Effect of pH and temperature on enzymatic activity

1. General setup of enzyme activity measurements

Aim

To find the conditions (pH and temperature) in which laccase activity is optimal.

Materials

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

Procedure

COMPONENT	CONCENTRATION
Buffer	100 mM
Laccase	≥0.5 U/mg
ABTS™	0,05 mg/ml

1. Add the ABTS to the citric acid – Na_2HPO_4 buffer solution.
2. Measure it as the blank.
3. Add 0.05 mg of laccase to the ABTS – buffer solution.
4. Mix thoroughly by inverting it.
5. Measure at 420 nm for 2 minutes right after mixing the content of the cuvette.
6. Calculate the slope (abs/min) of this continuous curve.
7. Repeat steps 2-6 at different pH and temperature conditions and compare.

Notes

The reaction is done in 1 mL cuvettes and thus the quantities of the different components are adjusted to that volume of reaction.

References

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M. Enriqueta, Arenas et al. (2003) “Kraft Pulp Biobleaching and Mediated Oxidation of a Nonphenolic Substrate by Laccase from Streptomyces cyaneus CECT 3335” Applied and environmental microbiology, Vol. 69, No. 4 p. 1953–1958.

Cantor, Charles R. and Schimmel, Paul R. (1980) "Biophysical Chemistry, Part I: The Conformation of Biological Macromolecules" Freeman, San Francisco.

Stryer, Lubert (1995) "Biochemistry" 4th Ed. W.H. Freeman and Company, New York.

van Holde, Kensal E.; Johnson, W. Curtis; and Ho, P. Shing. (2006) “Principles of Physical Biochemistry” 2nd Ed. Pearson, New Jersey.

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2. Kinetics assay

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 mL cuvettes with ABTS concentrations ranging from 10 µM to 500 µM. We recommend to perform all calculations for each concentration prior to starting the experiment.

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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.

References

Cantor, Charles R. and Schimmel, Paul R. (1980) "Biophysical Chemistry, Part I: The Conformation of Biological Macromolecules," Freeman, San Francisco.

Stryer, Lubert (1995) "Biochemistry," 4th Ed. W.H. Freeman and Company, New York.

van Holde, Kensal E.; Johnson, W. Curtis; and Ho, P. Shing. (2006) “Principles of Physical Biochemistry,” 2nd Ed. Pearson, New Jersey.