Investigating the effect of temperature on the rate of an enzyme-catalysed reaction

Proteases are enzymes that digest protein in the alimentary canal. Environmental factors such as temperature and pH can alter the shape of the active site of the protease, affecting the likelihood of an enzyme-substrate complex forming.

The rate of digestion of a protein called casein, which gives milk its white colour, will be used to investigate the effect of temperature on an enzyme-controlled reaction.

A range of water baths are prepared for temperatures from 5 to 75 degrees Celcius. Two sets of test tubes are prepared. The first set is labelled with the temperature and a large cross. These tubes will contain the milk and will be referred to as the 'substrate tubes.' The other set are labelled only with the temperature, and are referred to as the enzyme tubes. Two further test tubes are prepared in this way for the control.

Before the protease is used, it is checked to make sure the pH is approximately 9. Protease is added to each enzyme tube.

To prepare the control, the enzyme tube labelled 'C' is placed into a beaker of boiling water for at least 5 minutes to denature the enzyme.

Milk is added to each 'substrate tube'. The pairs of test tubes are put in their respective water baths in order to reach the required temperature.

After 10 minutes, one pair of test tubes is removed from their water bath. The contents of the enzyme tube are carefully poured into the substrate tube. A bung is fixed to the top of the substrate tube, and the tube is inverted once to thoroughly mix the contents. The test tube is then placed with the marked cross hidden from view. The timer is started. The test tube is watched closely for the milk to decolourise. The timer is stopped when the cross becomes clearly visible through the solution.

This process is repeated for each pair of test tubes. The enzyme and substrate are kept in the appropriate water baths until the point immediately before they are mixed.

The method is repeated another two times for each temperature so that a mean can be calculated. The mean rate of reaction for each sample can be calculated by dividing 1000 by the mean time taken in seconds, this gives a quantitative value to compare the rate of each reaction. The larger the value, the quicker the reaction.

A suitable graph of the mean results can be used to make conclusions about the observed relationship between enzyme activity and temperature.

Understanding the relationship between environmental factors, such as temperature, and enzyme activity is important for optimising industrial and pharmaceutical processes that rely on enzymes.

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