Buffering in muscles

Many chemical systems are affected by changes in acidity. The human body normally has a pH of about 7.4, and illness or death can occur if pH moves outside a narrow range.

Large quantities of hydrogen ions in the form of lactic acid are continually produced in the body, but are consumed again by normal chemical processes. However, a burst of exercise, such as a 100 m sprint, produces an excess of H^+ ions in a short time. The body is unable to consume the excess hydrogen ions fast enough. Under these conditions muscles must stabilise pH in another way. This ability to stabilise pH is known as 'buffering capacity'.

Although we are interested in muscles' ability to buffer acid, this experiment investigates their ability to buffer base. This is done because acids cause muscle, which is made of protein, to change chemically. When acid is added, proteins unfold in a process called denaturing. Muscles that buffer a base well will also buffer an acid well, as both involve stabilising pH.

Aim

To test the buffering capacity of three different types of chicken muscle.

Equipment

For the class:

blender
7.5 g of chicken thigh
7.5 g of chicken breast
7.5 g of chicken heart
140 mL of 0.145 mol L^{-1} NaCl

For each group:

100 mL beaker
pH meter
glass stirring rod
pasteur pipette
graduated pipette that can deliver 0.2 mL
10 mL graduated cylinder
25 mL of 0.145 mol L^{-1} NaCl
a few drops of 0.05 mol L^{-1} HCl
a few drops of 0.05 mol L^{-1} NaOH
about 2 mL of 0.002 mol L^{-1} NaOH

Method

Your teacher will blend approximately 7.5 g of muscle (record actual weight and type of muscle) with 140 mL of 0.145 mol L^{-1} NaCl in a blender until there are no large pieces of muscle in the mixture. This mixture is called a homogenate. Repeat for each type of muscle. Note: This is enough for the whole class.

1. Pour 7.5 mL of the first homogenate into a 100 mL beaker.
2. Add 7.5 mL of the 0.145 mol L^{-1} NaCl and stir.
3. Measure the initial pH with a pH meter. Allow the pH meter to stabilise for a minute or two before taking the first reading.
4. Adjust the initial pH to about 5.9 (less than 6.0) by adding drops of 0.05 mol L^{-1} HCl or 0.05 mol L^{-1} NaOH, using a small pasteur pipette as required. Stir the homogenate well after each addition. Ensure that all the fluid goes into the solution, not onto the pH meter or the side of the beaker.
5. Add 0.2 mL of the 0.002 mol L^{-1} NaOH and stir well before measuring the pH again. Record the results in a table similar to the one below.

Total volume of NaOH added (mL)	pH	Total µmol NaOH added
0.00 mL	Initial pH =	0.00
0.20 mL		

6. Repeat step 5, and record the volume added and the pH. Continue repeating until pH 7 is reached.
7. Repeat steps 1–6 for the other two types of muscle.
8. Calculate the number of micromoles (μmol) of NaOH added after each addition and record in your table.
9. Plot results as pH vs total μmol NaOH added. All muscle types should be graphed on the same axis. Draw the line of best fit for each type of muscle. If possible, perform a linear regression and calculate the correlation coefficient (r) on your calculator for each muscle.

Questions

1. Look at your graph. Which type of muscle required the most base to be added before its pH changed from pH 6 to pH 7?

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2. Is this type of muscle a good buffer? Explain your answer.

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3. Buffering capacity is defined as the number of micromoles of acid or base needed to change the pH by one pH unit per gram of muscle. Calculate the buffering capacity of each of the three muscles you tested. (Hint: use your graph to see how many micromoles of base were needed to change from pH 6 to pH 7 for each muscle.)

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4. Compare the buffering capacities of the three muscle types. Which one had the highest buffering capacity?

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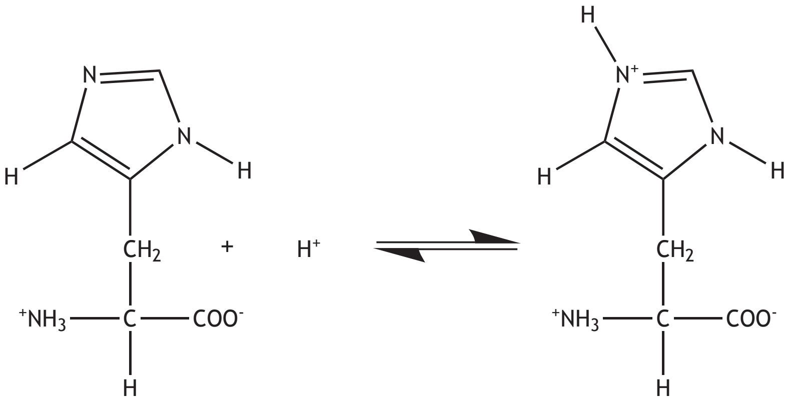
Vertebrates, including chickens and humans, have two types of muscle: red muscle and white muscle. Red muscles store plenty of oxygen and are used for endurance activity such as running a marathon. White muscles are used for high-energy, short-term activities like sprinting and therefore need a high buffering capacity. In chickens, breast muscles are used to assist flight in short bursts so are composed mainly of white muscle fibres. Heart muscles are similar to red muscle as they are used continuously.

Research scientists have found that vertebrates have different levels of a chemical called histidyl in the protein of different types of muscles in their bodies. They believe this may account for different buffering capacities. The reversible reaction for this buffer is shown below.



histidyl

5. Did you find that the breast muscle had the highest buffering capacity? Would you expect more or less histidyl to be present in white muscle fibre? Explain.

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6. Use the reversible equation to explain how high levels of histidyl would help buffer excess hydrogen ions produced during exercise.

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