

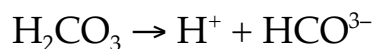
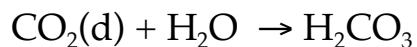
Problem Set #4 Answers

BMB 401 Spring 2004

Problem 1 – The proximal histidine is histidine F8, which coordinates the iron of the heme. This coordination plays a role in preventing oxidation of the heme in order for reversible oxygen binding to take place. This histidine is also a primary player in the structural changes that take place that allow allosteric regulation of oxygen binding. The distal histidine is that which hangs over the plane of the heme, and which helps to form the binding pocket for oxygen. It will allow oxygen to bind in its normal bent conformation; however, it lessens the ability of carbon monoxide to bind, because it prevents it from binding in its normal linear conformation. In addition, it forms a terminal hydrogen bond with the terminal oxygen atom in oxymyoglobin or oxyhemoglobin, thereby stabilizing the interaction of oxygen with the heme prosthetic group.

Problem 2 – From the two models of homotropic allosteric regulation that were discussed, the most important feature is that there must be two or more binding sites for the substrate. Because of the tetrameric nature of hemoglobin, the binding of oxygen at one site can influence the binding of oxygen at other sites. In the case of myoglobin, the protein is a single polypeptide chain with only one heme per polypeptide.

Problem 3 – As we have discussed previously, dissolved carbon dioxide is in equilibrium with carbonic acid. The approach to equilibrium is facilitated by the enzyme carbonic anhydrase.



The proton that is released can bind to hemoglobin. When it does, the deoxyhemoglobin form is stabilized, resulting in negative

heterotropic allostery. This results in a higher partial pressure of oxygen being required in order to bind to hemoglobin.

Problem 4 – At very high ligand concentrations, so many binding sites on the protein are occupied that for all practical purposes, the protein can be considered to exist as one having only one available binding site. Any binding which occurs to such a protein is effectively like that of the last ligand which becomes bound to a protein, and cooperative interactions can be considered to be negligible. At very low ligand concentrations, any binding to the protein can be considered to exist as one having no bound ligands. Cooperative effects are expressed only after most protein molecules have at least one ligand bound.

Problem 5 – If the slope of the Hill plot is 3.0, then the Hill coefficient is 3. If the ordinate intercept is $-.6021$ then the K_d is $10^{0.6021} = 4$