NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

The Mermaid and the Globins:

Hemoglobin Function and Regulation

by
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Part I – The Mermaid

It was on a pleasant Saturday afternoon that Joanna and Alex headed to their local aquarium to meet up with one of Joanna's old friends, Elaine. Elaine had told Joanna she would be performing at the aquarium but would not tell her exactly what she was doing. Joanna and Alex were running late so they figured they would meet Elaine after the show using the backstage passes she had given them. As soon as they walked into the entrance of the aquarium Joanna saw a huge picture of Elaine dressed as a mermaid surrounded by sea lions. The caption on the poster announced a special limited engagement aquashow featuring "Mermaid Elaine." Joanna almost fell over laughing that her friend had taken her skin-diving hobby and turned it into a career as a professional mermaid.

Alex thought it was cool that Elaine was going to swim with sea lions in the giant tank in front of them. The show started with the animal trainer, Mason, who brought the sea lions out to warm up the crowd. As the spectators watched the sea lions thread their way through a series of diving rings, Mason shared some interesting facts about these endangered mammals. Alex ignored most of what Mason said because he was watching the show but he thought he heard Mason say that sea lions could hold their breath for up to twenty minutes. Alex asked Joanna if he had heard correctly. She said he had, but she didn't understand how it was possible. Alex thought that maybe they were somehow able to get more oxygen than land animals.

Question

1. Think of at least three adaptations that might allow marine mammals to remain underwater for an extended time.

Mason the trainer asked the crowd if they were ready to meet Mermaid Elaine. The kids in the audience screamed out an enthusiastic *"Yes!"* Joanna laughed as Elaine waved to the crowd while being carried onto the stage in a shallow container of water. Mermaid Elaine was all smiles as she lifted herself out of the container and sat at the edge of the viewing tank. Joanna couldn't believe the elaborate mermaid tail Elaine was wearing. She pulled out her phone and starting taking pictures so she could post them later. As Elaine lowered herself into the tank, Mason told the crowd that an average human can hold her breath underwater for less than two minutes. Joanna used her phone to time how long Elaine could hold her breath. Mermaid Elaine swam through the same diving rings as the sea lions. She gracefully made her way around the tank taking time to wave and blow kisses to the crowd. Joanna and Alex were so entertained during the show they didn't want it to end. By the time Elaine surfaced she had held her breath for almost five minutes! Mason then brought out the sea lions to join Elaine. Alex couldn't believe Elaine could stay under water for so long; how did she do it?

Question

2. Think of three reasons to explain how Mermaid Elaine was able to hold her breath for so long.

Part II – Myoglobin

After the show ended Joanna and Alex had to wait before they could go backstage. While they stood at the door Alex thought about the lecture on oxygen transport from the biochemistry course he and Joanna were taking. He asked Joanna if she thought hemoglobin (Hb) or myoglobin (Mb) might explain how the sea lions and Elaine were able to remain underwater for so long. Joanna admitted that she really didn't remember much as she just crammed before their exam. At that moment the door opened and they were led backstage. They saw Mason and introduced themselves as Elaine's friends.

Alex: I was wondering if you could tell us what allows sea lions to hold their breath for 20 minutes?

Mason: Actually, it's not just sea lions but some other aquatic mammals as well.

Joanna: Is it due to hemoglobin differences? We know from our biochemistry class that hemoglobin is responsible for transporting oxygen.

Mason: I loved my biochemistry class in college. I thought it was so much fun because it combined many of my interests.

Alex: Uh, right… *[Said sarcastically.]*

Mason: *[Laughing a little.]* Well, a sea lion's ability to hold its breath for an extended period is not due to hemoglobin but rather myoglobin. They have an increased amount of myoglobin, which acts as a storage protein for molecular oxygen (O_2) in muscle tissue (Lee, 2013; Mirceta *et al.,* 2013).

Joanna: Then is it increased myoglobin that allows Elaine to remain underwater for so long?

Mason: No. Compared to sea lions, humans have a fraction of myoglobin in their muscle tissue. Elaine has a specific training regimen, but I'm sure she could tell you more about that.

Alex: Can you tell us anything else about myoglobin? It could be useful in our class.

Mason: Myoglobin can bind one oxygen molecule since it's monomeric and has only one heme group.

[Mason grabs a pad of paper off a nearby desk and starts writing.]

$$\mathrm{Mb} + O_2 \rightleftharpoons \mathrm{MbO_2}$$

Mason: Myoglobin binds one oxygen molecule per heme as represented by this equation. I can't remember what the O_2-dissociation curve looks like, but let's find out.

[Mason moves to the computer and types something.]

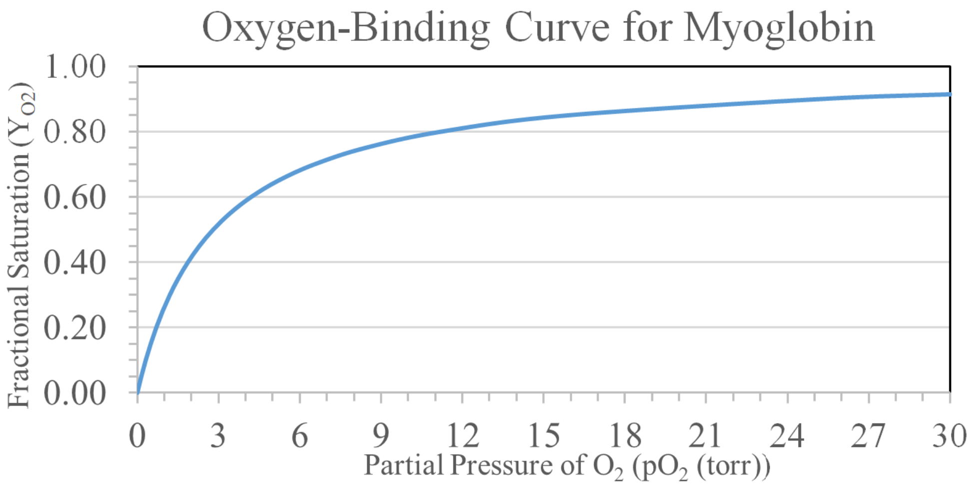


Figure 1. Oxygen-binding curve for myoglobin showing a hyperbolic curve.

Mason: Here we go! This website says the O_2-dissociation curve for myoglobin forms a hyperbolic curve (Figure 1), which indicates that myoglobin does not exhibit cooperative binding.

Alex: That's right! I remember now. You could calculate the dissociation constant, K, of myoglobin to determine the partial pressure of oxygen (pO_2) at which myoglobin is 50% saturated, which is also known as the p_{50}. Let me write the expression.

$$K = \frac{[Mb][O_2]}{[MbO_2]}$$

Joanna: Alex, isn't that related to the fractional saturation, Y_{O2}, which is the fraction of O_2-binding sites occupied by O_2 and can range from 0 to 1? Here, give me that pencil.

$$Y_{O2} = \frac{pO_2}{K + pO_2}$$

Alex: Right. I think you can figure out the p_{50} from the dissociation curve, but I don't remember how.

Mason: Sounds like you both know more about myoglobin than you thought. One of the cool things about myoglobin is that the physiological range of pO_2 causes myoglobin to be almost fully saturated with O_2.

Questions

3. Using the O_2-binding curve of Mb (Figure 1) show that the p_{50} of Mb is 2.8 torr.

4. Assuming the pO_2 in the blood ranges from 30 torr (venous blood) to 100 torr (arterial blood), calculate the fractional saturation of Mb in venous blood and arterial blood.

5. Myoglobin has a positive net-surface charge. Why would this allow for a larger [Mb] to occur in the cells of aquatic mammals, such as sea lions?

Part III – Hemoglobin

Joanna and Alex waited backstage in Elaine's dressing room as she finished signing autographs and taking pictures with fans. They decided since Elaine wouldn't have more Mb than other people, her ability to remain underwater longer than most people must depend on Hb. Alex knew Hb is a dimer of αβ subunits and that each molecule of Hb can bind four molecules of O_2 to a Fe^{2+} ion in each heme prosthetic group in the four subunits. Joanna knew the O_2-binding curve of Hb had a different shape compared to Mb, but didn't know how to describe it so she looked it up on her phone (Figure 2). She noted that the website said the O_2-binding curve of Hb is sigmoidal, which indicates a cooperative interaction between the four binding sites.

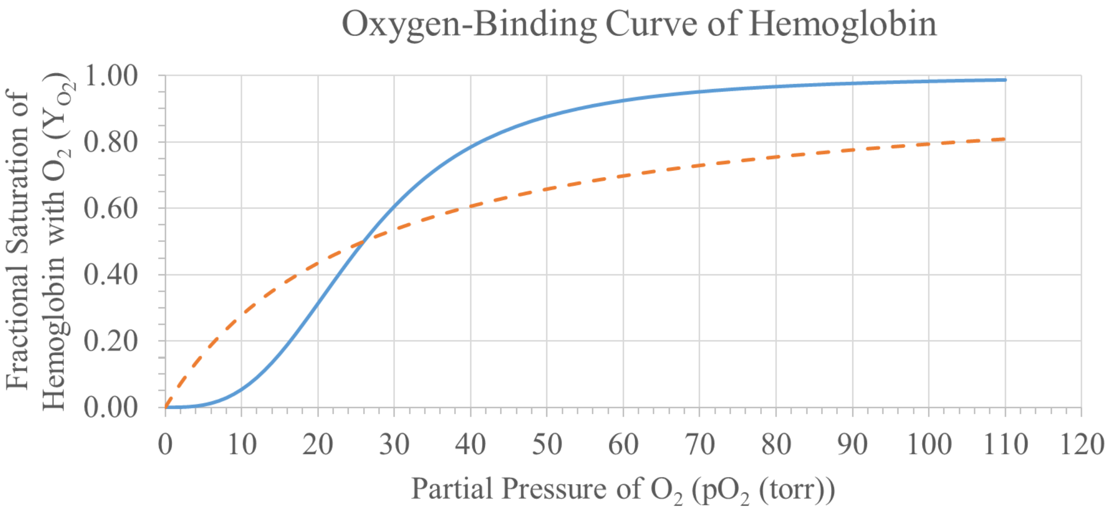


Figure 2: Oxygen-binding curve for hemoglobin showing a sigmoidal curve (solid blue). The dashed, red line shows a hyperbolic O_2-binding curve using the same p_{50} as hemoglobin.

Questions

6. Define cooperative binding.

7. Using the O_2-binding curve of Hb (Figure 2) show the p_{50} of Hb is 26 torr.

8. Refer to Figure 2 to estimate the amount of O_2 released from Hb as blood moves from the arteries (pO_2 100 torr) to the veins (pO_2 30 torr).

9. Refer to Figure 2 to explain why it is advantageous for Hb to have a sigmoidal binding curve rather than a hyperbolic curve.

Elaine entered her dressing room, carrying a large prosthetic tail, and ran to hug Joanna. Joanna introduced her to Alex and told her how much they had enjoyed her show.

Alex: Elaine, we were trying to figure out how you could hold your breath underwater longer than most people. Is it just because of your training?

Elaine: Sorry, it's a trade secret and I'll never tell.

Joanna: Come on Elaine! We're taking a biochemistry course that deals with oxygen transport, so we're really interested in how you do it.

Elaine: Okay, it's because of my training as a skin diver. I started diving for fun after I visited Japan and saw the Ama, a group of mostly women who dive for pearls. I thought it would be fun to try and before I knew it I was working as a professional mermaid. I actually learned some biochemistry when I started training so I understood what was happening in my body.

Alex: You mean you willingly learned this without it being for a grade?!?

Elaine: Of course; I wanted to be as good as I could be. Most skin divers, including me, train their bodies to dive for long periods of time by increasing their lung capacity through breathing exercises and learning to control the urge to breathe (Ostrowski *et al.*, 2012; Joulia *et al.,* 2003). I also practice meditation and don't eat for about five hours before a dive.

Joanna: Okay, that makes sense, but what does this have to do with biochemistry?

Elaine: One of the first things I learned about was the mammalian diving reflex. All mammals have it although it's stronger in aquatic mammals (Panneton, 2013). It's triggered once our faces contact cold water. The diving reflex is designed to allow mammals to stay underwater for an extended period (Speck & Bruce, 1978). I bet you can hold your breath underwater longer than you can on land.

Alex: I always thought that was true but I've never timed it before.

Elaine: The mammalian diving reflex does provide some benefits. Bradycardia happens first, which is the slowing of the heart (Ostrowski *et al.*, 2012; Speck & Bruce, 1978). Blood is also diverted from your extremities, generally by closing off capillaries. Humans don't have as much myoglobin in their muscles as some aquatic mammals and as a result we don't have as much oxygen stored in our muscle tissue. Once blood flow is constricted human muscles will run out of oxygen before the muscles in aquatic mammals causing them to fatigue earlier (Speck & Bruce, 1978).

Joanna: So meditation and the diving reflex explain your ability? What happens as your body builds up the concentration of carbon dioxide (CO_2)?

Elaine: They are certainly important because they help to make sure hemoglobin delivers oxygen, although I don't remember the specifics right now. I'm not sure about the role of CO_2. I also spent some time training in Denver, Colorado. Among other effects, the higher altitude increases the amount of something known as 2,3-BPG. Don't ask me what it stands for, but it helps my hemoglobin release more oxygen.

Alex: I went to Denver once, so do I have super hemoglobin too?

Elaine: Unfortunately, no. The effect is temporary, so I only go there for a few weeks before a major diving competition.

Questions

10. Compared to Hb, how could having a larger amount of Mb (assume K is 2.8 torr) allow aquatic animals to remain underwater for an extended time?

11. Even with bradycardia, Elaine indicated that she often would meditate and fast prior to a dive. What benefits do you think skin divers could receive from meditation and fasting?

Part IV – Hemoglobin Regulation

Joanna and Alex left the aquarium after their visit with Elaine. On their drive home they tried to think about how meditation, fasting, and 2,3-BPG affect oxygen affinity of Hb. Joanna recalled that Hb has two conformational states. The T state is deoxygenated while the R state is oxygenated, and the two conformations differ in their affinity for O_2. Alex knew Hb had a low affinity for O_2 in the T state (deoxy) and a high affinity for O_2 in the R state (oxy). As soon as Joanna and Alex got back to campus they pulled out their biochemistry textbook.

Joanna: *[Reading from the biochemistry text.]* "Oxygen binding triggers conformational changes in the tertiary structure of neighboring subunits causing them to transition from the T to R state. This increases the O_2 affinity of neighboring subunits and thus demonstrates cooperative binding."

Alex: We need to find out more about the effectors of hemoglobin so we can understand what happens when Mermaid Elaine dives.

[Alex takes the book and flips a few pages.]

Alex: *[Reading from the text.]* "Once O_2 binds to hemoglobin the pKas of several C-terminal residues decrease causing them to become more acidic (T to R transition). The Bohr effect states that O_2 dissociation is inversely related to the $[H^+]$.

Joanna: So as the acidity increases, O_2 is favored to be released from hemoglobin.

Questions

12. Many of the metabolites formed during respiration are acidic. How does Hb respond as your blood becomes more acidic?

13. What would happen to the O_2-binding curve of Hb (Figure 2, p. 4 above) if there was suddenly a large drop in pH?

Joanna and Alex understood how O_2 is transported into the body, but sought out their biochemistry TA, Kirk, to review how carbon dioxide (CO_2) produced in tissues through aerobic respiration could be removed. Since Elaine was generating CO_2, they knew it would play a role in her skin-diving ability. They filled Kirk in on their visit with their mermaid friend.

Kirk: Ultimately, an increase in CO_2 leads to the release of O_2. In red blood cells, carbonic anhydrase hydrates CO_2 to form carbonic acid (H_2CO_3), which ionizes to form H^+ and bicarbonate (HCO_3-). Most of the CO_2 is transported through the blood as HCO_3-.

Alex: Is it true some of the CO_2 can be transported directly by hemoglobin?

Kirk: Yes, many blood proteins can transport CO_2. The *N*-terminal amino groups on the T (deoxy) state of Hb can bind CO_2. Therefore, when the concentration of CO_2 is high the T state is favored and O_2 is released.

Joanna: When bicarbonate is formed aren't protons released?

Kirk: Yes.

Questions

14. Carbon dioxide is hydrated as follows: $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$. What effect does the release of a proton have on the O_2 affinity of hemoglobin?

15. Some amateur skin divers hyperventilate (breathe rapidly) prior to a dive to increase the $[O_2]$ in their blood. Would the $[O_2]$ in their blood increase? Would hyperventilation be advantageous for the skin divers based on Hb regulation?

16. The urge to breathe is a result of a build-up of CO_2. As Mermaid Elaine remained underwater the $[CO_2]$ would build up through cellular respiration. She would have to learn to suppress the urge to breathe, which skin divers often use meditation to accomplish. How would the increase of CO_2 affect the O_2 affinity of her Hb?

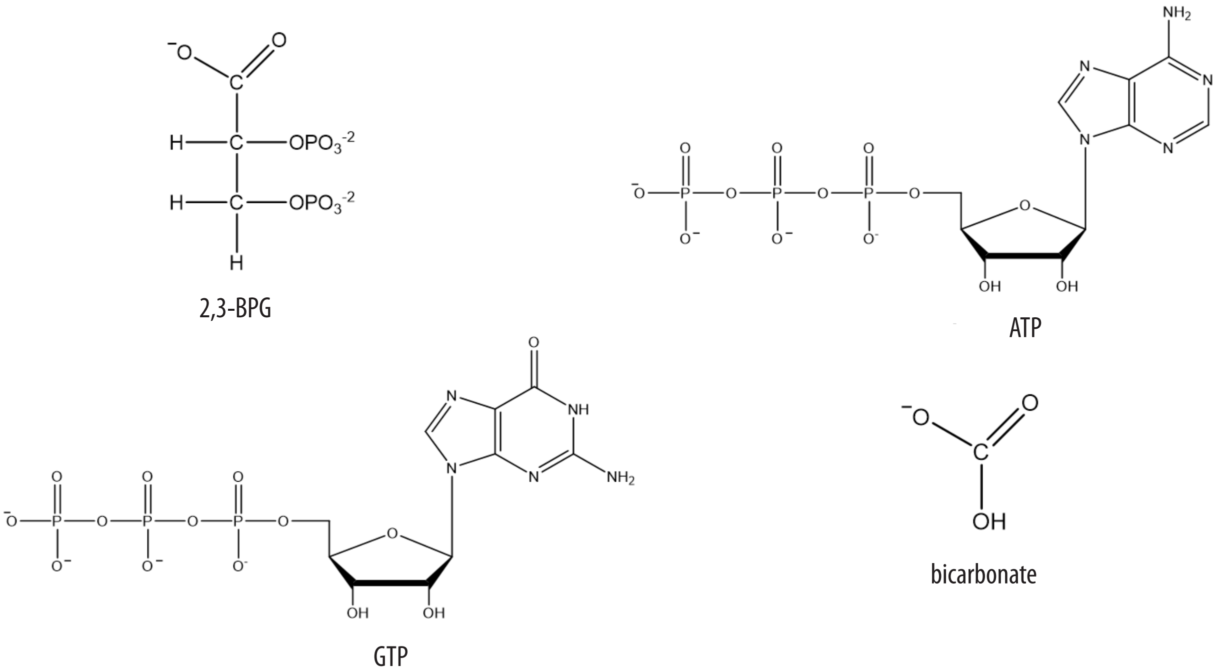
Joanna: This is starting to click. I can't believe we didn't recall this from class.

Alex: Kirk, when we met Mermaid Elaine she mentioned that an increase in 2,3-BPG helped her release more O_2 to her tissues. Can you tell us more about 2,3-BPG? She didn't know what it stood for.

Kirk: As we just discussed, H^+ and CO_2 affect the O_2 affinity of hemoglobin, but one of the main modifiers of hemoglobin is D-2,3-bisphosphoglycerate, or 2,3-BPG. 2,3-BPG decreases the affinity of hemoglobin for O_2 by keeping hemoglobin in the deoxy (T) conformation.

Joanna: Would 2,3-BPG have anionic groups to form hydrogen bonds and ions pairs with the *N*-terminal groups of the β subunits of hemoglobin?

Kirk: Correct! What is really cool is that at high altitudes (low pO_2), the concentration of 2,3-BPG increases and the hemoglobin affinity for O_2 decreases as a result. This causes more O_2 to be released in the tissues so that the ΔYo2 would essentially be the same regardless of elevation. Of course this isn't the only change in blood chemistry but it plays a role. Though 2,3-BPG is important in humans, in other vertebrates ATP, GTP, or HCO_3^- act as allosteric regulators of hemoglobin (Figure 3) (Komiyama *et al.,* 1995; Gronenborn *et al.*, 1984).



2,3-BPG

ATP

GTP

bicarbonate

Figure 3. Some common regulators of hemoglobin, which cause O_2 to be released.

Questions

17. Many long distance runners, and some skin divers, like to train at high altitudes even though they compete at (or below) sea-level. What would be the benefit of this?

18. Figure 3 shows some common allosteric modifiers of Hb. All of these modifiers bind to the T state of Hb favoring the release of O_2. What structural feature allows these molecules to bind to Hb?

19. Some animals do not use 2,3-BPG as an allosteric modifier of Hb. Instead, the T state binds HCO_3^-. As a result of this adaptation they can remain underwater for about an hour. How would this be advantageous compared to using 2,3-BPG as a modifier of Hb?

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