Candidate 3 evidence

Enzyme Inhibition

Aim

To investigate the effect of end product phosphate on the enzyme phosphatase

Underlying Biology

The active site of an enzyme is a flexible structure and when a substrate molecule enters the active site, the enzyme molecule and the enzymes active site changes so that it fits very closely around the substrate molecule. This is called induced fit. Induced fit makes sure that the active site comes extremely close in contact with the substrate molecules which increases the chance that a reaction will take place.

The molecules of a competitive inhibitor compete with substrate molecules for the active site of an enzyme as the molecular structure of the inhibitor is very similar to the substrates molecular structure. The competitive inhibitor is able to attach itself easily to the active site because of the similarities and blocks the substrate molecules from the active site. Therefore, the rate of reaction decreases if a competitive inhibitor blocks the active site as the substrate molecules cannot bind with the active site.

Non-competitive inhibitors do not bind directly to the active site of an enzyme, instead they bind to a non-active site known as an allosteric site. When a non-competitive inhibitor attaches to the allosteric site it changes the shape of the enzyme and results in the substrate being unable to bind to the active site. The larger the number of enzyme molecules affected by non-competitive inhibitors, the slower the rate of reaction will be.

Feedback inhibition is another way in which a metabolic pathway can be regulated as the end product in a metabolic pathway binds to an enzyme in at the start of the metabolic pathway. Feedback inhibition stops the metabolic pathway and the higher the concentration of the end product then the quicker the metabolic pathway will stop.

Brief Description

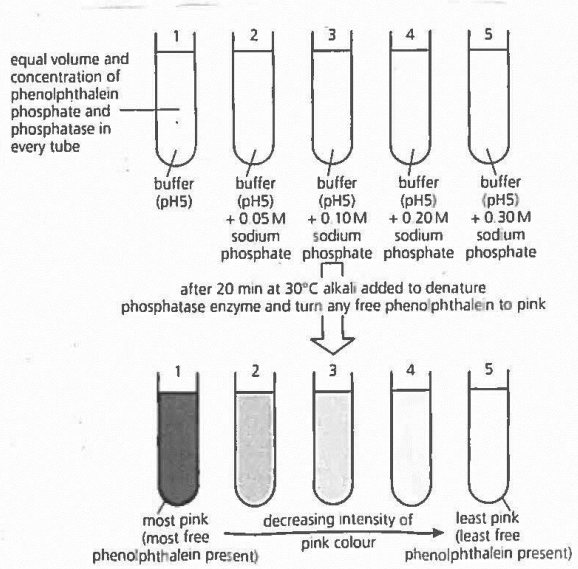
The enzyme was obtained from grinding up beansprouts and being placed in a centrifuge. 5cm^3 of different molarities of sodium phosphate were added to 5 different test tubes using a syringe and 1cm^3 of the substrate, phenolphthalein phosphate was added to all 5 test tubes. 1cm^3 of the enzyme solution was added to each test tube and all 5 test tubes were then incubated in a water bath at 30°C for 20

minutes. After the 20 minutes 5cm³ of sodium carbonate was added to each tube and mixed. The intensity of the pink colour in each test tube was measured using a colorimeter.

Test Tube (number)	Molarity of Sodium Phosphate (M)	Absorbance from experiment 1	Absorbance from experiment 2	Average absorbance
1	0	0.63	0.6	0.62
2	0.1	0.36	0.37	0.36
3	0.2	0.33	0.25	0.29
4	0.3	0.25	0.2	0.23
5	0.4	0.13	0.15	0.14

Literature Source

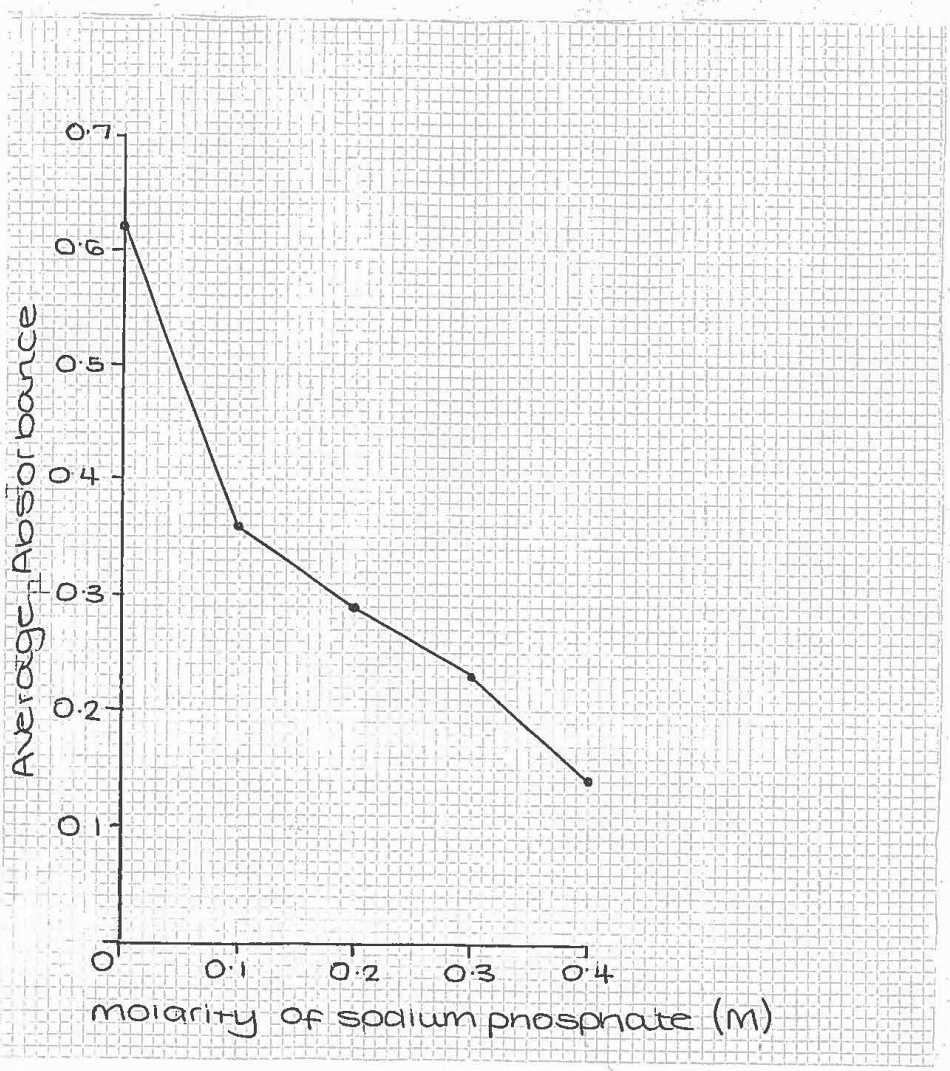
I chose this data as it is from an experiment that is the same as the experiment that I carried out. The results of this data only show the pink colours as the absorbency was not measured.



(1)

Figure 6.32 Investigating the effect of phosphate on phosphatase

Graphical Representation



Analysis

I have analysed my experiment by calculating the percentage decrease between my average results when the molarity of sodium phosphate was changed from 0 (M) to 4 (M).

0 (M) = 0.63 + 0.6 = 1.23
4 (M) = 0.13 + 0.15 = 0.28

Difference = 1.23 - 0.28 = 0.95.

$$\text{Percentage decrease} = \frac{\text{difference}}{\text{original}} \times 100$$

$$= \frac{0.95}{1.23} \times 100$$

$$= 77.2357$$

$$= 77.24\% \text{ (to 2 d.p)}$$

There was a 77.24% decrease in average absorbency when the molarity of sodium phosphate increased from 0 (M) to 4 (M). This suggests that when you increase the molarity of the end product it has an effect on the phosphatase activity.

Conclusion

In conclusion, my experiment and the literature source I have used both show that when you increase the molarity of sodium phosphate the absorbency decreases as the pink colour becomes less prominent. End product phosphate effects the enzyme phosphatase by slowing down the rate of reaction as the product acts as an inhibitor for the metabolic pathway.

Evaluation

The results gathered from the experiment I carried out are reliable as it was repeated so an average result could be taken from it. The results from my experiment are valid

as a control was used that had a molarity of 0 as it can be used as a comparison to other molarities. Throughout our experiment we used syringes instead of droppers to make sure that the measurements could be as accurate as possible to avoid inaccurate results. When incubating our test tubes we used a water bath to make sure that the temperature was kept the same at all times because the solutions used are controlled by enzymes and different temperature could impact how well they work. The reliability of the literature source used could have been improved by measuring the absorbance and also repeating to get a result for the average absorbency.

Reference

(1) – Higher Human Biology for CFE, James Torrance, page 95,
IBSN 978 1444 182 132
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