Effect of ph and temperature on enzyme activity in skeletal muscle essay

Introduction: The purpose of this experiment is to measure the effects of changes in temperatures and pH on enzyme activity in skeletal muscle, particularly the activity of lactate dehydrogenase (LDH). LDH is a glycolytic enzyme which converts pyruvate to lactate in the following equation: LDH Pyruvate+ NADH ? ————? Lactate + NADThe reaction above can move in both directions, forward (favored by Type II skeletal muscle) and reverse (favored by Type I skeletal muscle and the heart). Enzyme activity is important in this reaction because enzymes act as catalysts, they decrease the activation energy (the difference between the transition state and the energy of the reactants or products) needed to start the reaction and therefore speed up the reaction rate. A substrate molecule must bind to the enzyme at the active site before the substrate can be catalyzed.

The rate at which an enzyme can catalyze the reaction is affected by factors such as enzyme concentration, substrate concentration, the affinity of the enzyme for the substrate (how well the enzyme binds to the substrate), concentrations of cofactors and coenzymes, pH and temperature. In this experiment, we will be measuring enzyme activity in samples of muscle homogenate (skeletal muscle that has been broken up) by measuring how much light is absorbed by NADH in the above equation, which will be related to how much NADH is present. We will then determine LDH activity by using the formula: ([A/min]/6. 2) x 78, 780, where [A/min]=(initial absorbance-final absorbance)/3.

We will be testing the absorbance using a spectrophotometer, which simply generates light that passes through a filter that causes the light to have a specific wavelength. NADH absorbs light maximally at a wavelength of 340

nm. Therefore, the measurement of NADH degradation is used to monitor the rate of lactate formation and therefore the LDH activity. Enzyme activity is important in skeletal muscle because the enzymes help speed up the reaction rate of anaerobic respiration, taking place in the body. Anaerobic respiration is a process of cellular respiration when there is no oxygen present. It converts the pyruvate from glucose molecules, to lactate to create ATP, which is a form of energy used by cells in the body.

If the reaction is sped up by enzyme activity, then more ATP can be made in a shorter time period, and the cells can perform their work much quicker. Methods: The equipment used in this lab experiment is as follows: •Spectrophotometer •Stopwatch •Gloves •Parafilm •Cuvette of deionized water •3 Cuvettes with samples of muscle homogenate, each with 0. 9 ml NADH at specific pH of 5. 5, 7. 4, and 8. , and 0.

05ml of pyruvate. •3 Cuvettes with samples of muscle homogenate, each with 0. 9 ml NADH at specific temperatures of 0 C , 25 C, and 37 C, and 0. 05ml of pyruvate. •Calculator As stated before, the reaction we will be looking at is the following: LDH Pyruvate+ NADH ? ——–? Lactate + NAD Where LDH is the enzyme we will be monitoring and NAD is the coenzyme.

If the reaction goes forward, then pyruvate is the reagent and lactate is the product and if the reaction goes in the reverse direction then lactate is the reagent and pyruvate is the product. To start, the spectrophotometer must be calibrated using the cuvette of deionized water to set the scale to zero. Then, the cuvette of muscle homogenate with NADH (pH of 5. 5) is placed into the spectrophotometer, after being mixed, and the initial absorbance is

recorded (at 340 nm). The cuvette is left in the spectrophotometer the absorbance is recorded after 1 minute, 2 minutes and 3 minutes.

The cuvette is removed and the deionized water is used to set the spectrophotometer back to zero. Do this procedure for all of the remaining four cuvettes and record the absorbances. Then calculate the average change in absorbamce per minute by subtracting the final absorbance from the initial absorbance and dividing by 3. To calculate the LDH activity use the formula: ([A/min]/6. 22) x 78, 780, where [A/min] is the change in absorbance.

Results: Table 1: Absorbance readings for different pHs and temperatures of NADH Initial aborbance1 min2 min3 min ABS/minLDH activity pH 5. 50. 352 nm0. 347 nm0. 338 nm0.

329 nm0. 0077 nm97. 103 nm pH 7. 40. 492 nm0. 480 nm0.

467 nm0. 454 nm0. 0127 nm160. 431 nm pH 8. 50. 371 nm0.

370 nm0. 364 nm0. 359 nm0. 0040 nm50. 662 nm 0 C0. 930 nm0.

21 nm0. 906 nm0. 891 nm0. 0130 nm164. 653 nm 25 C0.

492 nm0. 480 nm0. 467 nm0. 454 nm0. 0127 nm160. 431 nm 37 C0.

768 nm0. 747 nm0. 719 nm0. 692 nm0. 0253 nm320.

861 nm *The readings for ph 7. 4 and 25 C are the same because this is the normal pH for room temperature (25 C) NADH. Graph 1: The relationship between pH and enzyme activity As you can see in the above graph, enzyme

activity increases with pH until it reaches the optimum pH, in this case 7. 4, and then the enzyme activity decreases.

The recorded data is in a lower range than the ideal data, but the two lines follow the same trend. Graph 2: The relationship between temperature and enzyme activity As you can see in the above graph, enzyme activity usually increases with an increase in temperature. The ideal data shows this trend. The recorded data follows this trend with the exception of the enzyme activity at 25 C, which decreases slightly.

This is probably due to an error in the recording. For both pH and temperature, the recorded data seemed to follow the ideal data, for the most part. The enzyme activities for pH for both the recorded and ideal data seemed to increase and then decrease when the optimum pH was reached. The enzyme activities for temperature seemed to generally increase when the temperature was raised. Discussion: After completing the experiment, it was found that both temperature and pH have a significant effect on enzyme activity.

By looking at the pH graph (Graph 1), we can see that the enzyme activity increases until the optimum pH is reached, in this case 7. 4 (about neutral), and then the enzyme activity decreases. The optimum pH is different for each enzyme. The enzyme activity decreases after the optimum pH is reached because after this point the enzymes, which are proteins, begin to denature, or break apart. The enzyme activity decreases because the enzymes are disintegrating.

If the pH is significantly less than the optimum temperature, for example a pH of 5. 5, then the enzyme activity also decreases because the pH is too low for the enzyme to function. The optimum temperature is neutral because our bodies have a neutral pH and skeletal muscle is found in our bodies. According to Graph 2, enzyme activity increases with an increase in temperature. This is different than the expected results, that there is an optimum temperature as well and that the enzyme activity would decrease after the optimum temperature is reached. This is because, like with pH, the enzyme begins to denature after the optimum temperature is reached.

Our results did not show this optimum temperature because the optimum temperature is 37 C, and this is where our data stops. If we were to measure the enzyme activity of the sample of a temperature higher than 37 C, like 45 C for example, then the enzyme activity would probably decrease. Just like optimum pH, the optimum temperature varies between different enzymes. The optimum temperature for the specific enzyme LDH is 37 C because this is the normal body temperature where skeletal muscles are usually found. Maintaining this optimum is important for maximum enzyme activity.

Our recorded data was similar to the ideal data in both pH and temperature. For pH, the trend of increasing and then decreasing at the optimum pH is the same, however, our recorded data was in a lower range than that of the ideal data. The enzyme activity for pH of 7. 4, the optimum pH was not as high in the recorded data than in the ideal data.

This is probably due to error in the recording of the data, which is explained below. For temperature, the increasing trend was followed by both the

recorded data and the ideal data, for the most part. The exception to this was at the temperature of 25 C, where the enzymatic activity decreased slightly. This was probably also due to some experimental error.

There were a few sources of experimental error in this experiment. The temperature and pH of the deionized water, used to set the spectrophotometer to zero, may have been off slightly because during the experiments it sat on the table, which could cause fluctuations in the temperature and pH when it was exposed to the air. This would cause fluctuations in the absorbance readings. Also, the time recorded may have been off as all stopwatches vary. The temperature and pH of each particular sample may have fluctuated during the time the sample was being carried to the spectrophotometer from where it was mixed.

This might have caused some of the differences shown in the recorded and ideal data. Finally, there may have been errors in the calculations with rounding. In conclusion, it was found that both pH and temperature have similar effects on enzyme activity- the enzyme activity increases until an optimum temperature and optimum pH is reached, and then the enzyme activity decreases because the enzymes begin to denature. The enzyme monitored, LDH, is found in skeletal muscles in our bodies and in the bodies of other animals. This experiment is important because it tells us a little bit more about why our bodies have to keep a constant temperature and pH. If the pH or temperature varies too much from the optimum pH or optimum temperature, then the enzyme activity decreases and the anaerobic respiration process, which includes LDH, slows down.

This therefore produces less ATP, which is the form of energy used in our bodies, and the cells cannot perform the work which they need.