Lecture Review

- In the previous lecture notes we studied base stacking. We looked at the experimental evidence for it, as well as its thermodynamic and structural basis. We concluded that the best stacking interactions can be as important to stability as formation of weaker Watson-Crick base pairs.

Ionization of Nucleic Acids

Background

A hydrogen atom\(^1\) might seem a small and rather insignificant atom in relation to a large nucleic acid. However, hydrogen atoms play crucial roles in molecular recognition (base pairing) and in catalysis by nucleic acids.

- Following is the general formulation of proton equilibrium based on the Henderson-Hasselbalch equation we all learned in Freshman Chemistry.\(^2\)

\[
HA \leftrightarrow H^+ + A^- \quad \text{(or HA}^+ \leftrightarrow H^+ + A) \\
K_A = \frac{[H^+][A^-]}{[HA]} \\
pK_A = -\log K_A \\
pH = pK_A + \log \left( \frac{[A^-]}{[HA]} \right)
\]

\[\therefore \text{If } pH > (pK_A + 1), \text{ have } > 90\% \text{ A- form} \]

If pH \(< (pK_A - 1), \text{ have } < 90\% \text{ HA form} \quad 3\)

If pH \(= pK_a, \text{ have } 50\% \text{ A-}, 50\% \text{ HA}\)

pK\(_a\) Values of the Nucleobases

- Generally the pK\(_a\) of a nucleobase is determined experimentally by an NMR titration, in which the chemical shift of a resonance (often a \(^{13}\)C label in large nucleic acids) is followed as a function of pH

- In the following figure, the major protonation state of the nucleobases at pH=7 are boxed.

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\(^1\) At times, I will refer to a hydrogen atom as a ‘proton’ since a dissociated hydrogen is simply a solvated proton (no electron and no neutron).

\(^2\) The triple line equal sign means it is a definition, not a derivation. You can arrive at the pH=pK\(_a\)+log([A]/[HA]) simply by taking the log of Ka and using the appropriate definitions. Prove it to yourself!

\(^3\) Note that a pK\(_a\) is a thermodynamic value. I think of two things with pK\(_a\)'s: 1.) The higher the pK\(_a\) value the tighter the proton is bound and the more basic it is. 2.) If the pH is high relative to the pK\(_a\), the function group is w/o a proton, and vice versa.

L16 p1
Due to environment, the $pK_a$ values of bases are most often perturbed from the values above.

Note that the $pK_a$ values of the bases are influenced by salt and temperature, as well as addition of a phosphate. Addition of a phosphate increases the $pK_a$ by 0.2 to 0.6 units since it stabilizes $HA^+$ or destabilizes $A^-$, depending on the case being considered.

The actual $pK_a$ is dictated by the local environment of the base, i.e.: whether the base is stacked, hydrogen bonded, or in a unique electrostatic environment. This will be handled in the L6 discussion paper and below.

Note in the following paper (next page)
- A8, A16-18 have unperturbed $pK_a$ values.
- A25 has a $pK_a$ value is shifted higher, towards neutrality. Why? Protonation might afford a hydrogen bond. Therefore this A desires a proton and becomes more basic. This might be interesting as $pK_a$’s near 7 allow optimal general acid-base catalysis. This type of coupling has positive cooperativity, or positive linkage.
- A4, A12 have $pK_a$ values that are shifted lower, away from neutrality. Why? Protonation of A would disrupt its Watson-Crick base pair. Therefore these A’s loathe this proton and become more acid. This type of coupling has negative cooperativity, or negative linkage.

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This last example is the general case for all Watson-Crick base pairs. Convince yourself that the imino proton of G would be shifted higher than 9, i.e., both the A and G are shifted even further from neutrality when engaged in Watson-Crick base pairs.


Unusual Dynamics and pKₐ Shift at the Active Site of a Lead-Dependent Ribozyme

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Figure 2. (A) pH titration curve of A25 (cross), A8 (open triangles), A16 (open circle), A17 (filled circle), A18 (open diamond), A4 (filled diamond), and A12 (open square) C2 chemical shifts. The y axis is the difference in ¹³C chemical shift between the unprotonated form and the chemical shift at the given pH. (B) Summary of the estimated pKₐ's in the leadzyme (see Materials and Methods). The sequence and secondary structure of the leadzyme are shown where the boxed region indicates the residues that are required for cleavage, and the arrow indicates the site of cleavage.¹⁸,¹⁹
Quantifying Coupling in $pK_a$ Shifting

In proteins or polypeptides, individual residues lose all their zwitterionic character in forming peptide bonds. One must be concerned only with the two chain termini and the amino acid side chains. Two effects will cause these to titrate differently than the corresponding sites in individual amino acids. These are (1) electrostatic interactions caused by the differences in the local chemical structure, and (2) thermodynamic interactions caused by coupling of ionization and conformational equilibria. Consider the difference between the N-terminus or C-terminus of a protein and the amino and carboxyl groups of free amino acids. A large electrostatic attraction between $\text{NH}_3^+$ and $\text{COO}^-$ is present at pH 7 in the free amino acids. This makes it more difficult to remove a proton from $\text{NH}_3^+$ or to add one to $\text{COO}^-$, as shown by the ionic equilibria for oligoalanines in Table 2-1. Although charge effects are very important in Ala or (Ala)$_2$, by (Ala)$_4$ the interaction between terminal charges has ceased to be significant. Box 2-1 reviews the principles of ionization equilibria and illustrates the use of titration data to estimate electrostatic interaction energies.

Box 2-1 PRINCIPLES OF IONIZATION EQUILIBRIA

The ionization reaction $\text{H—A} \rightleftharpoons \text{H}^+ + \text{A}^-$ will be governed by an acid dissociation constant

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{H—A}]}$$

It is convenient to express $K_a$ in a form analogous to pH:

$$pK_a = -\log K_a$$

When conditions are adjusted such that the acid is half ionized, $[\text{H—A}] = [\text{A}^-]$, and $K_a = [\text{H}^+]$ or $pK_a = p\text{H}$. The dissociation constant is related to the standard free energy of dissociation by

$$\Delta G^0 = -RT \ln K_a$$

$\Delta G^0$ is the free energy difference between products ($\text{H}^+$ and $\text{A}^-$) and reactant ($\text{H—A}$) when both are in their standard states (say, 1 M in an aqueous solution). The actual free energy change involved in carrying out the ionization reaction under some other conditions is

$$\Delta G_{\text{ioniz}} = \Delta G^0 + RT \ln \frac{[[\text{H}^+][\text{A}^-]]}{[[\text{H—A}]]}$$

Thus, whenever ($\text{H}^+$), ($\text{A}^-$), and ($\text{H—A}$) satisfy the conditions of the equilibrium constant $K_a$, then $\Delta G_{\text{ioniz}} \leq 0$.

Suppose that the ionization reaction is coupled to some other process or interaction—for example, binding of a proton to $\text{A}^-$ changes the interaction of $\text{A}^-$ with some other group in the molecule. Let the free energy change involved in this interaction be $\Delta G_c$, where the subscript $c$ refers to coupling. Then the total free energy change involved in the ionization reaction is

$$\Delta G_{\text{tot}} = \Delta G_{\text{ioniz}} + \Delta G_c = \Delta G^0 + \Delta G_c + RT \ln \frac{[[\text{H}^+][\text{A}^-]]}{[[\text{H—A}]]}$$

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These next two pages come from Cantor & Schimmel Vol 1.
Equilibria

\[
\begin{align*}
\text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COOH} & \rightleftharpoons \text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- + \text{Ala}^+ \\
\text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- & \rightleftharpoons \text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- + \text{Ala}^- \\
\text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- & \rightleftharpoons \text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- + \text{Ala}^- \\
\text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- & \rightleftharpoons \text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- + \text{Ala}^- \\
\text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- & \rightleftharpoons \text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- + \text{Ala}^- \\
\end{align*}
\]

If the system is allowed to equilibrate, \( \Delta G_{\text{tot}} = 0 \). The \( H^+ \) concentration at which the acid is half ionized is

\[
[H^+]_{1/2} = e^{-\frac{(\Delta G^0 + \Delta G_c)RT}{RT}}
\]

The apparent \( pK_a \) is

\[
pK_a' = -\log (H^+)_{1/2} = (\Delta G^0 + \Delta G_c)/2.303 \ RT
\]

If a model system is available in which there is no coupling, the \( pK_a \) determined in that system is

\[
pK_a = \Delta G^0/2.303 \ RT
\]

Therefore, from the difference in the two \( pK_a \) values, the coupling energy can be evaluated as

\[
\Delta G_c = 2.303 \ RT \left( pK_a' - pK_a \right)
\]

Let us use this result to analyze the electrostatic interaction between the COO\(^-\) and NH\(_3\)\(^+\) groups in alanine, using data given in Table 2-1. The unperturbed \( pK_a \) for dissociation of an \( H^+ \) from COO\(^-\) can be estimated from the titration of (Ala)\(_4\) as 3.42. The perturbed \( pK_a \) for Ala is 2.34. Because \( RT \) is \( \sim 0.6 \) kcal mole\(^{-1}\) at room temperature,

\[
\Delta G_c = (2.303)(0.6)(2.34 - 3.42) = -2.5 \ \text{kcal mole}^{-1}
\]

Thus, for the reaction

\[
\text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COOH} \rightleftharpoons \text{NH}_3^- + \text{CH}(	ext{CH}_3)_2 - \text{COO}^- + H^+
\]

interactions between the NH\(_3\)\(^+\) and COO\(^-\) lower the free energy of the molecule by 2.5 kcal mole\(^{-1}\). This effect promotes easier dissociation, which is expressed as a lower \( pK_a \).
pK\textsubscript{a} of (a) is 3.5. pK\textsubscript{a} of (b) is 6. Therefore, we can calculate

\[
\Delta G^o_{37,c} = 1.38 \text{ kcal/mol} \ \Delta pK\textsubscript{a} = 1.38 (6 - 3.5) = 3.45 \text{ kcal/mol}
\]

**pK\textsubscript{a} Values of the Phosphate Backbone**

- We need to be familiar with pK\textsubscript{a} values of the backbone as well. Here is the equilibrium for a phosphate monoester. Note that for the diester backbone, the equilibrium is pretty much the first (left-hand) one only.

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\textsuperscript{8} This equation comes from the previous pages from Cantor and Schimmel. This is my treatment of this paper, not the paper’s itself.
• Based on the very acid $pK_a$ for the phosphodiester backbone you almost never see it drawn protonated. This means counterions play a very important role in Nucleic Acid stabilization. (more later in the semester).