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THE OXYGEN DISSOCIATION CURVE OF HEMOGLOBIN: BRIDGING THE GAP BETWEEN BIOCHEMISTRY AND PHYSIOLOGY

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The sigmoidal dissociation curve of hemoglobin (Hb) and cooperativity are very difficult concepts to grasp for the students of biochemistry in the health sciences (such as undergraduates, chemistry and biology pre-medical majors). The teaching of Hemoglobin serves multiple purposes in the biochemistry classroom, among them the illustration of protein binding and stability, transport and kinetics. In this sense, the concept of the origin of protein function and its regulation in the cell is one of the key points of proposed innovative course sequences based on the logic of chemistry [1].

The fact that the Hb molecule is composed of 4 subunits ($\alpha_1, \alpha_2, \beta_1, \beta_2$) makes oxygenation easier as Hb becomes more saturated with oxygen. To explain this, it is important to remember that hydrophobic as well as ionic and hydrogen bond interactions between subunits determine the final quaternary structure of a complex protein. Specifically, a number of ionic bonds (classically referred to as "salt bridges") formed between highly hydrophilic or charged amino acids (e.g., Asp, Glu, Lys, Arg) hold together the four subunits in the quaternary structure of Hb. Oxygenation can occur only if the ionic bonds are broken and the polymer switches from a "tense" (T) or low oxygen affinity conformation to a "relaxed" (R) form. When this happens, the iron atoms can adopt the optimal stereochemical position in the porphyrin plane that allows for the proper binding of the oxygen molecule(s). The more bridges that are broken the easier it is (i.e., the binding affinity or $-\Delta G^0$ increases) for the entry of new molecules of oxygen, up to four per molecule of Hb. This was, in simplistic terms, a structural mechanism proposed by M.F. Perutz in the 70's that, although disputed by some investigators, is substantially correct based on new experimental evidence [2].

Since its first edition, the *Biochemistry* book by L. Stryer [3] has presented the analogy between breaking ionic bonds and ripping apart a block of four stamps as an explanation of the mechanism of cooperativity. I have found that even using this analogy, students are still confused, most likely because "breaking" (a stamp) is equated to "binding" (of oxygen), two verbs that have intrinsically contradictory meanings, which do not lead naturally to the formation of a mental association of two abstract ideas.

Further, the teaching of Hb often concentrates solely on the molecular mechanism and does not extend to its functionality in the body. I have successfully applied the analogy to the *releasing* of oxygen instead of to the binding, thus effectively bridging biochemistry (cooperativity) with chemical physiology (oxygen dissociation). As with all equilibria, the process is reversible and the sigmoidal curve can work either way as we consider the X-axis: Left to right for binding of oxygen or right to left for its dissociation (Fig. 1). In the latter case, the high O_2 tension of the lungs (100 mmHg) provides full saturation. As blood leaves the lungs for the peripheral tissues, Hb releases its load and the percent oxyhemoglobin decreases.

To work the "cooperativity in reverse", which is in actuality a *functional*, rather than a *structural*, analogy, I give some students a four post-office stamp square block and tell them that the stamps represent the four molecules of oxygen bound to Hb inside an erythrocyte in the lung, (at this point, I make sure that they understand that the oxygen molecules in real life are bound to the hemoglobin subunits, not to each other). Then I pretend to embark on a journey to the tissues where all this oxygen is needed. As I tell them to rip the first stamp apart, they do so by making two cuts, one horizontal and the other vertical. To release the second stamp only one cut is needed. And with one final cut the last two stamps are separated. Then I move to the dissociation curve graph (Fig. 2) and I tell them that cooperativity means that the release of every oxygen from Hb in regions of the body farther and farther away from the lungs, facilitates the release of each subsequent molecule, and with *less* energy each time (if 1 cut = 1 arbitrary unit of energy, then to separate the first, second, third and fourth oxygens we need, respectively, 2 units, 1 unit, 1/2 unit and 1/2 unit).

A closer look at the X-axis reveals that for the release of the first oxygen, the tension must drop from 100 to 40 mmHg. However, for the release of the second, the drop is smaller, down from 40 to 26 mmHg (the normal P_{50} for Hb). Further, the release of a third or a fourth oxygen molecule requires only the smallest drop in pressure (however, in real life Hb never unloads its oxygen content completely). At this point, I stress the functional meaning of "needing less energy each time" to unload oxygen: Just small drops in partial pressure do the trick in the right place (tissues) but not in the wrong one (lungs) where we need it tightly bound to the carrier.

This analogy describes the steps in the mechanism of Hb/O₂ interaction and provides a pictorial framework for getting into the traditional biochemical story (i.e., moving now to the binding of oxygen: left to right in the X-axis of Fig. 1). The model is therefore put into the general context of cooperativity in that the first molecule bound causes structural changes that influence the binding of the next molecule. This property (along with allosterism) is a direct consequence of the quaternary arrangement of the 4 polypeptides in Hb, akin of "molecular communication" that is lacking in other oxygen-binding molecules, such as myoglobin (Mb). The shape of the oxygen dissociation curve of Hb is sigmoidal, whereas that of Mb is not (it is hyperbolic); only the sigmoidal curve is characteristic of the cooperative process. Nature has come up with two clever molecular designs for particularly distinct purposes in the body. Hb ferries oxygen in blood stream from an extreme gradient of partial pressure from lungs (where it must remain tightly bound) to tissues (where it has to be easily released), but, on the other hand, Hb would be unable to provide all oxygen that the cell needs in a high ATP demanding situation (e.g., exercising). Mb, located in the cell cytoplasm, releases oxygen to the respiratory chain of the mitochondrion, an organelle that uses oxygen faster than the gas can diffuse into it. Since the binding affinity of oxygen to the cytochrome oxidase is ~10 times higher than that for Mb, a "hyperbolic" release works in this particular situation better than a "cooperative" release.

Going back to the "cooperativity in reverse" illustrated by the 4-stamp block analogy as described before, I have found that the learning of a difficult concept is further facilitated since the educator uses here the three main models of learning: association, discovery and mentoring [4] as follows:

- First, by comparing molecules of oxygen to post office stamps we can move from the abstract to the concrete. By evoking images in the mind of the learner close to the realm of everyday life, the process of *association* is set into motion (behavioral theory of Pavlov and Skinner). The teacher tells the story, painting pictures and transporting the students to scenarios of sound, shapes and colors and in doing so,

he seamlessly delivers the idea to be communicated. This is like sending a text e-mail (the concept) with a picture file "attachment" (the associated image).

- Second, the students are engaged in a little hands-on activity. In the model of development and *discovery* (based on the experiential theory of Piaget), the students experiment, touch, feel, ask questions about the outside world and try to extract the correct answers by themselves. Since the learner uses the senses in a search for the right answers, the educator serves here as a facilitator and the student assimilates them into his/her experience or inside world.
- Third, if the educator shares with the class how he or she felt when first encountered this difficult concept, it will establish a *mentoring* relationship (based on Bandura), in which teacher and students share experiences together. This would be practical only if a large class is broken down into small groups, and even so, some educators might feel reluctant to embrace an approach that could be perceived as "exposing" weakness in front of the students.

However, there is still another possibility to teach an abstract and difficult concept: by guiding them to discover the truth *by themselves*. The "Socratic Method" was done one-on-one, involved active learning, was learner-centered, and facilitated by the teacher. Skillful, appropriately-phrased, questions aimed at knowledge, comprehension and application of difficult disciplines can efficiently enhance the cognitive abilities of students from the various health professions [5].

In our example of the dissociation curve of hemoglobin, we can imagine walking alongside the disciple on the green of the University campus. The teacher could pose the question: "Let's say oxygen is bound to hemoglobin in the lung -will this be a tight binding?" The student would answer: "Possibly, since we don't want to lose any of it right there". The teacher, then: "Now let's imagine the erythrocyte is traveling farther away from the lung"; "It's time to release it"; "Yes, but what would happen if it is released as soon as the oxygen pressure drops just slightly?"; "Well, I suppose there won't be anything left for the most hidden tissues bathed in CO₂"; "Exactly"; "Then I imagine the release should be gradual, but, wait a minute, a mechanism should exist for that!"; "Indeed. Let's call that mechanism '*cooperativity*' for now". And so on.

Albeit impractical, this would be the learning method of choice and the use of online interactive tutoring is getting closer to that goal. In the mean time, I can't wait to start a new quarter and see the students faces glow when they discover the secrets of oxygen delivery with the aid of a 4×33-cents "device".

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FIGURE 1. THE CLASSICAL OXYGEN DISSOCIATION CURVE OF HEMOGLOBIN

The shape of the oxygen dissociation curve of Hb is sigmoidal, whereas that of other oxygen-carrying molecules (such as Myoglobin) is hyperbolic. Only the sigmoidal curve is characteristic of the cooperative process by which the release of one oxygen molecule alters the affinity for the remaining oxygens bound to the other proteic subunits. The 4 subunit arrangement in Hb ($\alpha_1, \alpha_2, \beta_1, \beta_2$), serves to accomplish a specific function in the organism of vertebrates, as it moves from an extreme gradient of oxygen partial pressure (or oxygen tension) from lungs to hypoxic tissues. The dashed diagonal lines in the figure indicate that oxygen molecules are bound to α/β subunits (to the sixth coordination positions of Fe^{2+} ions on the heme planes).

FIGURE 2. THE POSTAGE-STAMP ANALOGY

In order to release single stamps from a block of four, we have to make two cuts to release the first stamp, only one cut to release the second, and with the final cut we release the last two stamps, thus each time needing "less energy" to do the job. Similarly, oxygen remains tightly bound to hemoglobin (Hb) in the lungs but it will be progressively released as partial oxygen pressure drops in the different tissues of the body. The release of a second, and even more so the third oxygen molecule, require smaller drops in pressure as the Hb-carrying erythrocyte moves farther away to the lungs. In the analogy, $\text{Hb} \cdot 4\text{O}_2$ exists as "four stamps bound to the 4 Hb subunits"; $\text{Hb} \cdot 3\text{O}_2$ exists as "three stamps bound + 1 subunits free" and so on.