Lecture-19

Chapter 10

Protein binding

Kinetics of protein binding

- The kinetics of reversible drug-protein binding for a protein with one simple binding site can be described by the law of mass action, as follows:

  Protein + drug $\longleftrightarrow$ drug-protein-complex

  or

  [P] + [D] $\longleftrightarrow$ [PD] (10.15)

- From the equation 10.15 and the law of mass action, an association constant, Ka, can be expressed as the ratio of the molar concentration of the products and the molar concentration of the reactants. This equation assumes only one-binding site per protein molecule.

$$K_a = \frac{[PD]}{[P][D]} \qquad (10.16)$$

- The extent of the drug-protein complex formed is dependent on the association binding constant Ka. The magnitude of Ka yields information on the degree of drug protein binding.
- Drug strongly bound to protein have a very large ka and exist mostly as the drug-protein complex. With such drugs, a large dose may be needed to obtain a reasonable therapeutic concentration of free drug.

- Most kinetic studies in vivo use purified albumin as a standard protein source, because this protein is responsible for the major portion of plasma drug-protein binding. Experimentally, both the free drug [D]and the protein-bound drug [PD], as well as the total protein concentration [P] + [PD], may be determined.
- To study the binding behavior of drugs, a determinable ratio r is defined, as follows:

$$r = \frac{moles\ of\ drug\ bound}{total\ moles\ of\ protein}$$

➢ Because moles of drug bound is [PD] and the total moles of protein is [P] + [PD], this equation becomes

$$r = \frac{[PD]}{[PD] + [P]} \qquad (10.17)$$

- According to Equation 10.16, [PD] = Ka [P] [D]; by substitution into Equation 10.17, the following expression is obtained:

$$r = \frac{K_a[P][D]}{K_a[P][D] + [P]}$$

$$r = \frac{K_a[D]}{1 + K_a[D]} \qquad (10.18)$$

- This equation describes the simplest situation, in which 1 mole of drug binds to 1 mole of protein in a 1:1 complex. This case assumes only one independent binding site for each molecule of drug. If there are n identical binding site per protein molecule, then the following is used:

$$r = \frac{nK_a[D]}{1 + K_a[D]} \qquad (10.19)$$

- In terms of Kd, which is 1/ Ka, Equation 10.19 reduces to

$$r = \frac{n[D]}{K_d + [D]} \qquad (10.20)$$

- Protein molecules are quite large compared to drug molecules and may contain more than one type of binding site for the drug.
- If there is more than one type of binding site and the drug binds independently on each binding site with its own association constant, then Equation 10.19 expands to

$$r = \frac{n_1 K_1 [P]}{1 + K_1 [D]} + \frac{n_2 K_2 [P]}{1 + K_2 [D]} + \cdots \qquad (10.21)$$

- Where the numerical subscripts represent different types of binding sites, the K's represent the binding constants, and the n's represent the number of binding sites per molecules of albumin.
- These equations assume that each drug molecule binds to the protein at an independent binding site.
- In reality, drug-protein binding sometimes exhibits a phenomenon of cooperativity. For these drugs, the binding of the first drug molecule at one site on the protein molecule influences the successive binding of other drug molecules.
- The binding of oxygen to hemoglobin is an example of drug cooperativity.

Determination of binding constants and binding sites by graphic methods

- In-vitro method(Known Protein Concentration)

A plot of the ratio of r (moles of drug bound per mole of protein) versus free drug concentration [D] is shown in Figure 10-13.

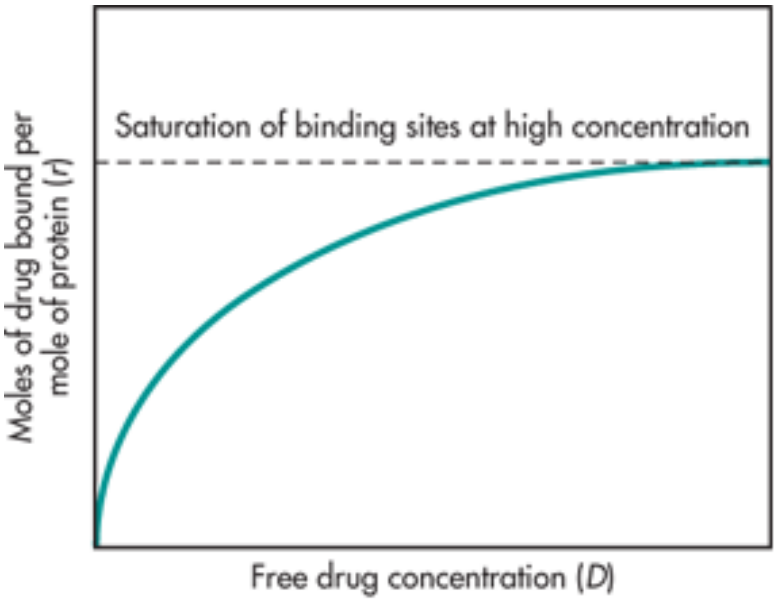

Figure 10-13. graphical representation of Equation 10.20, showing saturation of protein at high drug concentrations.

- Equation 10.20 shows that as free drug concentration increases, the number of moles of drug bound per mole of protein becomes saturated and plateaus. Because of nonlinearity in drug-protein binding, Equation 10.20 is rearranged for the estimation of n and Ka, using various graphic methods.

- The values for the association constants and the number of binding sites are obtained by various graphic methods. The reciprocal of Equation 10.19 gives the following equation:

$$r = \frac{nK_a[D]}{1 + K_a[D]} \qquad (10.19)$$

$$\frac{1}{r} = \frac{1 + K_a[D]}{nK_a[D]}$$

$$\frac{1}{r} = \frac{1}{nK_a[D]} + \frac{1}{n} \qquad (10.22)$$

- A graph of 1/r versus 1/[D] is called a double reciprocal plot. The y intercept is 1/n and the slop is 1/nKa. From this graph (Figure 10-14), the number of binding sites may be determined from the y intercept, and the association constant may be determined from the slope, if the value for n is known.

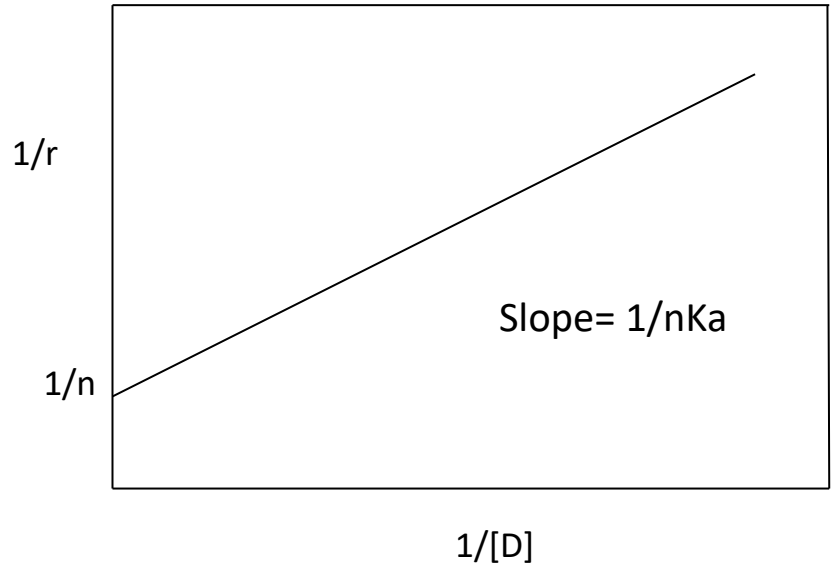

Figure 10-14. hypothetical binding of drug to protein. The line was obtained with the double reciprocal equation.

- If the graph of 1/r versus 1/[D] does not yield a straight line, then the drug-protein binding process is probably more complex.
- Equation 10.19 assumes one type of binding site and no interaction among the binding sites.
- Another graphic technique called the **Scatchard plot**, is a rearrangement of Equation 10-19. the Scatchard plot spreads the data to give a better line for the estimation of the binding constants and binding sites. From Equation 10-19, we obtain

$$r = \frac{nK_a[D]}{1 + K_a[D]} \qquad (10.19)$$

r + rKa [D] = nKa [D]

r = nKa [D] – rKa [D] (10-23)

r/D = nKa -rKa

- A graph constructed by plotting r/[D] versus r yields a straight line with the intercept and slope shown in Figure 10-15

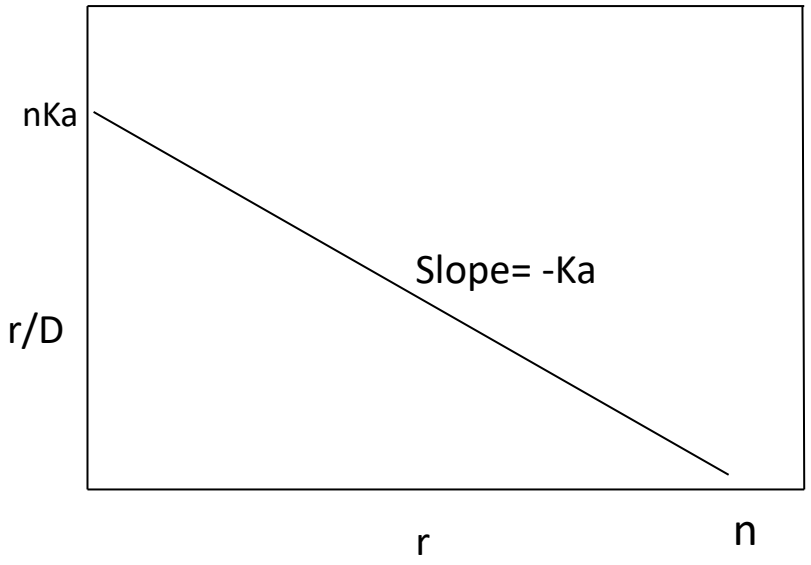

Figure 10-15. hypothetical binding of drug to protein.
The line was obtained with Scatchard equation

- Some drug-protein binding data produce Scatchard graphs of curvilinear lines (Figure 10-17). The curvilinear line represents the summation of two straight lines that collectively form the curve.
- The binding of salicylic acid to albumin is an example of this type of drug-protein binding in which there are at least two different, independent, association constant (k_1 and k_2). Equation 10-21best describes this type of drug-protein interaction.

$$r = \frac{n_1 K_1 [P]}{1 + K_1 [D]} + \frac{n_2 K_2 [P]}{1 + K_2 [D]} + \cdots \qquad (10.21)$$

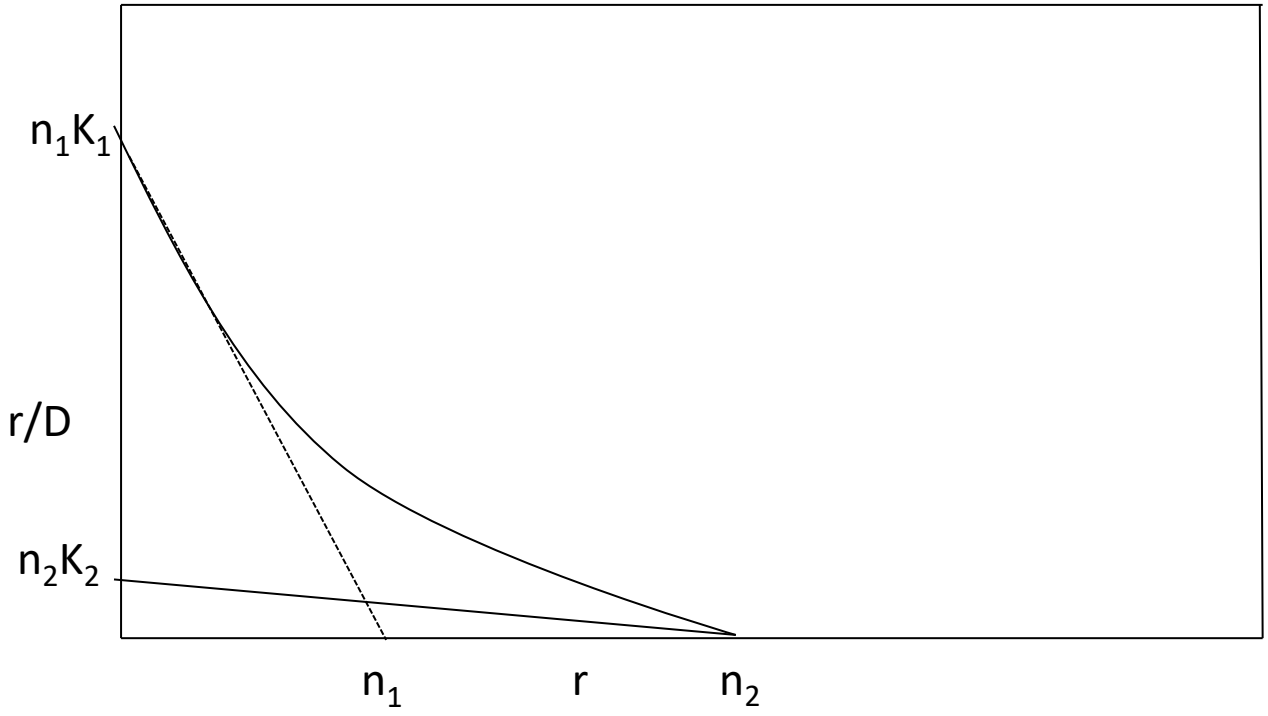

Figure 10-17. hypothetical binding of drug to protein.
The k's repesent independent binding constants and the n's represent the number of binding site per molecule of protein.