Although chemical shifts accuracy may vary depending on factors such as pH and chemical constituents of the culture medium, which are variable along the time course of the cultures, it was feasible to monitor the presence of Suc and determine the kinetics of its metabolization in M. velutina cell culture by measuring ^{1}H-NMR spectra of the culture medium. Higher reliability is obtained when using peak height ratios instead of peak areas of signals of the compounds of interest.*

NMR spectroscopy has become one of the most powerful tools for either *in vitro* and *in vivo* plant metabolism studies. Usually, quantitative analysis in ^{1}H-NMR is carried out by integrating the peak(s) area of the signal(s) selected for the compound of interest, considering that the accuracy of the measured data might suffer signal overlapping. Signal suppression is used as a subterfuge to overcome this constraint, but it affects integral values as well. As an alternative, quantitative analyses of compounds have been made by measuring peak height for any compound relative to peak height of the internal standard.[1] Thus, experiments were performed to compare the suitability of using peak-surface or peak height ratio in ^{1}H-NMR spectra for analyzing sucrose (**Suc**) metabolism in *Mandevilla velutina* (line A) cell suspension cultures.

The initiation and the maintenance of the cultures were performed as previously described.[2] For the purpose of **Suc** analysis in culture medium, 15 mL of the culture medium were collected at 5th, 10th, 15th, 20th, 25th, and 30th day after inoculation, centrifuged and freeze-dried. Thus, 750 μL of D_2O was added to the lyophilized samples, mixed in a Vortex and centrifuged. Subsequently, 650 μL of the supernatant was transferred to a 5-mm NMR tube. Sucrose content was monitored by measuring 300 MHz ^{1}H-NMR (D_20, $TSPA_{d4}$ - 3 mg/10 mL as internal standard) spectra on a

Bruker WM-300 spectrometer, equipped with an Aspect 2000 data system, operating at 300 MHz hydrogen resonance frequency. 20 scans were recorded for each extract, using 16 K data points. Calibration was done with standard solutions of **Suc** (1 mg/mL, pH 5.4; Sigma, MO), measured under identical conditions. The quantitative analysis of **Suc** was performed by measuring peak heights ($\delta_{sucrose}$: 5.42 – H1; – H1 relative to the peak height of the internal standard, δ_{TSP}: 0.00) and also by calculating integral surface areas of the mentioned peak. Both integral and peak height ratio data were further analyzed by polynomial regression (3rd order – *Origin 5.0 version, statistical package*) to check for the reliability of these parameters. The ^{1}H-NMR spectrum data analysis of the time-course experiment monitoring sucrose content in the culture medium revealed a hyperbolic profile for the metabolization of that sugar in *M. velutina* cell cultures. By measuring peak area or peak height, similar results were obtained (Figure 1). Similar values were found for coefficient of determination (r^2) regardless of the parameter used; however the error found for sucrose quantitative analysis was significant as measured by integral area ($\delta_{sucrose}$: 5.42 – H1) relative to peak height ratio.

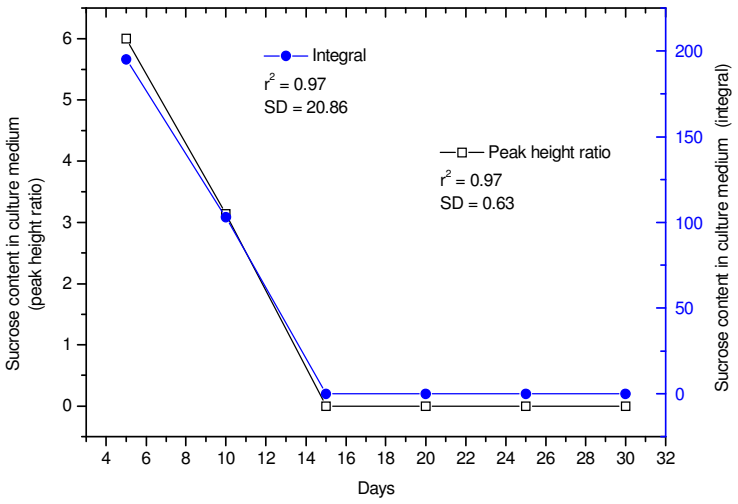

Figure 1. Sucrose uptake by *Mandevilla velutina* cell line *A* monitored by measured ^{1}H-NMR spectra in a time-course experiment (30 days). The curve shown in squares is referred to values obtained using peak height ratio as line curve in spots was calculated by using integral values. For details see Material and Methods.

Although chemical shifts accuracy may vary depending on factors such as pH and chemical constituents of the culture medium, which are variable along the time course of the cultures, it was feasible to monitor the presence of Suc, as well as to determine the kinetics of its metabolization in *M. velutina* cell culture by measuring ^{1}H-NMR spectra of the culture medium. Higher reliability is obtained when

using peak height ratios instead of peak areas of signals of the compounds of interest.

References

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