Enzymatic activity - Michaelis-Menten model

Aim

Characterize an enzyme by measuring the initial reaction rates for different substrate concentrations. With this data you can calculate the kinetic constants k_{cat} and K_M using the Michaelis–Menten equation.

Materials

ABTS™ buffer (11204530001 Roche)
ABTS ™ (10102946001 Roche)
Citric Acid – Na_2HPO_4 Buffer Solution (pH 4)
Laccase from T. versicolor (38429 Sigma)

Procedure

1. Prepare ABTS solution at concentration 1mg/mL by mixing ABTS in ABTS solution as recommended by the supplier.
2. Prepare laccase stock solution (10mg/ml) by mixing the laccase with citric acid – Na2HPO4 buffer.
3. Add the desired ABTS volume to the desired citric acid – Na2HPO4 buffer solution volume (total volume should be 995 µL if you add 5 µL enzyme in step 5) into a 1,5 mL polystyrene cuvette.
4. Measure it as the blank.
5. Add 5 µL of laccase from the stock (10mg/ml) to the ABTS – buffer solution.
6. Mix thoroughly by inverting it.
7. Measure at 420 nm for 1 minute right after mixing the content of the cuvette.
8. Calculate the slope (ΔAbs/min) of this continuous curve in the initial region (approximately the first 15 seconds if it looks linear).
9. Repeat steps 3-8 with different concentrations of substrate. We recommend doing at least duplicates

Notes

The reaction is done in 1,5 mL cuvettes with ABTS concentrations ranging from 0,01 mg/ml to 0,3 mg/ml. <We recommend to test some concentrations in a high, low and middle range and then choose concentrations to catch the K_M where the rate increases approximately linearly.

Convert the slopes from (ΔAbs/min) to (mM/min) by dividing by the extinction coefficient (the extinction coefficient of the oxidized product is 36 mM^{-1} cm^{-1}). Plot substrate concentrations as the x values and the reaction rates in mM/min as the y values. If it looks like a smooth saturation curve you can plot the data in a Hanes-Woolf plot and calculate the kinetic constants.

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References

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