

Determining which temperature is more efficient to breakdown protease enzyme, a l...

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INTRODUCTION

There are various type of laundry detergent. Most older types of laundry detergent would use very strong soaps to wash away dirt, many of the more modern brands of detergent are trying ways that are more “ green” for getting rid of stains. These detergents have fewer harsh chemicals and are easier on skin and the environment.¹ These types of detergents generally use differing types of enzymes to break down stain molecules into a form that is easily rinsed away. The reason that this is a more “ green” way is because the enzymes used degrade much more quickly than soaps and therefore stay in our water systems for far less time.² In this set of experiments we have taken protease enzyme and used varying tests to determine under what temperature this enzyme operates most efficiently.

HYPOTHESIS

We hypothesised that the rate of enzymatic breakdown would change in response to change in the temperature of the protein.

MATERIALS & METHODS

We used three groups of six test tubes. Each group had been designated for cystine, glycine, or buffer solution. We pipetted 1 ml of the corresponding solution into each of a group’s six tubes. We then placed one tube of each kind into each of the five temperature settings for fifteen minutes. The temperatures used were 0, 4, 27, 37, 59, and 95 °C. After the allotted time frame had elapsed, we took the tubes and pipetted 10 µl of the P₁ enzyme into each of the tubes. After doing so, we waited another fifteen minutes. After the second period of fifteen minutes had elapsed we added the testing

solution. The testing solution used was ninhydrin, of this we added 2 ml to each test tube, we then placed the test tubes in a bath of 95 °C water for ten minutes.

After the ten minutes had elapsed we removed the test tubes from the water bath and any color change was observed. Once we had observed the existing color changes the tubes were then placed into the spectrometer to obtain a quantitative measurement of the enzymatic breakdown. We calibrated the spectrometer absorbance at a level of 570 nm. We used a test tube containing a high glycine concentration to calibrate for maximum absorption. We carefully noted the levels of absorbance for each tube. We then discerned based on this data whether or not our hypothesis was correct.

DATA

Absorption data for cystine breakdown to glycine

Temperature Absorption (570 nm) Appx Glycine Conc ($\mu\text{g}/\text{nm}$)

0°C 1. 35 70

4°C . 378 35

27°C . 660 45

37°C 1. 27 68

59°C 1. 46 76

95°C 1. 18 63

*Note that the 0°C data point has been disregarded as an outlier in this graph.

RESULTS

The resulting data points are as follows:

0°C-1. 35, 4°C-. 378, 27°C-. 660, 37°C-1. 27, 59°C-1. 46, and 95°C-1. 18

The 0°C data point has been considered an outlier and we have disregarded this point in our conclusion.

Conclusion

We saw a marked difference in the rate of enzymatic breakdown depending on the temperature of the protein. With a general trend of increased temperature causing a faster rate of breakdown. We noted that the fastest breakdown/highest absorbance was at 59°C. We theorized that this may be the optimal temperature for maximum efficiency of breakdown. We also noted the slight slowing upon reaching 95°C, we theorized that this temperature could be the breaking point for enzyme and thus would result in a slower reaction. Conclusively, our hypothesis was confirmed, as there was a difference in enzymatic breakdown depending on temperature.