Computer Bulletin Board

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A Maple Program That Illustrates the Effect of pH on Peptide Charge

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Early in the first semester of a biochemistry course the concept of acid–base chemistry is presented in the context of understanding the structure of amino acids. The Henderson–Hasselbalch equation (more generally referred to as the buffer equation) is used to determine the ionization of these biologically important molecules. Given the structure of an amino acid and the associated pK_a values for the N-terminus, C-terminus, and R-group (if an acidic or basic side chain is present), a student can calculate the charge of the molecule. This is a relatively simple and straightforward arithmetic procedure, but the proper substitution into the equation and the interpretation of the numerical result can be confusing.

$$pH = pK_a + \log([\text{conjugate base}]/[\text{acid}])$$

Henderson–Hasselbalch equation

Initially a student must overcome the preconceived notion that the neutral molecule is always the acid and thus the denominator in the Henderson–Hasselbalch equation. It is imperative that students recognize how structure and protonation dictate the correct definition of acid and conjugate base. For the 20 commonly occurring amino acids the neutral acidic moieties are the protonated C-terminus and the protonated R-groups of aspartic acid (D), glutamic acid (E), threonine (T), serine (S), cysteine (C), and tyrosine (Y). In these cases the functional group changes from an uncharged state to a negatively charged state as the pH increases. The cases in which the neutral unprotonated functional group is the conjugate base and thus the numerator in the Henderson–Hasselbalch equation are the N-terminus, and the R-groups of histidine (H), lysine (K), and arginine (R). In these cases the functional group goes from the positively charged protonated form at low pH to a neutral group as pH is increased.

Another problem arises when students try to predict the charge not of single amino acids, but of short peptide chains. Owing to differences in pK_a, the contribution made by each functional group to the peptide charge is not necessarily canceled by the ionization of an R-group of another oppositely charged functional group. For example, when an acid moiety like aspartic acid is completely deprotonated, a basic amino acid such as histidine may be partially protonated and thus the net charge is negative. The difficulty of calculating the charge of a peptide at different pH values becomes increasingly complex as the number of amino acids in the peptide grows. The quantity of calculations necessary to estimate the charge of the peptide can overwhelm a student, and thus the understanding of peptide charge as it relates to pH is lost in a multitude of calculator entries that need to be performed in order to arrive at "the answer".

The Program Described

To address these problems, I and an undergraduate student, Jay Neidermeyer, coauthored an IBM/PC-compatible computer program for use with the Maple software package (Maple V, release 3, Waterloo Maple Software, Waterloo, ON, Canada) that will calculate the fractional charge of each acidic/basic functional group on an amino acid or peptide chain. The user enters the one-letter codes for the amino acids and then simply strikes the "enter" key. A histogram will then be displayed which animates the change in functional group charge over the pH range 0 to 14 in increments of 0.25 pH units. A second graph is also displayed, which shows the net charge of the peptide over the entire pH range. The second graph can be printed.

As an example, Figure 1 shows three of the animation frames for the peptide sequence alanine–lysine–tyrosine–aspartic acid–glycine (AKYDG). The pH for the particular frame is given in the title line, the amino acid is listed above each bar of the histogram (or on the *x*-axis if the R-group is neutral), and the extent of the ionization from zero to 100 percent is indicated on the *y*-axis. In color, the positive charges are blue bars in the positive half of the graph and negative values are red bars on the lower half. Figure 2 is the second graph of the program. It indicates the total charge of the peptide over the entire pH range (0 to 14). This is the summation of each ionized functional group (total charge on the *y*-axis) at the different pH values (pH on the *x*-axis).

In-Class Use of the Program

In an introductory biochemistry course this program is used, with the appropriate and commonly available computer/overhead projection system, when amino acids are first introduced. For single amino acids the computer calculations are relatively quick (approximately 10 seconds) thus allowing the instructor to demonstrate ionization for any amino acid and describe the values in terms of structural equilibria. Once demonstrated and discussed, the program is then used to look at the more complex situation of several amino acids in a peptide. Here the static plot indicating overall peptide charge at a single pH is especially useful in demonstrating the effect of each functional group in the peptide on the total charge of the peptide.

[W]Supplementary materials for this article are available on *JCE Online* at *http://jchemed.chem.wisc.edu/Journal/issues/1998/Nov/abs1500.html*.

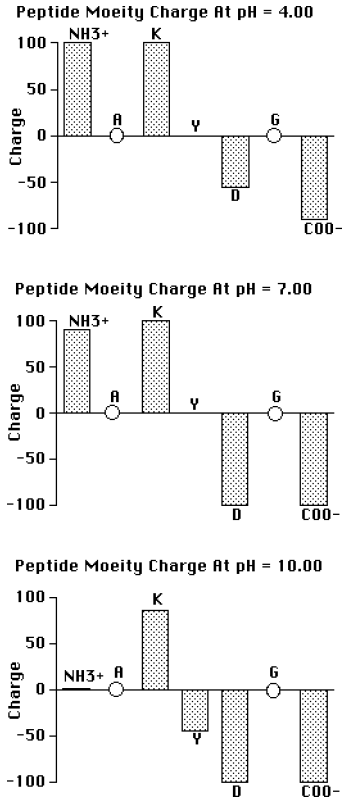

Figure 1. Three frames (pH = 4.0, 7.0, and 10.0) of the animated histogram showing the charge of ionized moieties as a function of pH. As pH increases the fraction of each group (NH_3^+, K) that is positively charged decreases, while the fraction of each group (Y, D, COO^-) that is negatively charged increases. Groups that are not ionized (A, G) are indicated by open circles.

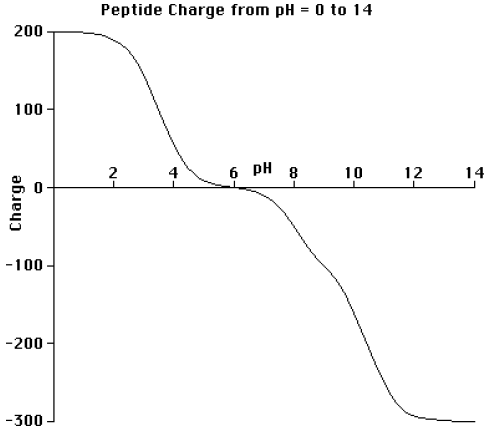

Figure 2. Peptide charge calculated over the range of 0 to 14 pH units. The curve represents the sum of the charges of each ionized group at the pH indicated on the *y*-axis.

The program can also be used as a tool in individual and small-group projects. Assignments are made in which each student or group is given an amino acid that has an R-group from the basic, acidic, and nonionizing category. They are asked to calculate the fractional charge of each functional group of the amino acids at three pH values and provide structural equilibria expressions. They then check their calculations with the program, assuring themselves that they are using the Henderson–Hasselbalch equation correctly and that the charged species in their equilibrium expressions correspond to the charges calculated and displayed in the histogram generated by the program. The fractional charge values are easily verified by clicking on the histogram bar corresponding to the appropriate functional group. The fractional charge of the group, a positive or negative number, is displayed at the bottom of the histogram. The students are then asked to construct a peptide that contains at least one amino acid from each of five categories: acidic, basic, aliphatic, alcohol, and sulfur-containing. For this peptide they use the program to generate an animated histogram and static plot of peptide charge over the entire pH range. In writing, or as a brief class presentation, they can describe their results, answering the following questions:

> Which functional groups contribute to the total charge at a particular pH value?
>
> What are the structural equilibria at a particular pH?
>
> What are the fractional charges at that pH value? (Here the program is especially useful in shortening the time required to perform the calculations with the Henderson–Hasselbalch equation; thus students are able to concentrate on the relationship between the peptide structure and the fractional charges of the functional groups that contribute to the overall charge of the peptide.)

The in-class use of the program combined with student assignments in and out of the class have been well received by students. Students display more confidence when speaking about pH and pK_a values and how they relate to amino acid and peptide structure and partial charges. They have also demonstrated a more profound awareness of the importance of these factors when course discussions expand into enzyme structure, function, and mechanism. Solid evidence of an increase in understanding on the students' part has been demonstrated by an approximately 5 to 10 percent grade increase on exam questions that cover this topic. However, this program has been in place for only one year, and as more classes are exposed its use will continue to be evaluated.

Concluding Remarks

In summation, this program can help students to understand amino acid/peptide chemistry in at least three ways. First, it provides an animated view of the relationship between an acid/base functional group charge and pH. This relationship is grasped quickly by some students, but for others an additional means by which to illustrate this point can help greatly in overcoming the apprehension they might encounter when faced with mathematical relationships. Second, once a student has mastered the relationship between the Henderson–Hasselbalch equation and peptide charge, the program will minimize the repetition of calculation, which can become quite tedious. With this program, getting to the crux of understanding peptide charge and its relationship to

structure–function is quicker because the initial calculations are performed by the computer, thus allowing the student more time to interpret the values. Third, the theoretical charge calculated at various pH values can lead to further explorations and discussions about why a peptide's charge might deviate from the calculated value. The reasons for such deviations are described briefly in the program introduction; the effect of primary and secondary structure on pK_a values of individual functional groups can be significant. Additional insight can be gained by testing the values in a laboratory experiment or by comparing the theoretical ionization of amino acids in a short peptide with that of a similar sequence at the active site of a well-studied enzyme. In the latter case a catalytic acid/base group in an enzyme active site may have a pK_a value that deviates one unit or more from the theoretical value.

This program written for the Maple software system has been used with success in an introductory biochemistry course. Students find the program easy to use, since it does not require any knowledge of the Maple software itself. Once the peptide sequence is entered, the animated graph and static graph are returned to the student for analysis. In the program's introduction the directions for entering the peptide sequence are clearly explained. Although Maple software can be used to analyze complex mathematical operations, it can also be successfully employed to illustrate the connection between the mathematical and the physical world, in this case peptide chemistry. The peptide charge program, written for an IBM/PC operating system, is available through the Internet. It can be downloaded from *JCE Online* at

http://jchemed.chem. wisc.edu/Journal/issues/1998/Nov/abs1500.html.

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