APPLICATION NOTE

UV/Visible Spectroscopy

Measurement of Tyrosinase Activity Using the LAMBDA 465 UV/Vis Spectrophotometer

Introduction

Enzymes are biological catalysts. Without their presence in a cell, most biochemical reactions would not proceed at the required rate. The physiological and biological properties of enzymes have been investigated since the early 1800s. The unrelenting interest in enzymes is due to several factors - their dynamic and essential role in the cell, their extraordinary catalytic power, and their selectivity. Two of these dynamic characteristics will be evaluated in this experiment, namely a kinetic description of enzyme activity and molecular selectivity.

This application note describes an enzyme activity determination using tyrosinase. Rapid data acquisition and processing are achieved using the LAMBDA™ 465 UV/Vis Spectrophotometer and the UV Lab™ software.

Principle

Tyrosinase, a copper-containing oxidoreductase, catalyzes the orthohydroxylation of monophenols and the aerobic oxidation of catechols. The enzyme activity will be assayed by monitoring the oxidation of 3, 4-dihydroxyphenylalanine (dopa) to the red-colored dopachrome.

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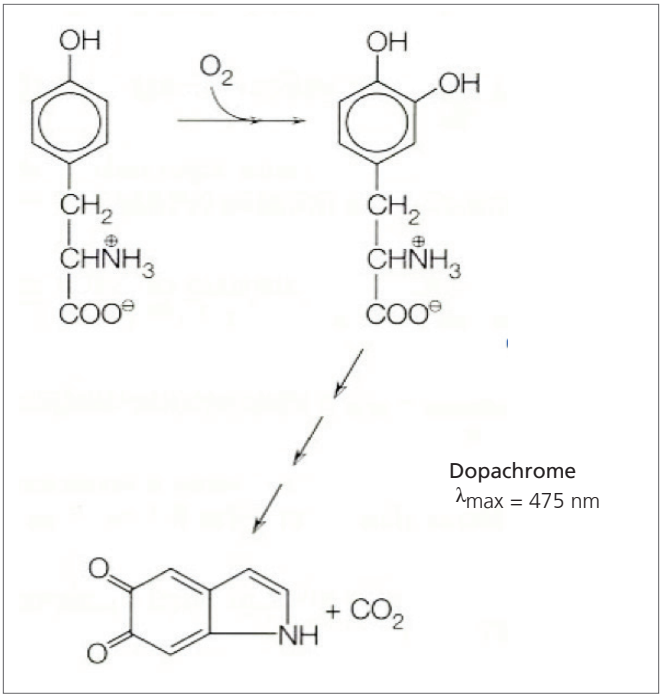

Figure 1. The oxidation of tyrosine and dopa as catalyzed by tyrosinase.

Properties of Tyrosinase:

Tyrosinase, also commonly called polyphenol oxidase, has two catalytic activities; o-hydroxylation of monophenols and aerobic oxidation of o-diphenols.

$$\text{monophenol} + O_2 \longrightarrow \text{catechol} + H_2O$$

$$2\ \text{catechol} + O_2 \longrightarrow 2\ o\text{-quinone} + 2\ H_2O$$

Mushroom tyrosinase is tetrameric, with a total molecular weight of 128,000. There are four atoms of Cu^{+} associated with the active enzyme. Two types of substrate binding sites exist in the enzyme, one type for the phenolic substrate and one type for the dioxygen binding site; hence, chemicals that form complexes with copper atoms are potent inhibitors of tyrosinase activity.

Units of Enzyme Activity:

The amount of a specific enzyme present in solution is most often expressed in units of activity. Three units are in common use, the *international unit* (IU), the *ketal,* and *specific activity*. The International Union of Biochemistry Commission on Enzymes has recommended the use of a standard unit, the international unit, or just unit, of enzyme activity. One IU of enzyme corresponds to the amount that catalyzes the transformation of 1 µmole of substrate to product per minute under specified conditions of pH, temperature, ionic strength, and substrate concentration.

$$\text{Units} = \frac{\mu\text{mol produced}}{\text{min}} = \frac{\Delta A}{\Delta T} * \frac{1}{\varepsilon\,(M^{-1}cm^{-1})} * 1(cm) * \frac{10^6\ \mu m}{M} * V_f(l) \quad \textbf{...(i)}$$

Where, ε = molar absorption coefficient ($M^{-1}cm^{-1}$)
v_f = final volume in the cuvette (l)
A = absorbance
t = time (min, sec etc)

Reagents and Apparatus

Sodium phosphate buffer , 0.1 M, pH 7.0

Tyrosinase from mushroom (Sigma), 5 units/uL in sodium phosphate buffer

L- 3,4-dihydroxyphenylalanine (L-dopa), 2 mg/mL in sodium phosphate buffer

LAMBDA 465 UV/Vis Spectrophotometer

UV Lab software

Cuvettes (10 mm pathlength)

Procedure

1. Prepare the 0.1 M sodium phosphate buffer.
2. Dissolve tyrosinase and L-dopa in 0.1 M sodium phosphate buffer.
3. Determine the amount of tyrosinase.

   3-1. Set up the reagent as follows:

Table 1. General procedure for the tyrosinase assay *.

Reagent	1	2	3	4	5
Phosphate buffer	1.995	1.990	1.980	1.960	1.940
L-Dopa	1	1	1	1	1
Tyrosinase	0.005	0.01	0.02	0.04	0.06

** units are ml*

   3-2. Read the absorbance at 475 nm

   3-3. The chosen enzyme levels should cover the ΔA/min range about 0.025/min to 0.25/min.

4. Calculate the tyrosinase concentration and enzyme activity factor.

   4-1. Measurement of tyrosinase concentration :Record the absorbance at 280 nm (Calculate the tyrosinase concentration as described in the Results section)

   4-2. Calculation of enzyme activity factor (see equation **(i)**)

   :ε = 3600 M^{-1} cm^{-1} (molar absorption coefficient for the product dopachrome)

   v_f = 0.003 liter (final volume in the cuvette)

   By substituting these values in equation **(i)**, we get

   Unit = $\Delta A/\Delta t$ x 0.83

   Thus, 0.83 is the tyrosinase activity factor

5. Instrument parameters are as follows:

Experiment Setup

Data type: Absorbance
Sampling: Single cell
Mode: Faster (Spectra No: 1, Scan No.:10, Integration No: 1, Gain No: 1)

Experiment Type: Enzyme Activity

(see Figure. 2)

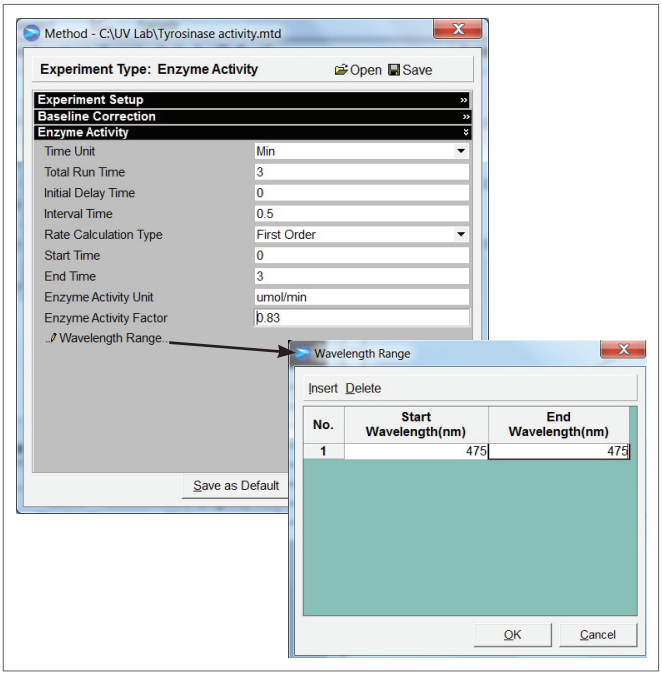

Figure 2. Experimental setup for measurement of tyrosinase activity.

6. Measure the A_{475} of the final sample solutions.

Results

1. Calculation of tyrosinase concentration

The absorption coefficient, $E_{280}^{1\%}$, for tyrosinase is 24.9. That is, the absorbance of the pure solution of 1% (w/v) tyrosinase in a 1 cm cell at 280 nm is 24.9. Beer's law states that

$$A = E\,l\,c$$

Therefore, the concentration of tyrosinase in solution is calculated from a simple ratio,

$$\frac{c_1}{A_1} = \frac{c_2}{A_2}$$

Where, c_1 = concentration of a standard solutio tyrosinase = 1%

A_1 = absorption of the standard solution of tyrosinase at 280 nm = 24.9

c_2 = concentration of the unknown solution of tyrosinase =X %(w/v)

A_2 = absorption of the unknown solution of tyrosinase at 280 nm = **0.045**

Thus, **X = 0.0018 %(w/v)**

Convert the concentration to mg of tyrosinase/mL and multiply the dilution factor(final volume/sample volume)

Tyrosinase conc. = 0.18 mg/mL

2. Determination of tyrosinase activity

(see Figure 3, Table 2, and Table 3)

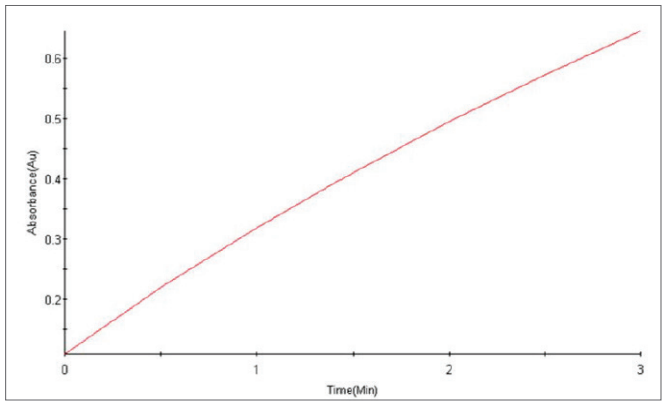

Figure 3. The change in A_{475} per minute.

Table 2. Absorbance values measured every 30 seconds.

Time (Min)	475.000 nm
0	0.1091
0.5	0.219
1	0.3186
1.5	0.4106
2	0.4956
2.5	0.5724
3	0.6451

Table 3. Cholesterol level in human serum.

Name	Start (Min)	End (Min)	Rate (Au/Min)	Activity Factor	Activity (µmol/min)
Tyrosinase	0	3	0.1780	0.8300	0.1477

3. Specific activity

The specific activity of tyrosinase is 82 units/mg.

Conclusion

Measurement of tyrosinase activity was performed using the LAMBDA 465 UV/Vis Spectrophotometer and UV Lab software. Rapid acquirement of spectra and good sensitivity were obtained and the software was used to quantify and to process the data efficiently.

References

1. Maniatis, F.L., Firitsch, E.F., Sambrook, J., Molecular Cloning : A Laboratory Manual, Cold Spring Harbor Press, New York, 1982.
2. Rodney Boyer, Modern Experimental Biochemistry, 3rd ed.: Benjamin/Cummings, 2000.

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