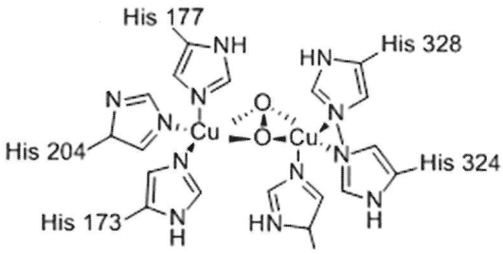
Haemoglobin – winning the equilibrium game

Haemoglobin is a protein that carries the oxygen in the blood and allows it to be transported from the lungs to cells all around the body. Haemoglobin is actually made of four units, called haem groups, each containing an iron ion that can carry an oxygen molecule. The success of haemoglobin can be judged from the fact it is common to almost all vertebrates (apart from some fish), and, therefore, clearly iron's ability to reversibly bind to oxygen is something that was made use of a long time ago in the history of life's evolution.

In some invertebrates, such as lobsters, different molecules are used, such as haemocyanin – a copper-based molecule that gives lobster blood its famous blue colour – but these creatures are much smaller and haemocyanin does not bind to blood in the same way as haemoglobin. The fact that Fe^{2+} ions can be oxidised to Fe^{3+} fairly easily is important to haemoglobin's success in being used across the majority of vertebrates, but other relatively common transition metal ions can also move quite easily between oxidation states, so what makes haemoglobin special? The answer is the way it manipulates equilibrium.



Haemocyanin *– note the two copper ions binding the oxygen in the molecule to 'carry' it*

Haemoglobin and Le Chatelier

Haemoglobin, Hb, binding to an oxygen molecule to form oxyhaemoglobin, $Hb(O_2)$, is a reversible reaction which can be represented as:

$$Hb + O_2 \leftrightarrows Hb(O_2)$$

The first way that Le Chatelier's principle is used is making use of the different oxygen pressures at different points in the body (called partial pressures because oxygen is not the only gas dissolved in the blood). The way that partial pressure affects the position of equilibrium is essentially the same as concentration. In the lungs the oxygen partial pressure is high, which pushes the equilibrium to the right, forming oxyhaemoglobin. When this oxyhaemoglobin reaches the muscles, away from the lungs, the oxygen partial pressure is much lower as the oxygen has all been used by the muscles for respiration. This causes the position of equilibrium to shift back to the left, releasing the oxygen that had been bound and freeing up the haemoglobin. The oxygen can then be used for respiration, and the haemoglobin is free to return to the lungs, carrying some carbon dioxide with it at the same time.

Le Chatelier's principle also explains why substances such as carbon monoxide are toxic. Carbon monoxide binds more strongly to haemoglobin than oxygen does. If carbon monoxide is inhaled into the lungs it forms carboxyhaemoglobin. The position of equilibrium for this reaction is far to the right. Because the position of equilibrium is to the right, the concentration of free haemoglobin (Hb) is significantly lowered. This means that in the oxyhaemoglobin equation:

$$Hb + O_2 \leftrightarrows Hb(O_2)$$

the equilibrium shifts to the left as haemoglobin is removed, meaning that not enough oxyhaemoglobin forms and cells do not get enough oxygen for respiration, and a person is likely to die unless they quickly get enough oxygen to push the position of equilibrium back to the right to form enough oxyhaemoglobin.

The oxygen dissociation curve

The change in how much haemoglobin is bound to oxygen can be represented by an 'oxygen dissociation curve'. The values plotted in this curve are measured by experiment.

Towards the left of the diagram, where oxygen partial pressure is low (such as in the muscles) it is the situation where the equilibrium has shifted to the left and most haemoglobin is free (low saturation). Conversely, on the right-hand side, oxygen partial pressure is high and most haemoglobin is bound to oxygen (high saturation).

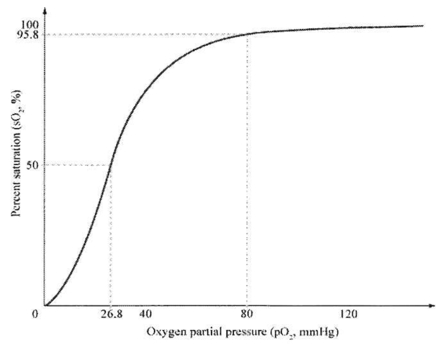


Figure 1*: An oxygen dissociation curve*

 © ZigZag Education, 2021

The experiment that is used to plot the dissociation curve can be carried out at different conditions, such as temperature or pH, in people with different health conditions or in different animals. By doing these experiments we can find out that the shape of this curve is changed by different factors, which shows that <u>the equilibrium can be altered by different factors</u>.

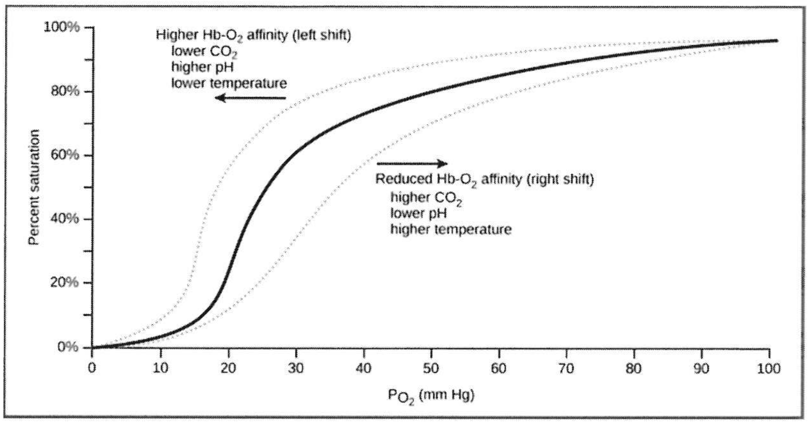


***Figure 2**: The effect of a change of conditions on the oxygen dissociation curve*

The shape of the curve for higher pH and lower temperature shows that haemoglobin becomes saturated much more easily in these conditions. Look at the values at a 20 mmHg partial pressure of oxygen (on the x-axis). The curve for lower temperature / higher pH has a much higher saturation of haemoglobin, i.e. more haemoglobin is able to carry oxygen and the position of equilibrium is shifted to the right in our equation. This is the case in fish such as sharks, which live in cold temperatures and have a higher blood pH. This is essential because it means sharks can carry just as much oxygen in their blood, even though the oxygen pressure is lower. How clever is that!

This curve also shows the risk to marine life of increased CO_2 levels and global warming. Higher CO_2 acidifies the sea and contributes to global warming, which raises sea temperatures. This risks causing a condition called 'acidosis' in fish, whereby the shape of the oxygen dissociation curve is flattened. In this case, higher oxygen levels are needed to saturate the haemoglobin, and it is harder for equilibrium to be shifted to the right in the way it is in our lungs. Fish blood (and all blood) contains **buffers** which help minimise the change in pH in the blood, even if the sea becomes more acidic. However, these buffers only work up to a certain point, and many fish species may have to adapt to the lowering of sea pH or risk extinction as the haemoglobin in their blood cannot carry enough oxygen to their muscles.

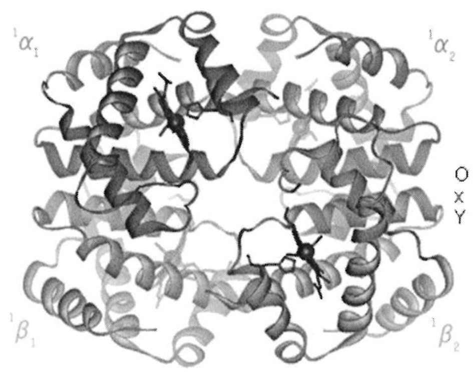
Why is it even a curve? The cleverest bit of all...

What the shape of the curve tells us is that once some oxygen is bound to haemoglobin, it then becomes much easier to add a lot more (the curve becomes steeper). The reason for this is because there are two different forms of haemoglobin binding to oxygen – relaxed (HbR) and tensed (HbT).

- Relaxed haemoglobin binds to one oxygen molecule and becomes tensed haemoglobin at the same time. (Position of equilibrium more to the left.)
- Tensed haemoglobin then easily binds to three more oxygen molecules. (Position of equilibrium more to the right.)

This is a phenomenon called **cooperative binding**. A haemoglobin molecule has four binding sites containing Fe^{2+}. Once the first Fe^{2+} binds to oxygen, a pull is exerted on the rest of the haemoglobin molecule (making it tensed), and moving the other three Fe^{2+} sites into a position where they bind more easily.

This is a fantastic manipulation of equilibrium – taking a situation where the position of equilibrium lies too much to one side (like the binding of O_2 to HbR, with the equilibrium further to the left meaning little oxygen can be transported) and changing the situation to one where the position of equilibrium lies more in the desired direction (more O_2 can bind – as with HbT).



***Haemoglobin** – Illustration of the four haem groups in haemoglobin, giving it four binding sites*

 © ZigZag Education, 2021

So why not just have HbT all the time if it makes it easier to bind all four oxygen molecules? We must remember the oxygen needs to be released again at the muscles. In Figure 2, oxygen release happens as we move from right to left, and the degree of saturation of Hb (y-axis) decreases. It is much harder to release oxygen in the steepest curve with a high Hb-O_2 affinity – the partial pressure of O_2 (x-axis) has to drop to around 20 mmHg for most of the oxygen to be freed. If HbT turns back into HbR, however, HbR has a flatter curve, and we can see, moving from right to left, that the oxygen is released at an earlier stage.

This is incredibly clever! The conversion between tensed haemoglobin and relaxed haemoglobin allows both easy binding and easy release of oxygen – equilibrium is truly being manipulated! Nature has played with this relationship over millions of years, and makes use of the different shapes of curves to adapt to conditions where more oxygen or less oxygen needs to be absorbed, or when an animal's environment is a different temperature or pH. This ability to adapt is the basis of natural selection, and it is, therefore, not a stretch to say that the control of the position of equilibrium in different reactions has played a central role in evolution.

Comprehension questions

1. Define the following terms:
   a) Haem group
   b) Partial pressure
   c) Dissociation
   d) Le Chatelier's principle
   e) Buffer
   f) Cooperative binding
2. Draw a flow diagram to illustrate what happens at each stage of the oxygen dissociation curve during binding of O_2 and release of O_2.
3. Explain how acidosis can cause fish to suffocate, using the oxygen dissociation curve to help.
4. Write a series of bullet points to explain how haemoglobin/blood exemplifies Le Chatelier's principle.
5. Why is the ability of iron to form multiple stable oxidation states important for its role in carrying oxygen?

Application questions

6. Sketch and annotate an oxygen dissociation curve to explain the phenomenon of alkalosis (the opposite of acidosis).
7. How would the oxygen dissociation curve appear different if there was no cooperative binding? Explain why this would be a problem.
8. How would you expect the equilibrium between HbR(O_2) and HbT(O_2) to be affected by the partial pressure of O_2?

Taking it further

9. Research the different oxidation states of iron and how they link to iron's orbitals. Write a summary of why iron has multiple different oxidation states.
10. What other examples of equilibrium reactions can you find in the human body or natural world? How do the conditions in the human body or natural world affect the position of equilibrium in these examples? Write at least half a page on your chosen example(s).

 © ZigZag Education, 2021