Protocol

Horseradish Peroxidase Assay

Introduction

Horseradish peroxidase (HRP) is an enzyme that when in the presence of hydrogen peroxide, oxidizes a substrate (oxygen donor), resulting in a color change that is quantifiable. There are about 40 HRP isoenzymes. However, the most commonly used is HRP-C, which consists of 308 amino acids and a single protoporphyrin IX prosthetic group, two calcium ions, and four disulfide bridges. During catalysis, a single two-electron transfer occurs, forming an enzyme intermediate, which is then reduced by two single-electron transfers from substrate molecules. These characteristics have been exploited and used in many fields including biochemistry, molecular biology, and neuroscience. This protocol describes the measurement of the rate of peroxidase activity using 4-aminoantipyrine (Am-NH2) as a hydrogen donor and the corresponding increase in absorbance at 510 nm, resulting from the decomposition of hydrogen peroxide catalyzed by horseradish peroxidase. This allows for a quantitative speed to be measured and compared to the oxidation of various other substrates, allowing for an efficient and affordable method of determining reaction rates and successful oxidation.

Materials

- Horseradish Peroxidase (GoldBio Catalog # P-100)
- 0.2M Potassium phosphate buffer, pH 7.0
- 30% Hydrogen Peroxide
- Phenol
- 4-aminoantipyrine
- Molecular biology grade water

Preparation of 0.0017M Hydrogen Peroxide

- Add 1 ml of 30% hydrogen peroxide to 100 ml molecular biology grade water.
- Further dilute 1 ml of the solution to 50 ml with 0.2M potassium phosphate buffer.

Preparation of 0.0025M 4-Aminoantipyrine

- Dissolve 810 mg phenol in 40 ml molecular biology grade water.
- Add 25 mg of 4-aminoantipyrine and dilute to a final volume of 50 ml with molecular biology grade water.

Preparation of Horseradish Peroxidase

- Dissolve 1 mg of Horseradish Peroxidase in 1 ml of molecular biology grade water.
- Immediately prior to use, dilute further to obtain a rate of 0.02-0.04 ΔA/min.

Storage and Handling

- This product should be stored desiccated at -20°C. Protect from light.
- Working solution should be prepared fresh for every use.

Method

1. Set spectrophotometer to 510 nm and 25°C.

2. Pipette into each cuvette as follows
   a. Phenol/aminoantipyrine solution 1.4 ml
   b. 0.0017M Hydrogen peroxide 1.5 ml

3. Incubate in a spectrophotometer at 25°C for 3-4 minutes to achieve temperature equilibration and establish a blank rate. Add 0.1 ml of diluted enzyme and record the increase in A_{510} for 4-5 minutes. Calculate ΔA_{510}/minute from liner portion of the curve.

Calculations

Decomposition of hydrogen peroxide in µM per minute (at 25°C), referred to in Units.

$$\frac{Units}{mg} = \frac{\frac{\Delta A_{510}}{minute}}{6.58 \times \frac{mg\ enzyme}{ml\ reaction\ mixture}}$$

Associated Products

- Horseradish Peroxidase (GoldBio Catalog # P-100)

References

Mogharrab, N., Ghourchian, H. and Amininasab, M. (2007). Structural Stabilization and Functional Improvement of Horseradish Peroxidase upon Modification of Accessible Lysines: Experiments and Simulation. *Biophysical Journal,* 92(4), 1192-1203. Doi:10.1529/biophysj.106.092858.

Morawski, B., Quan, S. and Arnold, F. H. (2001). Functional expression and stabilization of horseradish peroxidase by directed evolution in *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering,* 76(2), 99-107. Doi:10.1002/bit.1149.

Morishima, I., Kurono, M. and Shiro, Y. (1986). Presence of endogenous calcium ion in horseradish peroxidase. *The Journal of Biological Chemistry,* 261(20), 9391-9399.