## Amino Acid and Peptide Net Charges: A Simple Calculational Procedure

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Recently, Aronson[1] discussed a problem that beginning students often have in using the Henderson–Hasselbalch equation to estimate the net charge at any pH for biological molecules such as amino acids and peptides. He quite correctly, in my opinion, defined the source of such difficulties as the often hazy understanding of the use of logarithms; an understanding that grows hazier as students rely more frequently upon electronic calculators. In order to help such students, I have derived from the Henderson–Hasselbalch equation a simple expression that will allow the calculation of net charges at any pH without a knowledge of logarithmic manipulations. The sole prerequisite for the use of the expression is a standard scientific calculator.

The simple expression is easily derived from the rearranged ionization equilibrium equation (the Henderson–Hasselbalch equation):

$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{[A^-]}{[HA]}$$

It is important to remember in the derivation that the charge of the *HA* form is positive for a Bronsted acid such as the protonated amino group while the unprotonated group, in the $A^-$ form, is neutral. Thus, for such a group, the goal is to calculate the fraction of protonated species and thereby the *fraction of positive charge*. For an acid such as the carboxyl group, the goal is to calculate the fraction of *un*protonated species and thereby the *fraction of negative charge*. The molecular net charge is then obtained by summing the charge contributions from the various groups. Thus, by following standard procedures for determining the fraction of charge, a general expression, derived from the Henderson–Hasselbalch equation, for the fraction of negative charge, $Q^-$, for functional groups (eg, −COOH, −SH, −PhOH) is as follows:

$$Q^- = \frac{(-1)}{1 + 10^{-(\mathrm{pH}-\mathrm{p}K_a)}}$$

A general expression, derived from the Henderson–Hasselbalch equation, for the fraction of positive charge, $Q^+$, for functional groups (eg, $-NH_3^+$, $=NH_2^+$, $\equiv NH^+$, etc) is as follows:

$$Q^+ = \frac{(+1)}{1 + 10^{+(\mathrm{pH}-\mathrm{p}K_a)}}$$

Thus, one has to remember only two terms of very similar form. The general expression for the molecular net charge at any pH is then given by

$$Q_{\mathrm{molecule}} = \Sigma Q^- + \Sigma Q^+$$

As an example of a calculation, say, one wants to find the net charge of the peptide Asp-Lys-Asp-Lys-Asp-Asp, at any pH. Here the ionizable groups (acid forms) are the *N*-terminus $-NH_3^+$ ($pK_a \sim 8.5$), *C*-terminus −COOH ($pK_a \sim 3.2$), four (4) Asp −COOH ($pK_a = 3.9$), and two (2) Lys $-NH_3^+$ ($pK_a = 10.5$). The $pK_a$ values for the *N*- and *C*-terminal groups will, in general, vary from one peptide to the next; the values used here represent approximate average values. The $pK_a$ values for the peptide side-chain groups are those commonly reported in biochemistry textbooks for the side-chain groups of the respective amino acids. Thus, the general net charge expression for the peptide (neglecting any interactions between the groups) is

$$Q_{\mathrm{peptide}} = \underbrace{\frac{(+1)}{1 + 10^{(\mathrm{pH}-8.5)}}}_{N\text{-terminus}} + \underbrace{\frac{(+2)}{1 + 10^{(\mathrm{pH}-10.5)}}}_{\mathrm{Lys}} + \underbrace{\frac{(-1)}{1 + 10^{-(\mathrm{pH}-3.2)}}}_{C\text{-terminus}} + \underbrace{\frac{(-4)}{1 + 10^{-(\mathrm{pH}-3.9)}}}_{\mathrm{Asp}}$$

If one considers the fraction of protonated or unprotonated species as a function of $(\mathrm{pH}-\mathrm{p}K_a)$, eg, Table 1 of ref 1, one finds that a calculator is needed, for a particular term, only if the exponent of ten is in the range −2.0 to +2.0. That is, if $\pm(\mathrm{pH}-\mathrm{p}K_a)$ is greater than +2.0, the denominator of that particular term is very large relative to the numerator and the term is approximately 0.0. If $\pm(\mathrm{pH}-\mathrm{p}K_a)$ is less than −2.0 then the denominator of that particular term is approximately 1.0, so the term reduces to the numerator. Thus, for example, at pH 7.0 the above general net charge expression becomes

$$Q_{\mathrm{peptide}} = \frac{(+1)}{1 + 10^{-1.5}} + \frac{(+2)}{1 + 10^{-3.5}} + \frac{(-1)}{1 + 10^{-3.8}} + \frac{(-4)}{1 + 10^{-3.1}} = -2.0$$

A calculator would be used on the first term (to determine the value of $10^{-1.5}$) while, by inspection, it is observed that the last three terms simply reduce to their respective numerators. Thus, at pH 7.0, one finds that the net charge of the hypothetical peptide is −2.0.

It has been shown by Tanford and coworkers[2,3] that the hydrogen ion titration curves of proteins can be completely predicted from the unperturbed $pK_a$ values of the ionizable groups when the interactions (eg, electrostatic, hydrogen bonding and hydrophobic bonding) between these groups in the native proteins are rendered negligible by a denaturing solvent medium such as 6 M guanidine hydrochloride (Gdn·HCl); ie, the polypeptide chains exist

in solution as random coils. The above simple net charge expression can be shown to be equivalent to the more elaborate equations used by Tanford and coworkers[2,3] to predict titration curves of proteins in 6 M Gdn·HCl. Accordingly, I have used this simple expression with moderate success to emphasize to students that isoelectric points (pI) determined by dominant-charge procedures, found in many biochemistry textbooks,[4–6] are only estimates of the actual pI values. When such procedures are used for amino acids or simple peptides (ie, peptides with compositions that do not have more than one of any particular residue represented), it is usually found that the calculated net charge is actually zero at the estimated pI. However for more complicated compositions, the dominant-charge procedures often fail readily to yield a good estimate of the pI.

For example, the titration curve of lysozyme in 6 M Gdn·HCl has been measured.[3] The lysozyme sample on which measurements were made contained 129 amino acid residues among which are the following numbers of ionizable groups ($pK_a$ values were determined[3] on reference compounds in 6 M Gdn·HCl): *C*-terminus $-COOH$ ($pK_a = 3.4$), 7 Asp $-COOH$ ($pK_a = 3.9$), 2 Glu $-COOH$ ($pK_a = 4.35$), 1 His $\equiv NH^+$ ($pK_a = 6.5$), *N*-terminus $-NH_3^+$ ($pK_a = 7.6$), 3 Tyr $-OH$ ($pK_a = 9.9$), 6 Lys $-NH_3^+$ ($pK_a = 10.35$), 11 Arg $= NH_2^+$ ($pK_a = 12.5$). Using dominant charge procedures, the pI is usually estimated from the average of the two $pK_a$ values that usually form the boundaries of the zero net dominant charge range. Application of the procedure for lysozyme in 6 M Gdn·HCl shows that the dominant net charge is +3 in the range $9.9 < pH < 10.35$ and −3 in the range $10.35 < pH < 12.5$. Since a dominant net charge of zero does not appear in any of the ranges considered, it is usually not obvious to students which $pK_a$ values one should average to get a good estimate of the pI. Most students will select pI = 10.35, others will select pI = ½(9.9 +12.5) = 11.2, some may even select pI = ½[½(9.9 + 10.35) + ½(10.35 + 12.5)] = 10.8. Using the simple net charge expression the students can calculate which of these would be the best estimate to the actual pI. Using pH = 10.35, 10.8, and 11.2 one calculates net charges of +1.8, −0.3, and −1.6, respectively. Thus, pI = 10.8 would be the best estimate to the measured pI of 10.7 for lysozyme in 6 M Gdn·HCl. In addition to the above instructional uses, the simple net charge expression is well-suited for use in computer programs.

## References


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## Molecular Drama in Biochemical Education


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In medical schools, biochemistry is rarely a favorite course. The freshman medical students find it 'too abstract' and oftentimes 'irrelevant'. Definitely it is not what they regard as 'fun' or 'exciting'. Worse, they come with negative preconceptions (gained from experiences in undergraduate chemistry courses) which are reinforced by well-meaning senior medical students who make it their business to brief the new students on the subjects they are about to take.

Lately, the Department of Biochemistry and Molecular Biology in the College of Medicine, University of the Philippines, has been reaping a lot of good-will from the student population. We would like to attribute this mainly to the teamwork of a cohesive and enthusiastic faculty in making the course more meaningful. Some features of our medical biochemistry are:

(*a*) *Distribution of general instructional and specific behavioral objectives for each of the four units*: 1. Proteins and Enzymes, 2. Energy Metabolism, 3. Nitrogen Metabolism, and 4. Biochemical Aspects of Nutrition and other Special Topics.

(*b*) *Short lectures* Lectures to the entire class of around 140 students last only for an hour to, at most, an hour and a half, three times a week.

(*c*) *Emphasis on preceptorships* These are group sessions of 20 students with a faculty-in-charge. Problem sets, journal reports, case discussions, textbook sessions, laboratory protocols and laboratory reports are discussed during these periods. Preceptorships are scheduled for 2 hours, twice a week.

(*d*) *Rotation of preceptors* A group is assigned to a different preceptor each unit. Thus, a group will have had four preceptors by the end of the semester.

(*e*) *Few laboratory exercises* There are only three laboratory exercises, each of which is treated as a mini-research project. The students prepare the protocols, do the actual experimentations, organize and interpret data and prepare for an oral presentation of results and conclusions.

(*f*) *Structured consultation hours* Consultation with the faculty-in-charge of a unit is part of the printed schedule.

Three years ago, we introduced a 'free-wheeling' learning activity, which we then called "role playing". This activity has become one of the most popular of our educational strategies. We feel that it has contributed a lot to the growing acceptability of biochemistry among the medical students. Role playing is a group presentation at the end of the course. Theoretically, the students have the whole semester (5 months) to prepare for this project. In practice, however, the whole act is conceptualized, rehearsed and presented in a span of a few weeks. Each