Determination of acid phosphate activity from potato extract

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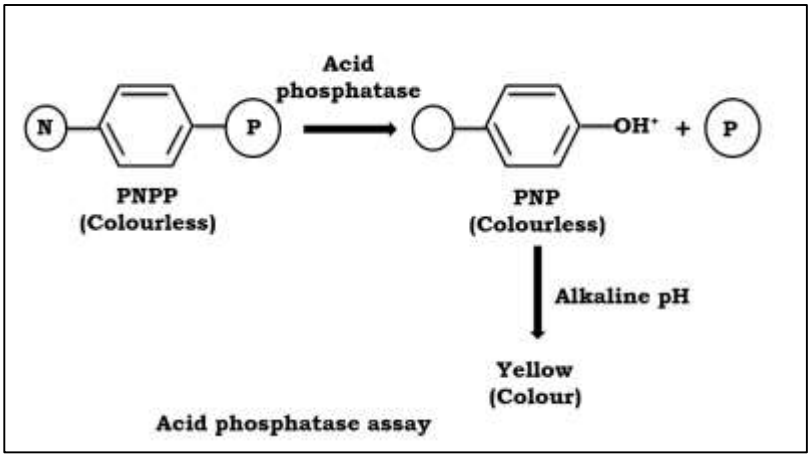
Background

The presence of an enzyme is generally recognized by the occurrence of the chemical reaction that it catalyzes, and the amount of enzyme present may be determined by measuring the rate of this reaction. Phosphatases are a family of enzyme belonging to the hydrolases class. Phosphatases catalyze removal of a phosphate group from different substrates. Within a living cell, acid phosphatases are lysosome localized enzymes, and are activated when the lysosome fuses with an endosome. The optimal pH for acid phosphatase activity is usually in the acidic range.

For acid phosphatase assay, a potato is an easily available source of the enzyme. The enzymes of a potato are fairly stable *in vivo*, during long-term storage of the potato. Para-nitro-phenyl-phosphate (PNPP) is used as a non-proteinaceous substrate for the acid phosphatase assay. During the enzyme catalyzed reaction, the PNPP loses phosphate and is converted into p-Nitrophenol (PNP). The p-Nitrophenol itself is colourless in the acidic pH. But in the alkaline pH, its chromogenic phenolic salt is formed having a bright yellow colour. Using a colorimeter, the intensity of yellow color



can be measured at 410 nm wavelength where p-nitrophenol has an absorption maximum in its spectrum.

[Teachers in the school may plan a connecting experiment for the students to make them learn how to determine absorption maxima for different compounds such as copper sulphate, methylene blue and potassium per magnate, etc. By plotting the graph of absorbance against wavelength, students can find out the wavelength at which a particular compound absorbs maximum light].

The molar extinction coefficient is frequently used in spectroscopy to convert the absorbance units onto concentration units and it, thus, measures the concentration of a chemical (substrate or product) in the solution. The millimolar extinction coefficient of p-nitrophenol is 18.1 mM^{-1} cm^{-1}, which can be used to directly quantify the product formed during the reaction. From there, one can also quantify the unit activity of an enzyme.

[The international Union of Biochemistry defines **a standard unit of enzyme activity** as that amount which will catalyze the transformation of 1 micromole of the substrate into product, per minute under standard conditions. To quantify an enzyme, one can thus either measure the rate of decrease in the substrate or the rate of increase in the product. This definition can also be asked as a question to the students and hence it is up to the teacher to decide.]

The aim of the present experiment is to determine the unit activity of acid phosphatase enzyme from the potato extract. It can be represented as units (U), where, 1U of acid phosphatase = the amount of enzyme required to form 1 micromole (µ mole) of the P-Nitrophenol per min. (µ mole min^{-1}).

Requirements

1) 10% potato extract supplied in a 15 ml tube

[*Potato is pealed, cut in to pieces, weighed and then is homogenized with minimum amount of distilled water. The homogenate is filtered through 4 layers of cheese cloth. The filtrate is diluted with distilled water to make 10% potato extract and stored in ice until use*].

2) Test tubes (5)
3) 15 mM PNPP in a 5 ml tube
4) 0.1 M citrate pH # 5 in a 5 ml tube

   [*16.968 g of sodium citrate dehydrate and 8.127 g of citric acid is dissolved in 900 ml distilled water, and the pH is adjusted to 5 by adding HCl/ NaCl and the final volume is adjusted to 1 L with distilled water.*]
5) 0.2 N NaOH in a 50 ml tube
6) Distilled water in a beaker
7) Test tube stand (1)
8) 1 ml Insulin syringe (3)
9) 5 ml syringe (1)
10) Colorimeter with cuvettes
11) Wash bottle
12) Tissue paper
13) Water bath

Procedure

1. Label the five test tubes supplied as 1, 2, 3, 4 and 5 and place them in a test tube stand.
2. To the labelled test tubes add water, citrate buffer, potato extract and the PNPP substrate as indicated in the table A.

   [**Precaution:** Use a different syringe for each reagent. Add water first. You can use the same syringe for adding the second component. While adding the components, try to inject from the top of the tube, without touching the solution.]

3. Place the test tubes containing the reaction mix in the 37° C water bath and incubate for 30 min.
4. Add NaOH solution as indicated in the "table A" and keep the tubes in room temperature for 15 min.
5. Now switch on the colorimeter and adjust the filter to 410 nm.
6. Pour the content of the tube number 1 (BLANK) to the glass cuvette of the colorimeter.
7. Wipe the external surface of the cuvette with tissue paper and place the cuvette in the slot of the colorimeter by aligning the marks on the cuvette and colorimeter.
8. Note the reading. Now rotate the "ZERO" knob of the colorimeter so that the reading is adjusted to zero.
9. Take out the cuvette and pour the solution back to tube 1.
10. Wash the cuvette with water and place it inverted on a tissue towel to remove extra fluid.
11. Now pour the solution of tube 2 into the cuvette, wipe as above and place in the slot of the colorimeter.
12. Record the reading and write in the space provided in table A.
13. Repeat step 10 to 13 for all the remaining test tubes.

Table A

SN	Citrate buffer (ml)	PNPP substrate (ml)	Potato extract (enzyme source) (ml)	Distilled water (ml)		NaOH (ml)	O. D. (A_{410})
1	0.5	0.4	0	0.6	Incubate at 37° C for 30 min	2.5	
2	0.5	0.4	0.1	0.5		2.5	

3	0.5	0.4	0.2	0.4		2.5	
4	0.5	0.4	0.4	0.2		2.5	
5	0.5	0.4	0.6	0		2.5	

1. Plot a graph by taking the volumes of enzyme on the X axis and OD (A_{410}) on the Y axis. Indicate the linear range of the graph.

2. Calculate the amount of substrate PNPP added into the reaction. [In the reaction you have used a PNPP stock of concentration 15 mM]

3. Calculate the enzyme activity of the acid phosphate (in tube 3) by using the following formula:

$$\text{Enzyme Units/ml extract} = \frac{(A_{410nm}\ \text{Test} - A_{410nm}\ \text{Blank})\ (4.0)\ \ (df)}{(18.1)\ \ (30)\ \ (0.5)}$$

where

A_{410nm} Blank = 0 as it was adjusted ZERO

4.0 = Total volume of assay
df = Dilution factor =1
30 = Conversion factor for 30 minutes to 1 minute
18.1 = Millimolar extinction coefficient of p-Nitrophenol at 410 nm
0.5 = Volume (in millilitre) of enzyme source (potato extract) used

4. Give one line justification for the reason why you divided it by 18.1.
