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chapter

5

Protein Function

1. **Relationship between Affinity and Dissociation Constant** Protein A has a binding site for ligand X with a K_d of 10^{-8} M. Protein B has a binding site for ligand X with a K_d of 10^{-6} M. Which protein has a higher affinity for ligand X? Explain your reasoning. Convert the K_d to K_a for both proteins.

Answer: Protein B has a higher affinity for ligand X. The lower K_d indicates that protein B will be half-saturated with bound ligand X at a much lower concentration of X than will protein A. Because $K_a = 1/K_d$, protein A has $K_a = 10^8$ M $^{-1}$; protein B has $K_a = 10^6$ M $^{-1}$.

2. **Negative Cooperativity** Which of the following situations would produce a Hill plot with $n_H = 1.0$? Explain your reasoning in each case.

- (a) The protein has multiple subunits, each with a single ligand-binding site. Binding of ligand to one site decreases the binding affinity of other sites for the ligand.
- (b) The protein is a single polypeptide with two ligand-binding sites, each having a different affinity for the ligand.
- (c) The protein is a single polypeptide with a single ligand-binding site. As purified, the protein preparation is heterogeneous, containing some protein molecules that are partially-denatured and thus have a lower binding affinity for the ligand.

Answer: All three situations would produce $n_H = 1.0$. An n_H (Hill coefficient) of 1.0 generally suggests situation (a)—the classic case of negative cooperativity. However, closer examination of the properties of a protein exhibiting apparent negative cooperativity in ligand binding often reveals situation (b) or (c). When two or more types of ligand-binding sites with different affinities for the ligand are present on the same or different proteins in the same solution, apparent negative cooperativity is observed. In (b), the higher-affinity ligand-binding sites bind the ligand first. As the ligand concentration is increased, binding to the lower-affinity sites produces an $n_H = 1.0$, even though binding to the two ligand-binding sites is completely independent. Even more common is situation (c), in which the protein preparation is heterogeneous. Unsuspected proteolytic digestion by contaminating proteases and partial

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Jianjun Gao



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