

Case 9

Allosteric Interactions in Crocodile Hemoglobin

Last modified 19 March 2004

Focus concept

The effect of allosteric modulators on oxygen affinity for crocodile hemoglobin is unique when compared with other species.

Prerequisite

Hemoglobin structure and function concepts.

Background

While most human beings are able to hold their breath for only a minute or two, other species are able to stay under water for much longer periods of time. In this case study we will examine the physiological adaptations that allow some organisms to deliver oxygen to tissues while submerged under water.

Deep sea-diving mammals, such as whales and seals, are able to stay under water for long periods of time. These mammals are able to stay submerged because their muscles contain many-fold higher concentrations of myoglobin (Mb) than humans.

Crocodiles are also able to stay submerged under water for periods of time exceeding one hour. This adaptation allows the crocodile to kill small mammals by drowning them. However, the crocodile doesn't have large amounts of myoglobin in its muscle as the deep sea-diving mammals do, so their physiological adaptation must be different. In 1995, Nagai and colleagues described in the British journal *Nature* a possible mechanism that allowed the crocodile hemoglobin to deliver a large fraction of bound oxygen to the tissues. They suggested that bicarbonate, HCO_3^- , binds to hemoglobin to promote the dissociation of oxygen in a manner similar to 2,3-bisphosphoglycerate (BPG) in humans.

This case is important because information gathered from experiments like those described here will allow scientists to design effective blood replacements.

Questions

1. In humans, oxygen is effectively delivered to the tissues because of the presence of several allosteric modulators. Name three of these modulators and explain how their presence allows oxygen to be delivered to the tissues.
2. Explain why having higher concentrations of Mb would allow whales and seals to stay submerged under water for a long period of time.
3. Let us consider the hypothesis that bicarbonate serves as an allosteric modulator of hemoglobin binding in crocodiles. What is the source of HCO_3^- in the crocodile tissues?

CASE 9 *Allosteric Interactions in Crocodile Hemoglobin*

4. Draw oxygen-binding curves for crocodile hemoglobin in the presence and absence of bicarbonate. Which conditions give rise to a greater p_{50} value for crocodile hemoglobin? What does this tell you about the oxygen binding affinity for hemoglobin under those conditions?
5. Komiyama *et al.* investigated the bicarbonate binding site on the crocodile hemoglobin by constructing human-crocodile chimeric hemoglobins in which amino acids in the human hemoglobin were replaced with amino acids found in the crocodile hemoglobin at the same location. (The investigators wanted to see if they could make a synthetic human hemoglobin that resembled the crocodile hemoglobin in terms of its ability to bind bicarbonate anions.) They found the bicarbonate binding site to be located at the $\alpha_1\alpha_2$ -subunit interface, where the two subunits slide with respect to one another during R \rightleftharpoons T transitions. Based on their results the authors modeled a *stereochemically plausible* binding site that included the phenolate anion of Tyr 41, the ϵ -amino group of Lys 38, and the phenolate anion of Tyr 42.

What kinds of interactions do you think the aforementioned amino acid side chains will have with the bicarbonate anion? (It might be helpful to draw the Lewis electron dot structure of bicarbonate).

6. In order to create an engineered human hemoglobin molecule that had the same bicarbonate binding properties as crocodile hemoglobin, twelve amino acid residues had to be changed. Not all of these residues directly interact with bicarbonate—perhaps only three of them do, as described in question 5. What might be the role of the other nine amino acid residues?
7. Other animals have similarly adapted to using small molecules as allosteric effectors to encourage hemoglobin to release its oxygen. Whereas humans use 2,3-BPG and crocodiles use HCO_3^- , birds use *myo*-inositol pentaphosphate (IP_5) and fish use ATP and GTP. The structures of ATP and IP_5 are shown in Figure 9.1. What structural characteristics do all of these molecules have in common and how would they bind to hemoglobin?

Reference

Komiyama, N. H., Miyazaki, G., Tame, J., and Nagai, K. (1995) *Nature* **373**, pp. 244-246.

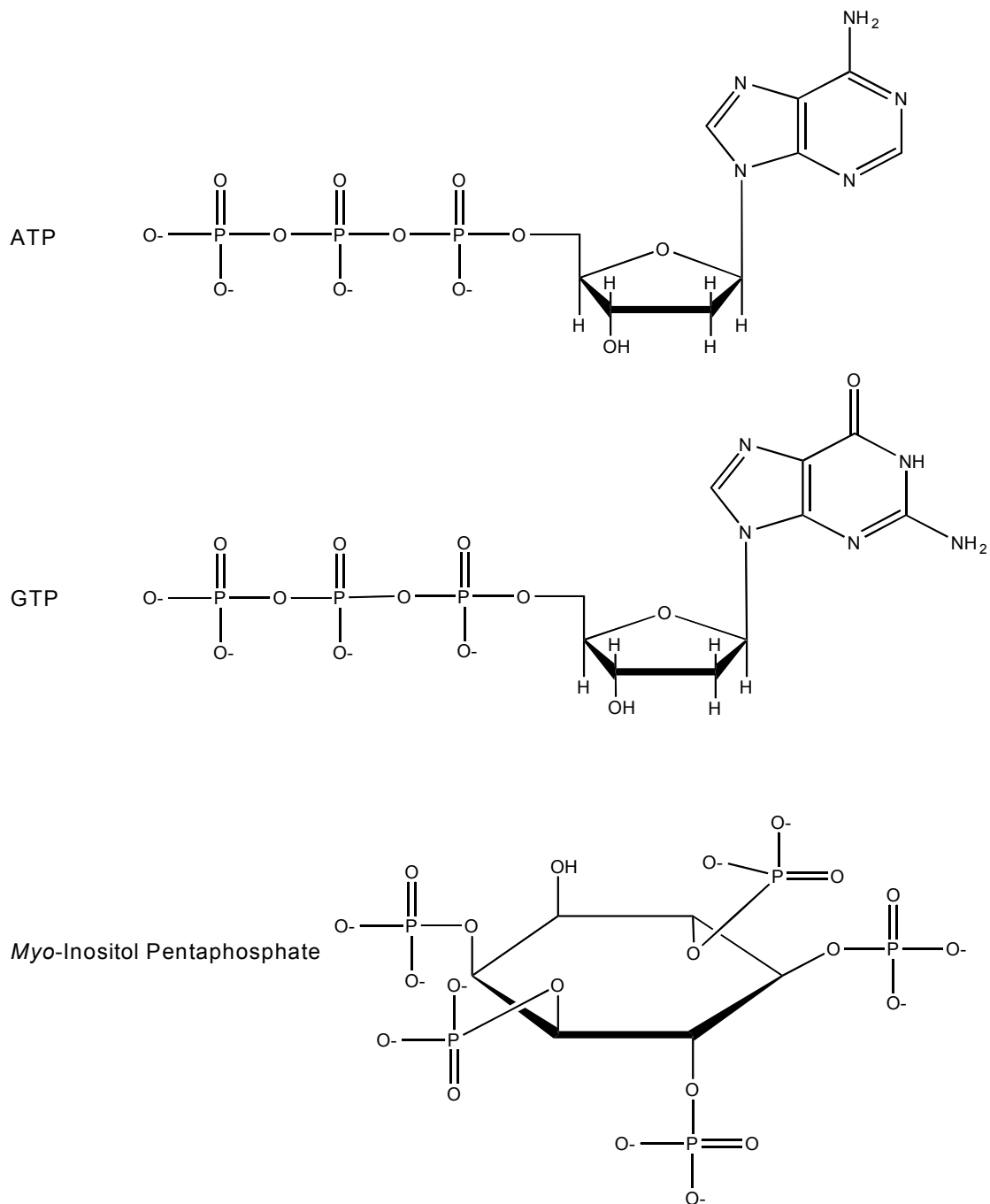


Figure 9.1: Allosteric effectors of hemoglobin in various species.