University of Sydney Library
Electronic Item

COURSE: MBLG1001

Lecturer: Dale Hancock
Amino acids: the building blocks of proteins

COMMONWEALTH OF AUSTRALIA
Copyright Regulation
WARNING
This material has been reproduced and communicated to you by or on behalf of the University of Sydney pursuant to Part VB of the Copyright Act 1968 (the Act).

The material in this communication may be subject to copyright under the Act. Any further reproduction or communication of this material by you may be the subject of copyright protection under the Act.

Do not remove this notice.
Charge.

All amino acids contain charged groups as part of their basic structure. The amino group and the carboxyl group attached to the alpha carbon are charged at certain pHs. Let’s consider the charge pattern of a standard simple amino acid, glycine.

If glycine is placed in a buffer at pH 1 (eg 0.1 M HCl) all the ionisable groups on the molecule will be protonated. The amino acid will look like this:

\[
\text{H}_3\text{N} - \text{CH} - \text{C} \quad \text{O} \\
\text{H} \quad \text{OH}
\]

Glycine, pH 1

The molecule has an overall charge of +1. If this solution is now titrated ie. hydroxide is added slowly (eg 0.1 M NaOH) the OH\(^-\) will draw or strip off the protons from the glycine molecule. The protons removed first will, naturally enough, be those which are not bound as tightly to the glycine molecule. Different chemical groups have different affinities for protons based on the chemical structure of the group and the local environment. The carboxyl group will lose its proton easiest, hence it will be stripped off after the addition of only a small amount of base.

How can we predict which proton will be removed first?

By the pKa. Before you all panic this term is simply the pH at which there is 50% dissociation ie. 50% of the glycine molecules in the –COOH form and 50% in the –COO\(^-\) form. Different chemical groups have different pKas and the quoted pKa for a chemical group will vary depending on the solvent, the ionic strength and the local environment ie other chemical groups around the moiety.

The pKa of the carboxyl group of glycine is ~2.2. That means at pHs below 2.2 most of the glycine molecules will be in the –COOH form and at pHs above 2.2 most will be in the –COO\(^-\) form. So as we progress with the titration to, say pH 7 (physiological pH) most of our glycine molecules will be in this form:
The glycine now has an overall charge of 0. If we continue to add hydroxide the proton will be eventually stripped off the amino group (pKa ~9.6). The glycine will eventually assume this form:

The amino group has now lost its proton

Glycine, pH 11

The glycine has an overall charge of -1.

How to determine the pI of glycine.

The isoelectric point or the pI is the pH where the molecule is electrically neutral ie. its overall charge is 0. The majority of glycine molecules will have an overall charge of 0 between the pHs of 2.2 and 9.6. The point where most of the molecules are neutral is the average of these 2 pKas: (2.2 + 9.6)/2 = 5.9.

The same process can be applied to the other uncharged amino acids; they will all have similar pIs. The pI alters dramatically when the amino acid has a charged side chain ie aspartate, glutamate, histidine, arginine and lysine.

In the case of the acidic amino acids, aspartate and glutamate, the pKas for these side chains occur around pH 4. This means the pH range where the molecule is neutral will be from ~2 to ~4. This gives pIs of ~3.

Basic side chains send the pI up by the same logic. Lysine and arginine have side chains with pKas well above 9 (10.5 and 12 respectively). This means the pH range where the molecule is neutral lies between 9 and 12.
Determining the overall charge of a protein.

When studying proteins it is usually not the pI of the isolated amino acid we are interested in but the overall pI of the protein. To do this we must first appreciate that only the charge of the side chain has to be considered when dealing with amino acid residues in a protein. The amino group and the carboxyl group attached to the alpha carbon have lost their charge in the formation of the peptide bond.

So when considering the pI of a protein it is mainly the proportion of acidic residues (asp + glu) to basic residues (his + arg + lys) that should be considered. If there are more acidic groups than basic groups the pI will be lower and if the ratio reverses the pI will be higher. Examples of proteins with high pIs are histones (pI ~10 – 12). These small proteins have a large proportion of arginines and lysines which give the protein an overall positive charge at pH 7. This property enables them to bind to DNA (-vely charged).

Protein charge at a given pH is used extensively in the purification of proteins. To predict the charge at a given pH you need to know the pI of the respective proteins.

Buffers

Most reactions in Molecular Biology and Biochemistry must be carried out under tight experimental conditions; temperature, ionic strength and pH must all be controlled. Enzymes only work in a very narrow range of temperature and pH. For this reason buffers are almost always used in any reaction. A buffer is a solution which resists a change in pH when challenged with either base (OH-) or acid (H+).

Most buffers are either weak acids or weak bases; mixed with their appropriate conjugate base or acid. These solutions buffer best ± 1 pH unit either side of their pKa. The ratio of the acid and its conjugate base (or base and its conjugate acid) will determine the pH within this range. To work out the ratio of acid to base you employ the Henderson Hasselbalch equation (this equation usually incites fear in most students). For the record the equation is:

$$\text{pH (that you require)} = \text{pKa} + \log \frac{[A^-]}{[HA]}$$

If you know the pH required and the pKa of the species the log term is the ratio of the protonated (HA) over the deprotonated (A-).

The concentration of the buffer will determine its buffering capacity but it is the ratio of HA:A- that determines the pH. If you check out the glycine titration curves you will see that they are flat around the pKa i.e. there is little change in pH despite the addition of H+ or OH-. The amount of acid (H+) or base (OH-) the buffer can handle before changing its pH dramatically is its buffering capacity. Once all the available HA or A- have been saturated with OH- or H+ there is no more buffering and the pH changes dramatically. In biological systems such as serum or inside your cells there are natural buffering systems to regulate pH. This is essential for the functioning of enzymes and life itself!