1.3.1. Charged Nature of Amino Acid

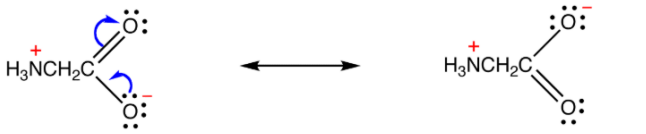
Amino acids are the building blocks used to make proteins and peptides. The different amino acids have interesting properties because they have a variety of structural parts which result in different polarities and solubility.

Each amino acid has at least one amine and one acid functional group as the name implies. The different properties result from variations in the structures of different R groups. The R group is often referred to as the amino acid "side chain".

Zwitterion

Amino acid physical properties indicate a "salt-like" behavior. Amino acids are crystalline solids with relatively high melting points, and most are quite soluble in water and insoluble in non-polar solvents. In solution, the amino acid molecule appears to have a charge which changes with pH. An intramolecular neutralization reaction leads to a salt-like ion called a **zwitterion**. The accepted practice is to show the amino acids in the zwitterion form:

1. The carboxyl group can lose a hydrogen ion to become negatively charged.
2. The amine group can accept a hydrogen ion to become positively charged.



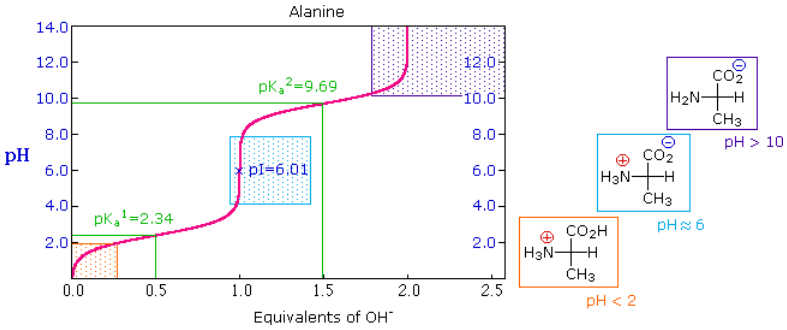
net charge = (+1) + (-1)
= 0

net charge = (+1) + (-1)
= 0

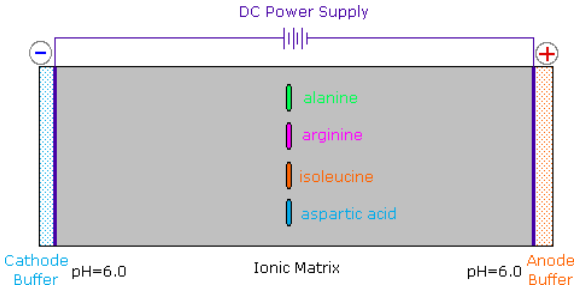
Since amino acids, as well as peptides and proteins, incorporate both acidic and basic functional groups, the predominant molecular species present in an aqueous solution will depend on the pH of the solution. In order to determine the nature of the molecular and ionic species that are present in aqueous solutions at different pH's, we make use of the **Henderson - Hasselbalch Equation**, written below. Here, the pK_a represents the acidity of a specific conjugate acid function (HA). When the pH of the solution equals pK_a, the concentrations of HA and A^- must be equal (log 1 = 0).

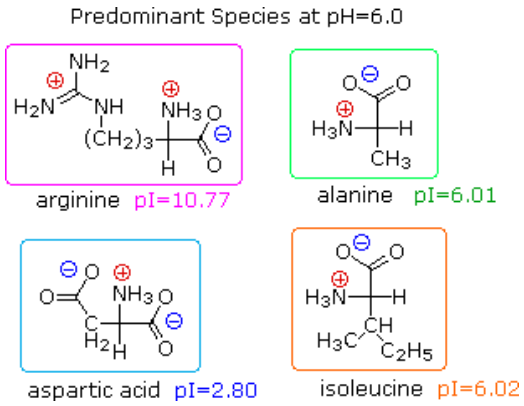
Henderson-Hasselbalch Equation: $pK_a = pH + \log \frac{[HA]}{[A^-]}$

The titration curve for alanine, shown below, demonstrates this relationship. At a pH lower than 2, both the carboxylate and amine functions are protonated, so the alanine molecule has a net positive charge. At a pH greater than 10, the amine exists as a neutral base and the carboxyl as its conjugate base, so the alanine molecule has a net negative charge. At intermediate pH's, the zwitterion concentration increases, and at a characteristic pH, called the **isoelectric point** (**pI**), the negatively and positively charged molecular species are present in equal concentration. Starting from a fully protonated state, the pK_a's of the acidic functions range from 1.8 to 2.4 for $-CO_2H$, and 8.8 to 9.7 for $-NH_3^{(+)}$. The isoelectric points range from 5.5 to 6.2. Titration curves show the neutralization of these acids by added base, and the change in pH during the titration.



The distribution of charged species in a sample can be shown experimentally by observing the movement of solute molecules in an electric field, using the technique of **electrophoresis**. For such experiments an ionic buffer solution is incorporated in a solid matrix layer, composed of paper or a crosslinked gelatin-like substance. A small amount of the amino acid, peptide or protein sample is placed near the center of the matrix strip and an electric potential is applied at the ends of the strip, as shown in the following diagram. The solid structure of the matrix retards the diffusion of the solute molecules, which will remain where they are inserted, unless acted upon by the electrostatic potential. In the example shown here, four different amino acids are examined simultaneously in a pH 6.00 buffered medium. Note that the colors in the display are only a convenient reference, since these amino acids are colorless.





At pH 6.00 alanine and isoleucine exist on average as neutral zwitterionic molecules, and are not influenced by the electric field. Arginine is a basic amino acid. Both base functions exist as "onium" conjugate acids in the pH 6.00 matrix. The solute molecules of arginine therefore carry an excess positive charge, and they move toward the cathode. The two carboxyl functions in aspartic acid are both ionized at pH 6.00, and the negatively charged solute molecules move toward the anode in the electric field.

The Isoelectric Point

As defined above, the isoelectric point, **pI**, is the pH of an aqueous solution of an amino acid (or peptide) at which the molecules on average have no net charge. In other words, the positively charged groups are exactly balanced by the negatively charged

groups. For simple amino acids such as alanine, the pI is an average of the pK_a's of the carboxyl (2.34) and ammonium (9.69) groups. Thus, the pI for alanine is calculated to be: (2.34 + 9.69)/2 = 6.02, the experimentally determined value. If additional acidic or basic groups are present as side-chain functions, the pI is the average of the pK_a's of the two most similar acids. To assist in determining similarity we define two classes of acids. The first consists of acids that are neutral in their protonated form (e.g. CO_2H & SH). The second includes acids that are positively charged in their protonated state (e.g. $-NH_3^+$). In the case of aspartic acid, the similar acids are the alpha-carboxyl function (pK_a = 2.1) and the side-chain carboxyl function (pK_a = 3.9), so pI = (2.1 + 3.9)/2 = 3.0. For arginine, the similar acids are the guanidinium species on the side-chain (pK_a = 12.5) and the alpha-ammonium function (pK_a = 9.0), so the calculated pI = (12.5 + 9.0)/2 = 10.75.

Contributors

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