Supporting Information

Multiple sampling in single cell enzyme assays using CE-LIF to monitor reaction progress

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Abstract

The supporting information section includes an explanation of how α-glucosidase I and β-galactosidase activity was determined for single Arabidopsis embryos. This calculation was complicated due to the co-elution of reaction products from the two parallel enzyme assays.

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Calculating α-glucosidase I and β-galactosidase activity:

In order to confirm that *Arabidopsis knf* mutants lacking the KNOPF gene which codes for the enzyme α-glucosidase I, were viable and only lacking α-glucosidase I activity, a control β-galactosidase assay was performed in parallel. Calculating percent conversion for the two enzyme reactions was complicated due to the co-elution of the β-galactosidase reaction product GN-TMR and any MG-TMR that is not completely hydrolysed to the linker arm (TMR). To calculate percent conversion, first a relationship for TG-TMR and LN-TMR, the substrates for the two enzymatic reactions, was obtained:

\[ I \quad \frac{T}{L} = \frac{\frac{TG_{\text{TMR}} + MG_{\text{TMR}} + TMR}{LN_{\text{TMR}} + GN_{\text{TMR}}}}{\frac{TG_{\text{TMR}} + \Delta TG_{\text{TMR}}}{LN_{\text{TMR}} + \Delta LN_{\text{TMR}}}} \]

\( T_0 \) and \( L_0 \) represent the initial peak areas of the two substrates, TG-TMR and LN-TMR respectively. Solving for \( \Delta TG_{\text{TMR}} \) gives the following expression:

\[ II \quad \Delta TG_{\text{TMR}} = \left( \frac{T}{L_0} \right) \left( LN_{\text{TMR}} + \Delta LN_{\text{TMR}} \right) - TG_{\text{TMR}} \]

The height of the co-eluted peak \( S \), contains MG-TMR and GN-TMR and can be related to \( \Delta L_{\text{TMR}} \) and \( \Delta TG_{\text{TMR}} \) through the following relationship:

\[ III \quad S = MG_{\text{TMR}} + GN_{\text{TMR}} = \Delta LN_{\text{TMR}} - TMR \]

Substituting the values for \( \Delta T_{\text{TMR}} \) from equation II into equation III gives an expression for \( \Delta L_{\text{TMR}} \).

\[ IV \quad \Delta LN_{\text{TMR}} = \frac{S + TG_{\text{TMR}} + TMR - \left( \frac{T}{L_0} \right) LN_{\text{TMR}}}{\left( 1 + \frac{T}{L_0} \right)} \]
Now to get an expression for $\Delta T$-TMR, the above value of $\Delta L$-TMR is substituted into equation II.

$$V \quad \Delta T_{TMR} = \frac{S + TG_{TMR} + TMR + LN_{TMR} - TG_{TMR}}{\left(\frac{L_o}{T_o}\right) + 1}$$

Finally the values of percent conversion for the $\alpha$-glucosidase I and $\beta$-galactosidase reactions were obtained using equations IV and V.

$$VI \quad \%TG_{\text{Conversion}} = \frac{\Delta TG_{TMR}}{\Delta T_{TMR} + TG_{TMR}} \times 100 = 100 - \frac{100 \cdot TG_{TMR} \left(\frac{L_o}{T_o} + 1\right)}{S + TG_{TMR} + TMR + LN_{TMR}}$$

$$VII \quad \%LN_{\text{Conversion}} = \frac{\Delta LN_{TMR}}{\Delta LN_{TMR} + LN_{TMR}} \times 100 = 100 \left(\frac{S + TG_{TMR} + TMR - \left(\frac{T_o}{L_o} + 1\right)}{S + TG_{TMR} + TMR + LN_{TMR}}\right)$$