

2.4: The Effect of pH

Hydrogen ions, or protons, are crucial supplies in various chemical and biological processes. The available level of these supplies, essentially, is what we mean by pH.

"Low pH" corresponds to a very high concentration of protons. "High pH" corresponds to a very low concentration of protons.

Mathematically, $pH = -\log[H^+]$. A chemically more correct statement in water is $pH = -\log[H_3O^+]$, since in water there will be no free protons; the protons will be bound to water molecules, forming hydronium ions. However, in some of our discussions we will simplify and refer to it as $[H^+]$. The use of pH rather than $[H_3O^+]$ allows for comparison of concentrations over a much larger range, since we are using a logarithmic scale.

$[H_3O^+]$ (mol L ⁻¹)	pH
0.1	1
0.01	2
0.001	3
0.0001	4
0.00001	5
0.000001	6
0.0000001	7

The log scale just highlights the number of decimal places in the number.

The concept of pH goes further than that, however. Water contains polar bonds that are able to ionize, forming a hydroxide ion and a proton. An individual water molecule does not ionize very easily, but given a very, very large number of water molecules, a few of them would be found in this ionized state. Water will contain a few hydroxide ions and a few protons.

Regular, garden-variety water typically has pH close to 7. It might range a little lower or higher depending on what minerals are dissolved in it. A very low pH, maybe 1 to 3, would be considered very acidic. A very high pH, maybe 12 to 14, is very basic. At low pH, water contains lots of protons and very, very few hydroxide ions, if any. That's because the equilibrium between ionized and non-ionized water gets pushed to the non-ionized side by the extra protons. That's Le Chatelier's principle. At high pH, there is actually an overabundance of hydroxide ions and essentially no free protons. That's because any free protons react with the hydroxide ions to re-form water. That's Le Chatelier's principle, again.

The pH has an influence on the redox potential of a metalloprotein because free protons or hydroxide ions alter the protonation state of the protein. Free protons can add to basic nitrogen sites, increasing the positive charge on the protein (or lowering negative charge). Hydroxide can remove protons from acidic sites, increasing negative charge (or lowering positive charge) on the protein.

Let's take a look at what happens to an amino acid when it undergoes a drastic change in pH. We'll use alanine as an example. Starting at pH 1, alanine actually has a plus charge. The amino end of the compound is protonated. As pH increases, the concentration of free protons drops further and further. Physically, we would carry out this change by adding a base such as hydroxide ion to consume the free protons. Eventually, the equilibrium shifts and the carboxylic acid group, the most acidic position in the molecule, releases its proton to replace the ones that were removed from solution. At that point, the charge on the alanine is overall neutral.

Going even further, eventually the protonated amino group loses its proton, too. At that point, the alanine has an overall negative charge.

Alanine has two pK_a values over the common pH range of 1-14. It has an acidic carboxylic acid group and a potentially acidic quaternary ammonium group.

? Exercise 2.4.1

Define the following equilibrium constants of alanine, in terms of concentrations of species.

a) K_{a1} b) K_{a2}

? Exercise 2.4.2

Define pK_a in terms of K_a .

? Exercise 2.4.3

Show that, for an amino acid such as alanine, pK_{a1} is equal to the pH at which the acidic group is 50% ionised; i.e. there are equal amounts of ionised and non-ionised molecules.

Several amino acids have acidic or basic side chains. In that case, there would be an additional protonation state. As a result, many amino acids have three different pK_a values. Examples are shown in the table below.

amino acid	pK_{a1}	pK_{a2}	pK_{a3}
arginine	2.03	9.00	12.10
aspartic acid	1.95	3.71	9.66
cysteine	1.91	8.14	10.28
glutamic acid	2.16	4.15	9.58
histidine	1.70	6.04	9.09
lysine	2.15	9.15	10.67

? Exercise 2.4.4

For each entry in the table of amino acids above, assign the pK_a value to the acidic/basic site in the structure

? Exercise 2.4.5

Draw the structure of each of the amino acids in the above table at neutral pH (pH 7).

? Exercise 2.4.6

Amino acids have an α -position, next to the carbonyl. Why doesn't that position have a pK_a value in the table above?

Answer

The pK_a of an α -position is typically about 20, although it can vary depending on what other groups are nearby. That is too high to build up a significant amount of the deprotonated species in water, which has a pK_a (in water) of 14.

As a result of these structural changes at different pH, proteins can change protonation states when the pH changes. Such a change would have a dramatic impact on the properties of the protein. One of these properties is reduction potential. As we have already seen, charge is one of the factors that has a strong influence on the reduction potential of a metalloprotein.

Because a protein might have lots of acidic or basic amino acids in the vicinity of the metal center, the effects of pH change could be very complicated. Some sites might become protonated during a change in the environment, whereas others might become deprotonated.

? Exercise 2.4.7

Predict whether the reduction potential of an Fe^{3+} center would increase or decrease in the following situations.

- There is a nearby histidine; pH changes from 7 to 5.
- There is a nearby aspartic acid; pH changes from 4 to 3.
- There is a nearby glutamic acid; pH changes from 4 to 5.

Answer a

a) The pK_a of the side chain of histidine is about 6.0; upon changing from pH 7 to pH 5, this group would become protonated and positively charged. The positive charge would be more stabilising (or less destabilising) toward Fe^{2+} than Fe^{3+} ; the reduction potential would increase.

Answer b

b) The pK_a of the side chain of histidine is about 3.7; upon changing from pH 4 to pH 3, this group would become protonated and positively charged. The positive charge would be more stabilising (or less destabilising) toward Fe^{2+} than Fe^{3+} ; the reduction potential would increase.

Answer c

c) The pK_a of the side chain of histidine is about 4.2; upon changing from pH 4 to pH 5, this group would become deprotonated and negatively charged. The negative charge would be more stabilising (or less destabilising) toward Fe^{3+} than Fe^{2+} ; the reduction potential would decrease.

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