# BINDING OF IONS TO OLIGOPEPTIDES

WILLIAM YAP

*From the Analytical Chemistry Division, National Bureau of Standards,*
*Washington, D.C. 20234*

ABSTRACT A model for the binding of ions to oligopeptides, in which nearest neighbor interactions are considered is developed. Equations for the titration curves are derived The apparent association constants are determined as a function of the degree of polymerization and of the interactions between nearest neighbors.

## INTRODUCTION

The binding of ions to polyelectrolytes has been described by many authors, using models which involve the electrostatic interactions between neighboring binding sites on the polyion (1–3). For polyion of high degree of polymerization (D.P.), several equivalent expressions for the hydrogen ion titration curves were obtained and these are independent of D.P. For polyions of small D.P., i.e. oligomers, one would expect that the titration curve and the apparent dissociation constants might depend also on the D.P., and this is indeed the case for oligopeptides of lysine (4). We shall here investigate the binding of hydrogen ions by oligopeptides as described by a model which takes account of nearest neighbor interactions.

## MODEL

Consider an oligomer consisting of $N$ identical units, and each unit having one binding site for hydrogen ion. In the case when the binding of proton by each unit is independent of the others, i.e. no interaction between the units, then the ratio of the probability that the binding site of the unit is occupied by the proton to the probability that it is unoccupied can be written as $k°[H^+]$. Here $[H^+]$ denotes the hydrogen ion activity and $k°$ is the intrinsic association constant for

$$A_i + H^+ \rightarrow A_iH^+$$

where $A_i$ denotes the $i$th unit. A given configuration of the oligomer is specified by giving the states of all the $N$ units, whether they are occupied (+) or empty (−). Thus the relative probability of occurrence of a configuration will be given by a product of $N$ factors, $\prod_{i=1}^{N} f_i$ ; where, when one considers only nearest neighbor interactions, $f_i$ assumes one of the following values: ($a$) $f_i = 1$ if $A_i$ is empty (−);

(*b*) $f_i = \alpha$ if $A_i$ is occupied (+), and $A_{i+1}$ is empty (−), where $\alpha \equiv k^\circ[\mathrm{H}^+]$; and (*c*) $f_i = \alpha\sigma$ if $A_i$ and $A_{i+1}$ are both occupied; here $\sigma$ is an interaction factor, i.e., $-RT \ln\sigma$ is the energy of interaction between two charged neighboring units.

The partition function $Z$ for this system will be the sum of all of these products, summing over all possible configurations. This sum can be obtained by a matrix method for the Ising model for a system of spins, and this method has been developed in detail by Zimm and Bragg for the mathematically equivalent problem of helix-coil transition (5–7). Thus we define a matrix

$$M = \begin{array}{ccc} & & A_{i+1} \\ & - & + \\ A_i \quad - & 1 & \alpha \\ + & 1 & \alpha\sigma \end{array},$$

where the four elements of $M$ give the relative probabilities of occurrence for the four possible pairs of neighbors, i.e. − −, − +, + −, and + +. Then the partition function is given by

$$\begin{aligned} Z &= [1 \quad 0]M^N\{1 \quad 1\}, \\ &= [1 \quad 0][TT^{-1}MTT^{-1}]^N\{1 \quad 1\}, \\ &= [1 \quad 0]T\begin{bmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{bmatrix}^N T^{-1}\begin{bmatrix} 1 \\ 1 \end{bmatrix}, \\ &= [1 \quad 0]T\begin{bmatrix} \lambda_1^N & 0 \\ 0 & \lambda_2^N \end{bmatrix} T^{-1}\begin{bmatrix} 1 \\ 1 \end{bmatrix}, \end{aligned} \tag{1}$$

where $\lambda_1$ and $\lambda_2$ are the eigenvalues of $M$;

$$\lambda_1 = [\alpha\sigma + 1 + \sqrt{(\alpha\sigma - 1)^2 + 4\alpha}]/2,$$

and

$$\lambda_2 = [\alpha\sigma + 1 - \sqrt{(\alpha\sigma - 1)^2 + 4\alpha}]/2,$$

and $T^{-1}$ is the inverse of $T$ which is a matrix of the similarity transformation which diagonalized $M$, i.e.

$$T = \begin{bmatrix} \lambda_1 - \alpha\sigma & \lambda_2 - \alpha\sigma \\ 1 & 1 \end{bmatrix},$$

and

$$T^{-1} = \begin{bmatrix} 1 & \alpha\sigma - \lambda_2 \\ -1 & \lambda_1 - \alpha\sigma \end{bmatrix} \Big/ (\lambda_1 - \lambda_2).$$

Therefore, performing the matrix multiplications, we obtain

$$Z = [\lambda_1^{N+1}(1 - \lambda_2) - \lambda_2^{N+1}(1 - \lambda_1)]/(\lambda_1 - \lambda_2). \tag{2}$$

### *Titration Curves*

The average fraction $\bar{y}_N$ of the binding sites on the oligomers which are occupied is given by

$$\begin{aligned} \bar{y}_N &= \frac{1}{N}\left(\frac{\mathrm{d}\ln Z}{\mathrm{d}\ln \alpha}\right), \\ &= \frac{(2\lambda_2/N + \lambda_2 - \lambda_1)\lambda_1^{N+1} - (2\lambda_1/N + \lambda_1 - \lambda_2)\lambda_2^{N+1}}{(\lambda_1 - \lambda_2)^2[(1 - \lambda_1)^{-1}\lambda_1^{N+1} - (1 - \lambda_2)^{-1}\lambda_2^{N+1}]} . \end{aligned} \tag{3}$$

For the case when there is no interaction, i.e. $\sigma = 1$, then $\lambda_1 = 1 + \alpha$, $\lambda_2 = 0$, and $\bar{y}_N$ reduces to the familiar expression

$$\bar{y} = \alpha/(1 + \alpha). \tag{4}$$

Eq. 3 also assumes the simple expression of Eq. 4 for $N = 1$. As $N$ becomes large, since $\lambda_1$ is greater than $\lambda_2$, $\bar{y}_N$ approaches

$$\bar{y}_\infty = \frac{1}{2} + \frac{1}{2}(\alpha\sigma - 1)\,[(\alpha\sigma - 1)^2 + 4\alpha]^{-1/2} .$$

Figs. 1 and 2 show a number of $\bar{y}_N$ vs. log $\alpha$ curves as calculated from Eq. 3.

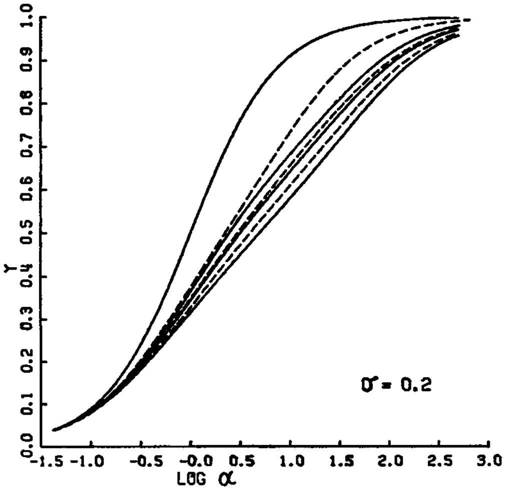

FIGURE 1

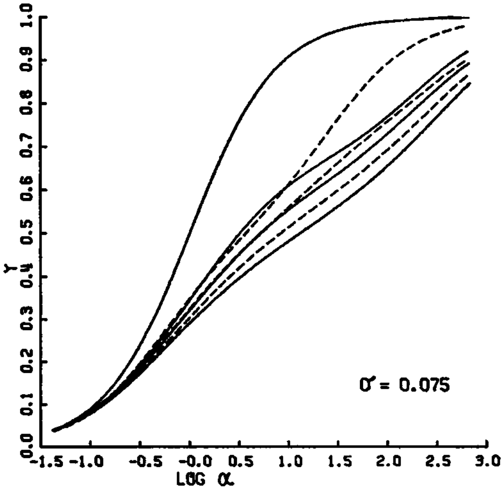

FIGURE 2

FIGURES 1 and 2 Titration curves as calculated from Eq. 3. From left to right: solid curves: $N = 1, 3, 5$, and $\infty$; dash curves: $N = 2, 4, 10$.

### *Apparent Association Constants*

The logarithm of the apparent association constant −pK is taken to be the negative logarithm of the hydrogen ion activity at which $p_N = \frac{1}{2}$. Therefore, setting $p_N = \frac{1}{2}$ in Eq. 3 gives

$$(\lambda_1-\lambda_2)^2\left[\frac{\lambda_1^{N+1}}{1-\lambda_1}-\frac{\lambda_2^{N+1}}{1-\lambda_2}\right] = 2[(2\lambda_2/N+\lambda_2-\lambda_1)\lambda_1^{N+1} - (2\lambda_1/N+\lambda_1-\lambda_2)\lambda_2^{N+1}]. \qquad (5)$$

For $N = 1$, the root of Eq. 5 is $\alpha = 1$, i.e. $\mathrm{pH}_{1/2} = \log k^\circ$ where $\mathrm{pH}_{1/2}$ denotes the negative common logarithm of the hydrogen ion activity at half-saturation of the binding sites. For $N = 2$, the root of Eq. 5 is $\alpha = \sigma^{-1/2}$ or $\mathrm{pH}_{1/2} = \log k^\circ + \frac{1}{2}\log \sigma$. For other values of $N$, the root of Eq. 5 for given $\sigma$ was determined by electronic computer and the $\mathrm{pH}_{1/2}$, which is also the logarithm of the apparent association constant, was calculated from the root. For a given $\sigma$, the roots (i.e., the values of $\alpha$ at $p_N = \frac{1}{2}$) for odd $N$'s form a converging sequence of number and those for even $N$'s form another, and these two sequences overlap at large $N$'s. This is especially obvious for large nearest neighbor interaction (small $\sigma$) as it is illustrated for $\alpha = 0.01$ in Fig. 3, where the change in the common logarithm of the apparent association constant, $-\Delta\mathrm{pK} \equiv \mathrm{pH}_{1/2} - \log k^\circ$, is shown as a function of the degree of polymerization. This difference between the even and the odd $N$'s is related to the fact that the configuration of a $N$-units oligomer, which has the maximum number of bound ions and yet without nearest neighbor interaction, is the one which has every alternating site occupied. For even $N$ this configuration corresponds to

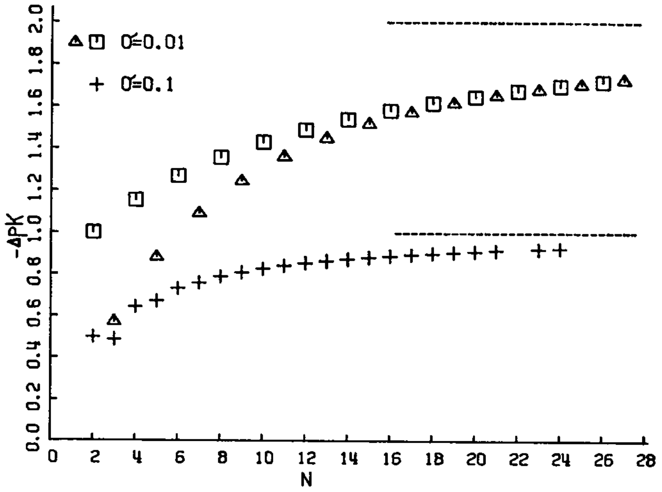

FIGURE 3 Plots of the change of the logarithm of the apparent association constants vs. the degree of polymerization, for $\sigma = 0.01$ and 0.1.

$N/2$ ions bound or a fractional saturation of the binding sites $y$ equal to $\frac{1}{2}$; while for odd $N$ this corresponds to $(N+1)/2$ ions bound or $y = \frac{1}{2} + \frac{1}{2}N$.

The average fraction of the total number of pairs of sites which has nearest neighbor interaction is given by

$$\bar{\xi}_N = \frac{1}{N-1}\frac{d\ln Z}{d\ln\sigma},$$

$$= \frac{1-\lambda_1-\lambda_2}{(\lambda_1-\lambda_2)^2}\cdot\frac{[(N-1)\lambda_1-(N+1)\lambda_2]\lambda_1^N+[(N+1)\lambda_1-(N-1)\lambda_2]\lambda_2^N}{(1-\lambda_1)^{-1}\lambda_1^{N+1}-(1-\lambda_2)^{-1}\lambda_2^{N+1}}.$$

TABLE I

CALCULATED VALUES OF $\bar{\xi}_N$ AT $\bar{y}_N = \frac{1}{2}$ FOR VARIOUS $N$ AND $\sigma$

| $N$ | $\sigma = 0.01$ | $\sigma = 0.1$ |
|---|---|---|
| 2 | 0.0455 | 0.1201 |
| 3 | 0.0055 | 0.0566 |
| 4 | 0.0298 | 0.0900 |
| 5 | 0.0091 | 0.0786 |
| 6 | 0.0274 | 0.0912 |
| 7 | 0.0126 | 0.0899 |
| 8 | 0.0269 | 0.0951 |
| 9 | 0.0160 | 0.0961 |
| 10 | 0.0271 | 0.0986 |

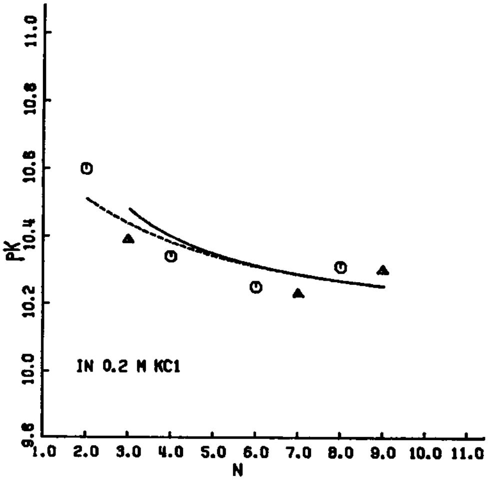

FIGURE 4

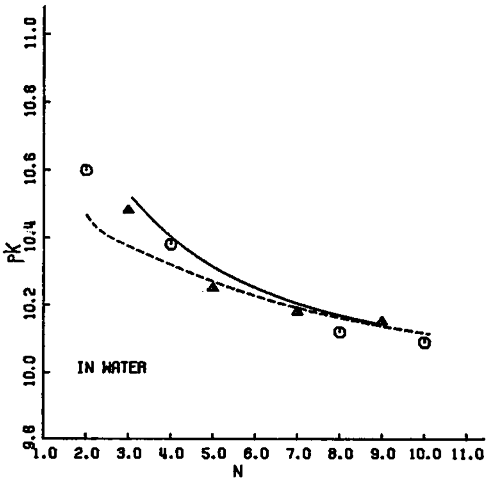

FIGURE 5

FIGURES 4 and 5 Apparent pK's of the ε-amino groups in oligomers of lysines as a function of the degree of polymerization. Experimental points are taken from Yaron et al. (4); △ for odd $N$'s and ⊙ for even $N$'s. Solid curves are for odd $N$'s, dash curves are for even $N$'s; they are calculated with the values of $k^\circ$ and $\sigma$ as given in the text.

Table I gives values of $\bar{\xi}_N$ calculated at $\bar{p}_N = \frac{1}{2}$ for several $N$'s and $\sigma$'s; it shows that for small $N$'s, $\bar{\xi}_N$ tends to be larger for even $N$ than for odd $N$, particularly for large interaction (small $\sigma$). For no interaction, i.e. $\sigma = 1$, $\bar{\xi}_N$ becomes equal to $\bar{p}^2$ and thus has the value of $\frac{1}{4}$ at $\bar{p} = \frac{1}{2}$.

### *Lysine Oligopeptides*

Yaron et al. (4) had done the potentiometric titration of oligomers of lysines. Using Eq. 5 to fit their values of the apparent association constants gives the following sets of values for the intrinsic constants $k°$ and the interaction parameter $\sigma$: (*a*) $k° = 11.05$, $\sigma = 0.0711$ in water; and (*b*) $k° = 10.91$, $\sigma = 0.161$ in 0.2 M KCl. Figs. 4 and 5 show the calculated and the experimental values of the apparent pK for the $\epsilon$-amino groups of the oligomers of lysines as a function of D.P. The fact that the $\alpha$-amino group of these oligomers also varies with D.P. suggests that physical factors other than the nearest neighbor interactions must also be considered. In this brief paper we were mainly interested in investigating the dependence of the binding of ions on the degree of polymerization as predicted by the nearest neighbor interaction model.

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