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The Identification of Amino Acids by Interpretation of Titration Curves: An Undergraduate Experiment for Biochemistry

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Abstract Undergraduate biochemistry students should have great familiarity with titration curves. These curves allow the prediction of protonation states, charges, and isoelectric points. Here we describe an experiment in which students identify four amino acids based on their titration behavior. Students make solutions of each unknown amino acid and monitor the change in pH upon adding aliquots of a strong base. They identify the amino acids based on the shapes of the curves. They annotate the plots with isoelectric points, pKas, buffering regions and the structures of the amino acids.

Keywords: *titration curve, amino acids, pH, biochemistry, pKa, isoelectric point*

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1. Introduction

Familiarity with amino acid chemistry including pKa values, pI values and protonation states is important for every biochemist. The protonation states of amino acids are important for understanding enzymatic catalysis, pH induced conformational changes, and the intermolecular interactions which stabilize tertiary and quaternary structure of proteins.

Creating a titration curve for a weak acid, such as phosphoric acid or an amino acid [1,2,3,4,6] is a typical undergraduate laboratory experiment. Indeed dozens of examples of titration curve exercises can be found by running a simple internet search. The amino acid titration described here is unique in that the students do not know which amino acid they are titrating. Instead students know they have one of four amino acids which they have to identify based on the shape of their titration curves. This experiment is valuable because students must fully exercise their understanding of amino acid chemistry.

This experiment is a beneficial introductory lab for students in biochemistry. It familiarizes them with how functional groups contribute to form a complex titration curve as well as how pH can influence protonation state [2,4,6,7,8,9]. This experiment requires only minimal laboratory equipment including pH meters and micropipettes. Other reagents include concentrated solutions of hydrochloric acid and sodium hydroxide and the four amino acids.

Students analyze lysine, glutamine, glutamic acid, and histidine (CAS numbers L5501, G3203, G1251 and H8000). These amino acids were selected because they have quite similar molar masses (155.1 for histidine, 146.2 for lysine, 146.1 for glutamine and 147.1 for glutamic acid [7]), so that equal masses of the amino acids will make solutions of approximately the same concentration. This is important so that the titration curves can be superimposed to assist with the correct identification of the amino acids.

Each of these amino acids has a different side chain chemistry (Figure 1). Lysine and histidine are basic amino acids, glutamic acid is acidic and glutamine is a neutral, polar amino acid. The differences in the type of amino acid (Table 1) produce different titration curve signatures.

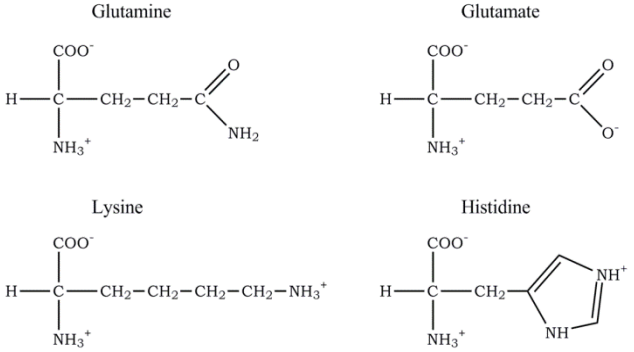

Figure 1. The predominate forms of glutamine, glutamic acid, lysine and histidine as they occur at physiological pH.

Table 1. pKa and pI values of the amino acids utilized in this experiment

Amino Acid	pKa –COOH	pKa $-NH_3^+$	pKa -R	pI
Glutamine	2.17	9.13	N/A	5.65
Glutamic acid	2.19	9.67	4.25	3.22
Histidine	1.82	9.17	6.00	7.59
Lysine	2.18	8.95	10.53	9.74

Each amino acid utilized in this experiment has a different side chain chemistry. Glutamine does not have a titratable R-group and therefore has a distinct titration curve signature from all of the other amino acids who have three titratable groups. Histidine contains an additional pKa value at around a pH of 6 and is thus able to be differentiated from the acidic residue Glutamic acid and the basic residue Lysine.

2. Experimental Procedure

Amino acid solids labeled A, B, C and D are provided and each student is instructed to make 25 mLs of a 20 mM solution of each amino acid. The amino acids in this experiment have comparable molar masses (155.1 for histidine, 146.2 for lysine, 146.1 for glutamine and 147.1 for glutamic acid [7]) and therefore the same mass can be used to prepare each amino acid solution irrespective of the identity. For consistency all students are directed to use the molar mass of lysine.

After calibrating the pH meter, a 60 mM HCl solution is used to bring the pH of each amino acid solution to 2 to ensure all the unknowns have the same starting point. Once a pH of 2 is achieved 1.000 mL aliquots of 50 mM sodium hydroxide are added and the resulting pH is recorded after each addition after each addition is recorded. This process is repeated for each amino acid. All amino acid titrations can be completed in a three hour laboratory period.

Titration curves (pH *vs.* equivalents of base) are graphed, and used to determine the identity of each amino acid. By overlaying at least three curves the identity of each amino acid can be determined. The resultant titration curves are analyzed for pKa values which assists in determination of the identity of each amino acid. In the final lab report students annotate each curve with the experimentally determined pKa values, any buffering regions present, and the structures of the amino acids at the different protonation states. Finally, based on the structures the experimental isoelectric point is determined. A graph generated from student data showing the superimposition of all four titration curves is shown in Figure 2.

2.1. Hazards

Hydrochloric acid and sodium hydroxide may be fatal if swallowed or inhaled, are extremely corrosive, and skin or eye contract may cause severe burns and permanent harm. The amino acids present minimal hazards.

3. Results

As can be seen in Figure 2, each amino acid has a distinct titration curve. The shapes of these curves allow the students to properly identify the amino acids. In addition, this experiment provides a solid foundation of characteristics of amino acids and how their protonation states change at different pH values. Amino acid titration curves are vital to understanding not only how pH influences amino acid chemistry, but are integral to understand protein structure.

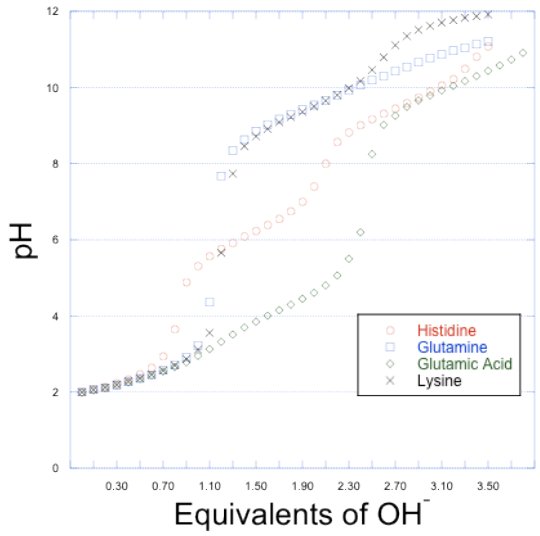

Figure 2. Titration curves of the amino acids histidine, glutamine, glutamic acid and lysine. Based on the shape of the titration curves students are able to differentiate the four amino acids

Each amino acid curve has distinct characteristics that make them easily interpretable. Histidine shows a distinct plateau near pH 6 due to the imidazole side chain. Glutamic acid is easily identified as it contains two acidic groups. The curves between lysine and glutamine are harder to distinguish. The glutamine has an additional inflection point at about pH 11, when the amino group becomes fully deprotonated, whereas the two amino groups of lysine blend together for a steady rise for two equivalents of base. Despite the challenges in identification, students are able to appropriately identify the amino acids based on the superimposition of the curves.

4. Conclusion

This experiment successfully demonstrates the ability to generate and analyze a titration curve to distinguish unknown amino acids. Titration curves are important for students to understand. This experiment takes an abstract concept presented in most General Chemistry and Biochemistry textbooks and makes it tactile. By matching the titration curves with the four amino acids students are required to demonstrate their understanding of side chain amino acid chemistry. By annotating their plots, students demonstrate their understanding of protonation states, buffering regions, and isoelectric points. In addition this experiment gives students practical skills such as making solutions and using pH meters.

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References

[1] Barnum, D., (1999). Predicting Acid-Base Titration Curves without Calculations. *Journal of Chemical Education* 76(7): 938-942.

[2] Bodner, G. (1986). Assigning the pKa's of Polyprotic Acids. *Journal of Chemical Education* 63(3): 246-247.

[3] Heimer, E. (1972). Quick Paper Chromatography of Amino Acids. *Journal of Chemical Education* 49(8): 1.

[4] Herman, D., Booth, K., et al. (1990). The pH of Any Mixture of Monoprotic Acids and Bases. *Journal of Chemical Education* 67(6): 501-502.

[5] Kuehl, L. (1978). A Novel Method for Presenting the Amino Acids in an Introductory Biochemistry Course. *Journal of Chemical Education* 55(11): 3.

[6] Kraft, A. (2003). The Determination of the pKa of Multiprotic, Weak Acids by Analyzing Potentiometri Acid-Base Titration Data with Difference Plots. *Journal of Chemical Education* 80(5): 554-559.

[7] Meister, A. (1965). *Biochemistry of the Amino Acids*. New York, Academic Press Inc.

[8] Sae, S. W. and B. A. Cunningham (1971). Chromatographic separation and identification of amino acids. *Journal of Chemical Education* 48(4): 275.

[9] Weiss, H. (2007). The Roles of Acids and Bases in Enzyme Catalysis. *Journal of Chemical Education* 84(3): 440-442.