Effect of temperature on the digestive enzyme pepsin biology essay

In this study an experiment was carried out to determine if varied temperatures affect the rate at which enzymes function. Enzymes are biological catalysts; catalysts are substances that increase the rate of chemical reactions without being used up (BBC, 2010), without these catalysts it would take an extremely long time for these reactions to take place. The enzyme used in this particular experiment was pepsin; pepsin is a zymogen of pepsinogen. Pepsinogen is activated by hydrochloric acid, which is released from parietal cells in the stomach lining. The hormone gastrin and the vagus nerve trigger the release of both pepsinogen and hydrochloric acid from the stomach lining when food is ingested. Hydrochloric acid creates an acidic environment, which allows pepsinogen to unfold and cleave itself in an autocatalytic fashion, generating pepsin. (Life Science Network, 2010)

A lot can be learnt about enzymes by studying the rate of enzyme catalyzed reactions, these rates of reaction can be studied in various ways. In this experiment, using a range of different temperatures, the enzyme pepsin will be mixed with egg albumen. This is high in protein and bound to the dye Coomassie blue to gain a light absorbance reading using a spectrophotometer and in effect see how much protein has been digested by the pepsin.

Egg albumen was used as the protein source in this study as although it is composed mainly of around 80% water it has about 15% of its total mass made up from approximately 40 different types of proteins, mainly Ovalbumin (54%) (Edin Formatics, 1999).

A spectrophotometer is a device used for measuring light intensity and will be used to determine the amount of protein in each mixture, it works by measuring the light intensity as a function of the colour or more specifically the wavelength of light (Global Water Instrumentation Inc, 2007) (Appendix 2). Therefore the lower the reading means less light has been absorbed by the solution being tested indicating in this case that more protein (egg albumen) has been digested by the enzyme (pepsin) and the lower the reading the faster the enzyme reaction rate.

If enzyme reactions are affected by temperature, then changes in temperature may bring about different absorbances of light readings related to how much protein has been broken down by the enzyme. As pepsin is found in the stomach it would seem only logical to assume the optimum temperature for this particular enzyme would be around 37°c, human body temperature.

Method

A cuvette was filled with 0. 5ml of distilled water and placed in a spectrophotometer, then the machine was calibrated by pressing the zero button with the spectrophotometer set to a wavelength of 595nm.

After calibration 0. 5ml of egg albumen (2mg. cm^{-3}) was added to a test tube using a glass pipette and incubated in a pre heated water bath at a temperature of 10°c (then 20°c, 30°c, 40°c, 50°c, 60°c, 70°c) for five minutes.

0. 5ml of pepsin (0. 1%) was then added to the test tube and incubated at the same temperature for a further ten minutes.

After this incubation period 2. 0ml of Coomassie blue reagent was added to the test tube and immediately mixed by capping the test tube with parafilm and inverting several times.

After the test tubes contents had been mixed they were carefully poured into a cuvette and placed into the spectrophotometer with the light absorbance levels being recorded at a wavelength of 595nm.

The experiment was carried out three times at each temperature to achieve reliable data.

Results

The results documented in Table 1 are the light absorbance reading averages of three separate experiments carried out at each temperature. A full set of results can be seen in Appendix 1. Figure 1 shows the averaged results plotted on a scatter graph.

Table 1

Temperature (Degrees Celsius)

Average light absorbance at 595nm

10

2. 501

20

2. 550

30

2. 516

40

2. 403

50

2. 543

60

2. 740

70

2. 806

Fig 1 Change absorbance

Discussion

The results in Table 1 as well as the bell curve graph (Fig 1) show that the optimum temperature as predicted in the hypothesis seems to be 40°c, close to human body temperature. These results also show that temperature has a definite effect on the rate the enzyme reacts to breakdown the protein in the egg albumen.

Table 1 shows that at 10°c, 20°c and 30°c the light absorbed by the solution is more than at 40°c, this is because the pepsin has not broken down as much of the egg albumen at lower temperatures as it has at 40°c so the spectrophotometer is picking up more undigested protein particles in these

readouts. The same applies to the temperatures above 40°c and indicates 40°c is the best temperature range for pepsin to be active.

Enzymes are made up of amino acids; amino acids are the basic building blocks of proteins consisting of a basic amino group, a carboxyl group, a hydrogen atom and an organic side group attached to the carbon atom (Biology Online, 2010). When an enzyme is formed it is made by stringing together between 100 to 1, 000 amino acids in a specific and unique order defining the three dimensional shape of the enzyme and its particular chemical reactivity (Brain, M. 2000).

The lock and key theory explains how an enzyme may work, it utilizes the concept of an active site on the enzyme. The theory is that a particular part of the enzymes surface has a strong affinity to the substrate (protein). The substrate is held in such a way that its conversion to the reaction products is more favorable. If you consider the enzyme is the lock and the substrate is the key, the key is inserted in the lock and turns it to open the door letting the reaction proceed (Worthington Biochemical Corporation, 2010) (Appendix 3). However, the induced fit theory expands on the rigid lock and key theory. This updated view of enzymology proposes that the substrate causes a conformational change in the enzyme so the active site achieves the exact configuration for a reaction to occur, the overall effect being a tighter binding between the enzyme and substrate (Allaby, M. 1999) (Appendix 4). The benefit of this tighter binding would be a faster reaction rate as more surface area of the enzyme would be in contact with the substrate.

The kinetic collision theory describes temperature affects on a system as the amount of kinetic energy it has, a lower temperature will provide less kinetic energy than a higher temperature. When molecules collide the kinetic energy can be converted into chemical potential energy, if the chemical potential is great enough the activation energy or energy required for an enzyme to work can be reached. The more chemical potential energy molecules have when they collide, a greater number of molecules per unit time will reach the activation energy needed to bind the enzymes active site to the protein resulting in a quicker rate of reaction. If the temperature gets too high some of the weak bonds that determine the shape of a protein and its active site could be broken resulting in the enzyme becoming denatured and decreasing the rate of reaction sometimes rendering the enzyme inactive (Brooklyn College, 2010). Figure 1 shows that after 50°c the enzyme reaction rate slows down considerably, the enzyme is denaturising at a faster rate than it is below 30°c. This change in enzyme reaction rate may be due to the fact that pepsins are stored at low temperatures to prevent the enzyme destroying itself, therefore pepsin is less active at lower temperatures until it reaches its activation energy around 30°c and anything beyond around 50°c – 55°c will rapidly denature the pepsin so the molecules in the active site can no longer bind to the protein and produce a reaction, rendering the enzyme inactive permanently.

Once the pepsin has digested the egg albumen it would still be difficult to analyse the amount of protein left at each temperature, this is why Coomassie was added before taking a reading. In an acidic environment the protein will bind to Coomassie causing a spectral shift from a reddish/brown

colour with a low absorbance maximum of 465nm to a light blue colour with a higher maximum absorbance of 610nm with the difference of the two colours greatest at 595nm, an optimal wavelength (Thermo Fisher Scientific, 2010). The binding of the Coomassie takes place when the red form donates its free electron to the ionisable groups on the protein causing a disruption of the proteins normal state and revealing its hydrophobic pockets. These pockets, via Van der Waals forces (attractive and repulsive forces between molecules) bind to the non polar region of the dye, putting the positive amine groups close to the negative charge of the dye, creating a strong bond. Binding of the protein stabilizes the blue form of Coomassie dye, thus the amount of complex present in solution is a measure for the protein concentration by use of an absorbance reading. (Bradford M, 1976, P248-254)

Although this experiment has produced reliable accurate data that has proven the hypothesis right, many things could affect the results and readings obtained. When using such an accurate way of recording the data such as a spectrophotometer a number of things could affect the reliability of the results. Things like minor differences in volumes of substances added to the test tubes or inattentive timing methods could be damaging to the results obtained. Simple human error could possibly influence any results with any fingerprints or water on the cuvette affecting the absorbance readings.

Conclusion

In conclusion, the study carried out was adequate for the data required and indicated that temperature definitely affects the rate at which an enzyme

reacts. As long as the method is executed well this is a great experiment to look at temperature and its effect on enzyme activity however as with any scientific study human input is a crucial factor and could affect the quality of results. Another experiment may need to be carried out to determine what the optimum temperature is on a more specific scale, something closer to body temperature would help to discover a more precise optimum temperature, 35°c – 40°c for example. As well as finding out an exact optimum temperature a further study to find out the optimum pH of pepsin could be done to further enhance the enzymes rate of reaction, focused around the acidic pH in the human stomach.

Appendices

Appendix 1

Temperature (°c)

Absorbance at 595nm (Reading 1)

Absorbance at 595nm (Reading 2)

Absorbance at 595nm (Reading 3)

10

2. 430

2. 550

2. 520

20

2. 480

2. 530

2. 640

30

2. 500

2. 510

2. 540

40

2. 360

2. 400

2. 450

50

2. 520

2. 560

2. 550

60

2. 660

2. 780

2. 780

70

2. 800

2. 820

2. 800

Appendix 2

http://commons. wikimedia. org/wiki/File: Spetrophotometer-en. svg

Appendix 3

http://www. chemistry. wustl.

edu/~edudev/LabTutorials/Carboxypeptidase/images/lockkey. jpg

Appendix 4

http://wpcontent. answers.

com/wikipedia/commons/thumb/2/24/Induced_fit_diagram. svg/450px-

Induced_fit_diagram. svg. png