A brewer's biochemistry

By **Professor Charles Bamforth**
Department of Food Science and Technology, University of California, Davis, USA.

This is the first of a series of articles aiming to position malting and brewing in biochemical terms for the benefit of those who have received no training in this area of science. In fact no formal scientific training is assumed in the reader so some basic chemical principles are included in an accompanying FactFile.

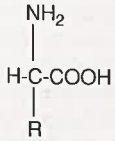
Part 1: Proteins

Proteins play a number of critical roles in nature. These include:

- structural roles (e.g. the collagen found in many tissues, including the swim bladders of fish that end up as isinglass finings)
- the carrying of other molecules around (e.g. the transport proteins which have a role in selectively moving substances across membranes, such as the membrane around brewing yeast)
- imparting mobility (e.g. in the whip-like flagella found on certain spoiling micro-organisms and which propel the beasts along)
- protection, as antibodies that are produced by higher organisms in response to the presence of "foreign" materials (such antibodies are increasingly widely used in selective tests for certain materials of interest to the brewer)
- catalysis. Here I am referring to the enzymes, including those from malt and yeast, which are critical in the brewing and fermentation processes.

Amino acids

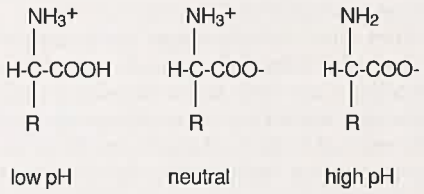
Proteins are polymers (see panel in FactFile) of amino acids. Amino acids are so-called because they have an amino group ($-NH_2$) and a carboxyl acid, (-COOH) group. There are twenty of them commonly found in proteins and they share a general formula:



Each amino acid has a different 'R' group. These are shown in Figure 1. The simplest, in glycine, comprises a solitary H atom. Others, such as that in tryptophan, are quite complex. The properties of these R groups impact greatly on the properties of the individual amino acids, but also on the properties of proteins in which they are found. The oddity is proline, which in the strictest terms is not an amino acid (so I show its full formula), but it obviously has close similarities to the amino acids.

Both the amino group and the carboxyl group of amino acids can become charged (see FactFile). Carboxyl is an acidic group, i.e. it releases hydrogen ions (H^+). Amino is a "basic" group because it accepts hydrogen ions:

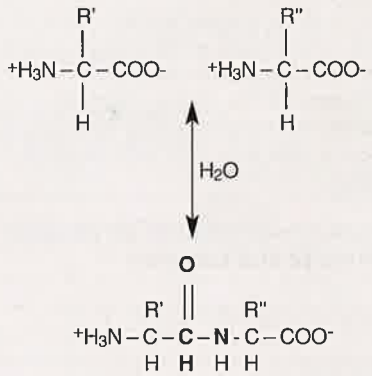
If the pH is very low (high concentration of H^+), then the carboxyl group will tend to be in the uncharged form (-COOH), whereas the amino group will tend to be in the positive ionic form ($-NH_3^+$). The converse applies at high pH. For intermediate pH's the majority of the molecules will have both a positive and a negative charge – they are *dipolar*.



This ability of amino acids to alternately "soak up" and release H^+ means that (within limits) they are able to regulate the pH of solutions that contain them – i.e. they are *buffers*. As such they play an important role in regulating the pH of wort and beer.

Peptide bonds

If two amino acids come together, then a molecule of water can be split out between them and a bond formed that joins them together. This is called a "peptide" bond (bold in the diagram):



The product is a *dipeptide*. This reaction is reversible: by adding water, the dipeptide will be split into its corresponding amino acids.

There are a very large number of possibilities for dipeptides. For any two amino acids, there are two dipeptides. Take glycine and alanine for instance. The first dipeptide will have the peptide bond formed between the $-COO^-$ of glycine and the $-NH_3^+$ group of alanine. The second has the peptide bond formed between the $-COO^-$ of alanine and the $-NH_3^+$ of glycine. They are quite distinct, have their own properties. Convention has it that peptide structures are written with the free (unbound) amino group to the left and the free carboxyl group (the one not tied up in a peptide bond) to the right. Thus the first of our dipeptides would be glycyl-alanine. The second would be alanyl-glycine.

There is essentially limitless scope for more and more amino acids to be joined on to the chain. Broadly speaking if there are between 2 and around 10 amino acids in the chain then we have a *peptide* or *oligopeptide*. If there are more then we have a *polypeptide*.

In these polymers only one of the amino acids has a free amino group (apart from the amino groups in the side-chains of certain amino acids, see Figure 1) and this is called the *N-terminus*. Similarly only one amino acid has a free carboxyl group (apart from the carboxyl groups in the side-chains of glutamate and aspartate, see Figure 1) and this is called the *C-terminus*.

Most polypeptides found in nature contain between 50 and 2000 amino acids. The average molecular weight of an amino acid is approx. 110, so the molecular weight of polypeptides may be as much as 220,000 (220K). (It seems to have become the norm to insert the word "daltons" after molecular weights, in honour of the Manchester-based professor who did the pioneering work on atomic structure. This is strictly incorrect, as molecular masses are unitless. They are ratios of size on a scale that sets the smallest atom,

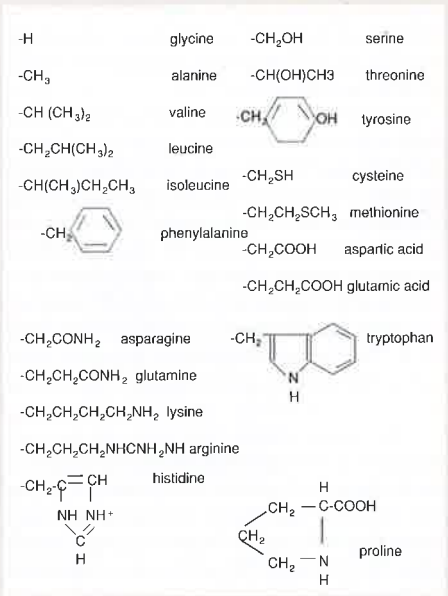


Fig 1 The side-chains in amino acids.

Fig 2 Ionic bonds.

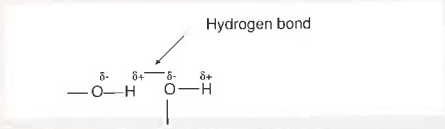


Fig 3 Hydrogen bonds.

hydrogen, with a molecular mass of one.)

The importance of amino acid sequence

The sequence of amino acids in a polypeptide chain is called its *primary* structure. It is this which determines the properties of the polypeptide, for the simple reason that the *folding* of the polypeptide chain is determined by the opportunity for amino acids in different regions of the chain to interact. This folding is referred to as *secondary* and *tertiary* structure.

Various forces dictate these interactions. There may be strong attractions between positively and negatively charged groups in the side chains of the amino acids – as every schoolchild knows, opposites attract, i.e. positive attracts negative.

Equally, like charges repel, so that positive repels positive and negative repels negative. And thus the impact of this on the shape of a protein molecule depends on how many of the amino acids are present that have these charges on their side-chains. It also depends on what the pH is, as this will influence the proportion of the groups that are present in a charged form or an uncharged form (see earlier).

If the conditions are right, then it may be that amino acids quite a long way apart in the molecule come close together and are "cemented" by these strong ionic interactions (Fig 2). In this way the polypeptide chain adopts a certain shape.

Other interactions can occur. Some of the most important are called *hydrogen bonds*. Suffice to say (and for reasons that we needn't go into) in the chemical grouping containing one oxygen and one hydrogen (i.e. –OH, the so-called *hydroxyl* group) the oxygen atom is slightly negatively charged and the hydrogen atom is slightly positively charged. Therefore if two separate –OH groups come close together there can be an interaction between their respective positive and negative regions (Fig 3).

Individually, these hydrogen bonds are weaker than the fully-fledged positive-negative bonds referred to above, but cumulatively they can be very significant. Similar bonds can occur with N-H groups. In fact hydrogen bonds involving –OH and –NH groups are responsible for the coiling of some regions of proteins rather in the way that the cord linking a telephone receiver to the main unit twists into a helix. Alternatively these bonds can link adjacent regions of proteins into sheet forms.

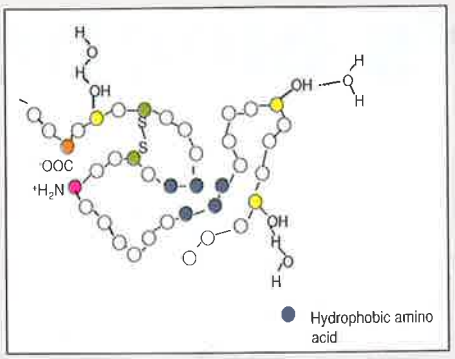


Fig 4 The types of interactions responsible for 3-dimensional structure of polypeptide chains.

This hydrogen bonding is most marked in water, where of course there are lots and lots of –OH bonds and whole network structures can be formed between adjacent molecules. It's a very cosy situation – and a party very difficult to break up by intruder molecules and groups. Examples of the latter types of group are the amino acids with hydrocarbon side-chains (i.e. those amino acids who's side-chains are entirely composed of C and H).

They can't make hydrogen bonds and so they are "excluded from the club". In fact they tend to be banished to the inside of protein molecules where they associate together. They are referred to as *hydrophobic* amino acids, because of this "water-hating" tendency. It is the *hydrophilic* groups that predominate on the outer surface of the proteins because they can "pal on" with the water molecules – for example the –OH group of serine is perfectly able to hydrogen bond with water.

Another type of bond that determines the shape of protein molecules is the so-called *disulphide* bridge. Amino acids containing –SH side chains may come together with the hydrogen atom of each being removed as the two sulphur atoms link. The bond formed is even more resistant to breakage than an ionic bond and in some proteins these bridges are very important for determining the overall shape.

Fig 4 gives a hypothetical picture of how these various types of interactions determine the shape of a protein molecule. You need to try to imagine these things happening in 3-dimensions - we are dealing with essentially spherical structures – i.e. footballs rather than Frisbees.

So why does protein structure matter to the brewer?

It matters for various reasons.

Enzymes

Let's start by considering the enzymes that are so important for producing brewer's wort and which are present in the yeast where they act in sequence to convert sugars into alcohol. Enzymes are all proteins, each of them capable of effecting its own specific reaction. Amylases exclusively break down starch, β-glucanases break down β-glucans, proteinases break down proteins, and so on.

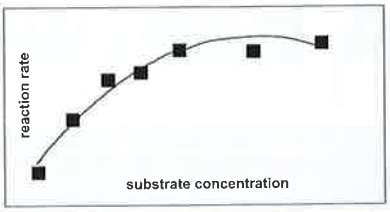


Fig 5 The relationship between

Enzymes are biological catalysts: molecules present in all living organisms that are responsible for speeding up the reactions occurring in those systems (see FactFile). The molecules that they act on are called *substrates*, the molecules formed are *products*.

The more enzyme available, the faster the reaction. We can liken them to turnstiles at a football ground: the more there are, the quicker can customers get through. The relationship between the speed of an enzyme-catalysed reaction and substrate concentration is not so simple and the relevant graph usually has a shape like that shown in Fig 5. At a certain substrate concentration the system becomes "saturated": increasing the substrate concentration no longer causes the reaction to go any faster.

The explanation is that the enzyme binds on to the substrate molecule to form an "enzyme-substrate complex". This then breaks down to re-form the enzyme (ready to grab another substrate) and release the product. This binding occurs at the so-called "active site".

There is a unique shape in each enzyme molecule that recognises the substrate. The shape of the protein molecules determines this niche, by the way in which it folds on itself through the interactions I described earlier. It's rather like a space module docking with the mother ship. A US module needs a US docking port, not a Russian one (by and large!)

The active site might comprise amino acids from quite distinct parts of the enzyme molecule (Fig 6), As enzymes are relatively flimsy, anything which will tend to drive these amino acids apart will disrupt the active site, prevent substrate binding and destroy enzyme activity.

Such factors include heat which causes the protein to jiggle about until it loosens up, and changes in pH, which alter the proportions of positive and negative groups in the proteins and therefore the opportunity for the charge-charge interactions which hold the shape of the molecule. These are the reasons why extremes of temperature and pH are to be avoided. Enzymes differ in their tolerance of temperature and pH.

As well as their effect on the integrity and "survival" of the protein molecule, temperature and pH also impact directly on the rate of the reaction that the enzyme catalyses. All chemical reactions are accelerated by heat, with the rule of thumb being that a 10°C rise in temperature speeds up a reaction by 2-3 fold.

This applies to reactions catalysed by enzymes, with the important rider that

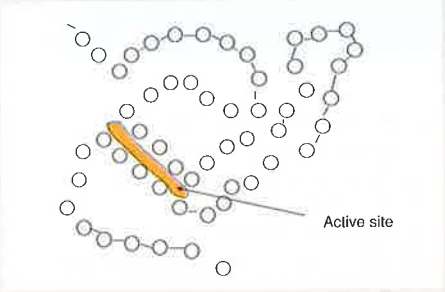


Fig 6 The concept of "active site."

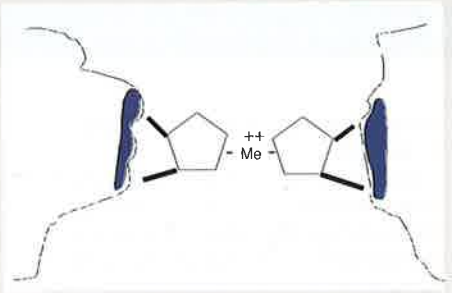


Fig 7 A model for iso-α-acid – metal ion – protein interaction in foam. The iso-α-acids are extremely stylised, depicting only two of their three hydrophobic arms (—), how these hydrophobic arms interact with hydrophobic regions in polypeptides and how the negative charges in adjacent iso-α-acids are neutralised by bridging through a divalent metal cation (such as magnesium, manganese or zinc). In this way large complexes between separate proteins and iso-α-acids are formed and strength is imparted to bubble walls.

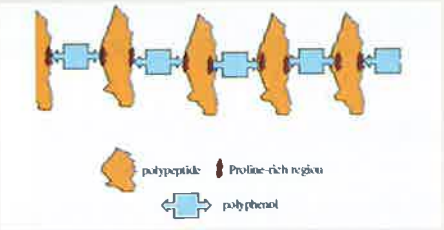


Fig 8 A model for polyphenol-protein interactions in haze.

increasing heat will also tend to disrupt the enzyme structure, deform the active site and therefore prevent the enzyme from doing its job. Thus the net rate of reaction observed is a balance dependent on how resistant the enzyme is to heat. Enzymes in mashes such as α-amylase and peroxidase are very resistant to heat, whereas others like β-glucanase and β-amylase are much more heat sensitive.

pH, too has impacts other than on the stability of the enzyme. We need to remember that the active site comprises several amino acids drawn from different parts of the overall polypeptide (and perhaps even several polypeptides – some enzymes contain 2 or 4 separate polypeptide chains and are said to have *quaternary* structure). Some of these amino acids, such as glutamic acid or lysine, have side chains that can become charged (see earlier).

It may be, for instance, that the enzyme uses the negative charge of a glutamic acid residue to bind the substrate. If that were the case, too low a pH, which would make the glutamic acid uncharged, would lead to a cessation of enzymic activity. Furthermore, some substrates can exist in uncharged or charged forms and may need to be in one or other form in order to become attracted to the enzyme. In fact most enzymes operate effectively only within a narrow pH range. Most of those of relevance in mashing happen to work best in the pH range of mashes (between 5 and 6)

It is not only changes in pH which can interfere with enzyme activity and stability. Enzymes are also susceptible to inactivation by other agents (*inactivators*). One such substance is the copper ion (Cu^{2+}). This snares –SH groups and screws up irreversibly those proteins that depend on –SH groups for their function.

Other molecules which can block enzyme activity do so reversibly (i.e. if you take them away enzyme activity is restored) and they are known as *inhibitors*. Basically there are four types of inhibition that we need to concern ourselves with. Remarkably, whilst they each almost certainly occur in brewery systems, they haven't been studied in great detail on an enzyme by enzyme case.

The first, *competitive inhibition*, results when a molecule is so similar to the substrate molecule that it can "recognise" the active site and bind to it, but is nonetheless sufficiently different that it just sits there and can't be tackled by the enzyme. It gets in the way of the substrate. However if the substrate concentration is high enough then it will squeeze out the inhibitor and inhibition is overcome.

In *non-competitive inhibition* the inhibitor binds to a site on the enzyme that is not the active site and, in so doing, distorts the shape of the molecule so that the active site is no longer available to substrate. Removing the inhibitor allows the enzyme to slide back into shape, but in this case increasing the substrate concentration cannot squeeze out the inhibitor.

At very high substrate concentrations we can sometimes encounter *substrate inhibition*. Once again we can turn to a football stadium for an analogy: if the crowd is dense outside and not forming an orderly line but rather jockeying to get through a single turnstile then nobody will enter comfortably. So it is with substrate inhibition: separate substrate molecules interfere with one another's ability to bind to the active site.

The fourth significant type is *product inhibition*. In this case it is departing product molecules that interfere with the substrate's ability to bind – rather as if a single turnstile was being used to let people leave the stadium as well as bring them in.

Interactions of proteins with other molecules

So we see that it is not only substrate molecules that can interact with proteins. Let's take two examples relevant to the brewer.

The first interaction is between the bitter compounds (iso-α-acids) and polypeptides. Former colleagues of mine, Paul Hughes and Bill Simpson proposed that the interactions involved here are of at least two types (Fig 7). In the first place the hydrocarbon hydrophobic parts of the iso-α-acids and those of polypeptides link. In turn the negative charges on adjacent iso-α-acids are bridged by the two positive charges on a metal ion such as magnesium.

The network formed stabilises proteins in bubble walls (i.e. in foam). Philip Slack and I had already shown that it was those proteins which are relatively hydrophobic that tend to give more stable foams. From understanding the rudiments of protein structure (as we have explored here) we can explain why: hydrophobic groups want to get together and get away from water. (In reality it's because the hydrogen-bonding water fraternity excludes them.) They associate together (and with other hydrophobic molecules such as the bitter acids) and will migrate to surfaces away from the body of the water (or beer): i.e. to the bubble walls.

The second interaction is between proteins and polyphenols. Karl Siebert found that the latter tanning molecules have a shape that fits comfortably with proline (see Fig 1) and that haze-forming polypeptides are rich in proline. Furthermore they hypothesised that each polyphenol can bind to more than a single polypeptide. These interactions build up and build up until large networks are produced that are so big that they are no longer soluble (Fig 8).

The result? Haze. In turn silica hydrogel is effective in removing haze proteins and polyvinylpolypyrollidone in removing polyphenols because they have surfaces which are similar to polyphenols and proline-rich proteins respectively.

Solubility

As we have seen, the delicate structure of proteins that presents a hydrophilic exterior with the hydrophobic groups on the inside ensures solubility but is easily disrupted. One of the main factors in brewing that will do this is heat. Thermal energy "blows" the structure wide open, the water-hating interior is exposed and these hydrophobic regions search one another out with the formation of insoluble clumps. This is what happens in wort boiling. Similarly proteins are sensitive to cold – the molecules rearrange and become insoluble (c.f. chilling before filtering).

Another agent that can come to bear is oxygen. Gerald Muts working at Heineken showed that adjacent -SH groups on proteins in a mash can be oxidised to form bridges:

$$-SH + -SH + O_2 \rightarrow -S-S- + H_2O_2$$

In this way separate protein molecules can join together and reach a size that makes them insoluble. In fact in a mash this type of reaction contributes to the formation of *teig* and the impeding of wort flow in lautering. The more oxygen present, the greater the opportunity for it to happen.

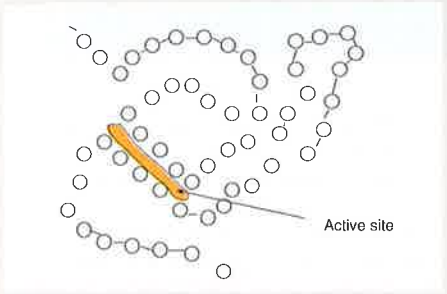


Fig 6 The concept of "active site."

increasing heat will also tend to disrupt the enzyme structure, deform the active site and therefore prevent the enzyme from doing its job. Thus the net rate of reaction observed is a balance dependent on how resistant the enzyme is to heat. Enzymes in mashes such as α-amylase and peroxidase are very resistant to heat, whereas others like β-glucanase and β-amylase are much more heat sensitive.

pH, too has impacts other than on the stability of the enzyme. We need to remember that the active site comprises several amino acids drawn from different parts of the overall polypeptide (and perhaps even several polypeptides – some enzymes contain 2 or 4 separate polypeptide chains and are said to have *quaternary* structure). Some of these amino acids, such as glutamic acid or lysine, have side chains that can become charged (see earlier).

It may be, for instance, that the enzyme uses the negative charge of a glutamic acid residue to bind the substrate. If that were the case, too low a pH, which would make the glutamic acid uncharged, would lead to a cessation of enzymic activity. Furthermore, some substrates can exist in uncharged or charged forms and may need to be in one or other form in order to become attracted to the enzyme. In fact most enzymes operate effectively only within a narrow pH range. Most of those of relevance in mashing happen to work best in the pH range of mashes (between 5 and 6)

It is not only changes in pH which can interfere with enzyme activity and stability. Enzymes are also susceptible to inactivation by other agents (*inactivators*). One such substance is the copper ion (Cu^{2+}). This snares –SH groups and screws up irreversibly those proteins that depend on –SH groups for their function.

Other molecules which can block enzyme activity do so reversibly (i.e. if you take them away enzyme activity is restored) and they are known as *inhibitors*. Basically there are four types of inhibition that we need to concern ourselves with. Remarkably, whilst they each almost certainly occur in brewery systems, they haven't been studied in great detail on an enzyme by enzyme case.

The first, *competitive inhibition*, results when a molecule is so similar to the substrate molecule that it can "recognise" the active site and bind to it, but is nonetheless sufficiently different that it just sits there and can't be tackled by the enzyme. It gets in the way of the substrate. However if the substrate concentration is high enough then it will squeeze out the inhibitor and inhibition is overcome.

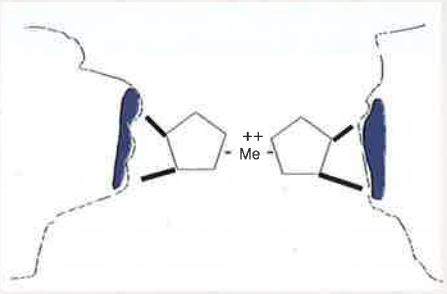


Fig 7 A model for iso-α-acid – metal ion – protein interaction in foam. The iso-α-acids are extremely stylised, depicting only two of their three hydrophobic arms (—), how these hydrophobic arms interact with hydrophobic regions in polypeptides and how the negative charges in adjacent iso-α-acids are neutralised by bridging through a divalent metal cation (such as magnesium, manganese or zinc). In this way large complexes between separate proteins and iso-α-acids are formed and strength is imparted to bubble walls.

In *non-competitive inhibition* the inhibitor binds to a site on the enzyme that is not the active site and, in so doing, distorts the shape of the molecule so that the active site is no longer available to substrate. Removing the inhibitor allows the enzyme to slide back into shape, but in this case increasing the substrate concentration cannot squeeze out the inhibitor.

At very high substrate concentrations we can sometimes encounter *substrate inhibition*. Once again we can turn to a football stadium for an analogy: if the crowd is dense outside and not forming an orderly line but rather jockeying to get through a single turnstile then nobody will enter comfortably. So it is with substrate inhibition: separate substrate molecules interfere with one another's ability to bind to the active site.

The fourth significant type is *product inhibition*. In this case it is departing product molecules that interfere with the substrate's ability to bind – rather as if a single turnstile was being used to let people leave the stadium as well as bring them in.

Interactions of proteins with other molecules

So we see that it is not only substrate molecules that can interact with proteins. Let's take two examples relevant to the brewer.

The first interaction is between the bitter compounds (iso-α-acids) and polypeptides. Former colleagues of mine, Paul Hughes and Bill Simpson proposed that the interactions involved here are of at least two types (Fig 7). In the first place the hydrocarbon hydrophobic parts of the iso-α-acids and those of polypeptides link. In turn the negative charges on adjacent iso-α-acids are bridged by the two positive charges on a metal ion such as magnesium.

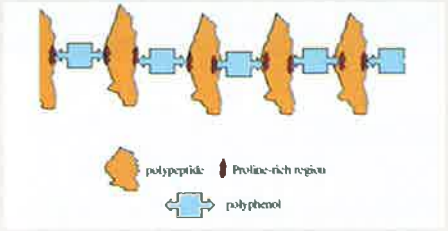


Fig 8 A model for polyphenol-protein interactions in haze.

The network formed stabilises proteins in bubble walls (i.e. in foam). Philip Slack and I had already shown that it was those proteins which are relatively hydrophobic that tend to give more stable foams. From understanding the rudiments of protein structure (as we have explored here) we can explain why: hydrophobic groups want to get together and get away from water. (In reality it's because the hydrogen-bonding water fraternity excludes them.) They associate together (and with other hydrophobic molecules such as the bitter acids) and will migrate to surfaces away from the body of the water (or beer): i.e. to the bubble walls.

The second interaction is between proteins and polyphenols. Karl Siebert found that the latter tanning molecules have a shape that fits comfortably with proline (see Fig 1) and that haze-forming polypeptides are rich in proline. Furthermore they hypothesised that each polyphenol can bind to more than a single polypeptide. These interactions build up and build up until large networks are produced that are so big that they are no longer soluble (Fig 8).

The result? Haze. In turn silica hydrogel is effective in removing haze proteins and polyvinylpolypyrollidone in removing polyphenols because they have surfaces which are similar to polyphenols and proline-rich proteins respectively.

Solubility

As we have seen, the delicate structure of proteins that presents a hydrophilic exterior with the hydrophobic groups on the inside ensures solubility but is easily disrupted. One of the main factors in brewing that will do this is heat. Thermal energy "blows" the structure wide open, the water-hating interior is exposed and these hydrophobic regions search one another out with the formation of insoluble clumps. This is what happens in wort boiling. Similarly proteins are sensitive to cold – the molecules rearrange and become insoluble (c.f. chilling before filtering).

Another agent that can come to bear is oxygen. Gerald Muts working at Heineken showed that adjacent -SH groups on proteins in a mash can be oxidised to form bridges:

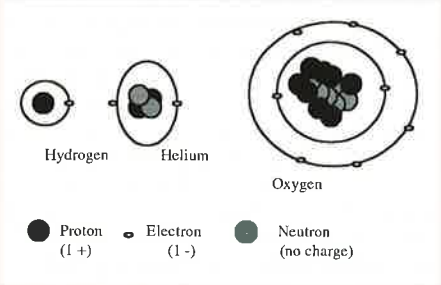
$$-SH + -SH + O_2 \rightarrow -S-S- + H_2O_2$$

In this way separate protein molecules can join together and reach a size that makes them insoluble. In fact in a mash this type of reaction contributes to the formation of *teig* and the impeding of wort flow in lautering. The more oxygen present, the greater the opportunity for it to happen.

FACTFILE – some necessary chemistry

Atoms

The basic unit of all matter is the atom. Modern thinking about the atom is all about waveforms, but it is still convenient to talk about the atom in terms of protons, neutrons and electrons.



At the heart of the atom (the nucleus) are the protons and neutrons, each of which has a mass of one. Protons are positively charged, neutrons have no charge. Together they comprise the mass of the atom.

Orbiting the nucleus, rather like the planets orbit the sun, are electrons. They are negatively charged, but have essentially no mass. In neutral atoms the number of electrons is exactly the same as the number of protons.

The electrons orbit the nucleus in defined orbits. There is a limit to how many electrons can occupy each orbit. The orbit nearest to the nucleus can accommodate just two electrons. Because they are so close to the nucleus, the strong positive-negative interaction means that these electrons are less free to move around than those further out – i.e. they have a relatively low energy. The next orbit holds eight electrons, which because they are that much further away are more energetic. The next orbit holds 18, then the next one 32...and so on.

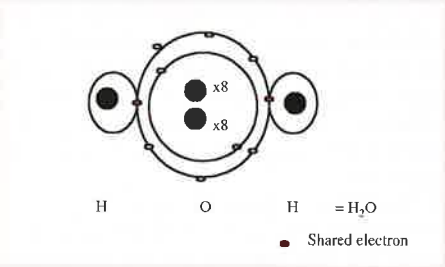
Each of the elements in nature consists of atoms. There are well over one hundred elements, each of them having successively one extra proton and therefore one extra electron. The simplest, hydrogen, has one proton and one electron. The next is helium: it has two protons (and also two neutrons, so it has a mass of 4 and not 2) and 2 electrons... and so on.

The most stable (least reactive elements) are those in which the outermost orbit is up to capacity with electrons (2, 8, 18 etc). Helium, then, is very unreactive – and that is why we can be rather more comfortable flying in airships filled with helium as opposed to hydrogen.

One way in which an atom can complete its outer shell of electrons is by donating electrons to another atom which in turn can complete is outer orbit by accepting electrons. For example sodium (which has one electron in its outer orbital) can lose a single electron and chlorine (which has seven electrons in its outer orbital) can gain an electron, each then assuming full outer orbits. Sodium acquires a net charge of 1^+ because it has lost one electron, chloride gains a net charge of 1^-, because it has one extra electron. The *compound* formed is NaCl. The sodium has become a positively charged *ion*, sometimes called a *cation* because it is attracted to a negatively charged electrode (the cathode). Chloride has become a negatively charged ion (an *anion*).

Magnesium needs to lose two electrons, which it can by donating one electron to each of two chlorines. Thus magnesium chloride consists of one magnesium and two chlorides, $MgCl_2$. This type of bond is called an *ionic* bond.

Alternatively an atom can complete its orbital by sharing electrons. Thus if the orbitals of two hydrogen atoms (one electron each) come into contact with the outer orbital of an oxygen atom (six electrons) then a pair of electrons can be shared between the oxygen and each hydrogen atom to make a much more stable molecule, water, H_2O. This type of bonding is called a 'covalent bond'.



The number of other atoms that an element can react with is known as its 'valence'. Thus hydrogen reacts with only one atom at a time, valence = one; oxygen reacts with two to make it more stable, valence = two. Ergo we have water with two atoms of hydrogen and one of oxygen, H_2O. Carbon has a valence of four. Sometimes one atom is linked to another by two links – this is called a 'double bond' and it is stronger than a single bond. An atom can use up two of its valences in this way Thus in carbon dioxide, CO_2, the carbon uses up its four valences by linking to two oxygens by double bonds, with each oxygen using up its two valences in a double bond to the carbon:

O=C=O

Oxidation and reduction

If oxygen is added to a substance then that substance is said to be *oxidised*. Conversely if hydrogen is added it is said to be *reduced*. Equally, removal of hydrogen is also oxidation. If you will allow one jump of logic without elaborate explanation, when a substance loses electrons, it is also said to have been oxidised. The substance that picks up those electrons is said to be reduced.

This is one type of chemical reaction. An example is the conversion of acetaldehyde to ethanol by yeast. Acetaldehyde is reduced, and the molecule NADH, which is the substance in living organisms which carries the electrons (reducing power), has become oxidised. If one component of a system is oxidised, another component or components must be reduced. The reducing power balances:

$CH_3CHO + NADH + H^+ \leftrightarrow CH_3CH_2OH + NAD$
Acetaldehyde Ethanol

Reactions

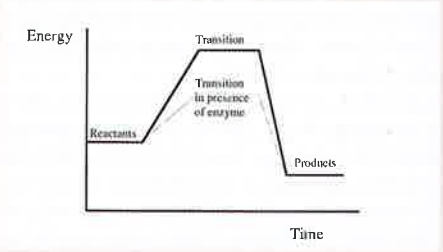
All chemical reactions also balance: the total number of atoms of a given element on the left side or a reaction must balance with that on the right. An example would be the conversion of glucose to ethanol and carbon dioxide, which is the overall reaction in alcoholic fermentation of yeast (of which more in a later article):

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2$$

A reaction happens because it is energetically favourable for it to occur. In other words, the total energy of the products is lower (more stable) than that of the reactants. The reaction may not proceed totally to the right hand side: equilibrium will be established.

This equilibrium may not be established rapidly, even if it is thermodynamically favourable. This is because bonds have to be broken in the reactants before new, more stable ones can be formed in the products. Liken it to a ball in a valley on one side of a hillock. If there was no hillock there and next to the valley with the ball in it was another valley that was lower down then the ball would tend (given a gentle nudge) to roll to the lower valley and stay there.

Because of the hillock some energy has to be put in (work done) to roll the ball over the hill. But more would effort would have to be exerted to move the ball in the other direction.



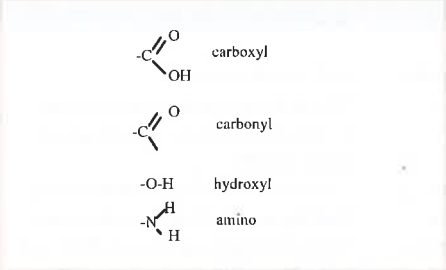
Now if a digger were moved in to shave the top off the hillock it would be considerably easier to move the ball from the first to the second valley (and vice-versa).

But the tendency would still be for the ball to move to the valley that was lower own. To return to our chemical reaction, a *catalyst* is a substance that allows chemical species to overcome the energy barrier and speed up the reaction. The catalyst is left unaltered at the end of the reaction, but may have been temporarily modified during the reaction.

Various other factors influence the rate of chemical reactions. If the reactants are more concentrated, they have an increased opportunity to interact. If the temperature is increased, the molecules collide with greater energy and bonds are broken more readily. A good rule of thumb, first coined by Arrhenius, is that reactions occur twice as fast for every 10°C rise in temperature. And reactions occur much more quickly in more fluid systems. Thus if you mix two powders together in a dry form they won't react, but if they are dissolved this allows the molecules to mix more freely and react together. The solvent often plays a key role in the reaction.

It is possible to group chemical compounds together into families, wherein the molecules have similar structures and similar reactivities. Three such families, which are of great importance to the brewer, are the proteins, the carbohydrates and the lipids.

It is also possible to make sense out of the complexity of chemistry by realising that the types of 'groups' that they contain determine the chemical properties of compounds. There are many types of groupings in chemistry, of which some relevant ones are:



Acids, bases, pH and buffers

The carboxyl group is *acidic*, in that it can furnish a hydrogen ion (H^+), i.e. a hydrogen atom without its electron, i.e. a proton.

$$-COOH \leftrightarrow -COO^- + H^+$$

The symbol ↔ indicates that the reaction is reversible (can move in both directions). If the pH is low (high H^+ concentration) then this will tend to push the reaction towards the left. If the pH is high (low H^+ concentration) then the reaction will tend to move towards the right, in order to produce more H^+.

The amino group can pick up a hydrogen ion and is said to be *basic*. Another base is the hydroxide ion, OH^-.

$$-NH_2 + H^+ \leftrightarrow -NH_3^+$$

$$-OH^- + H^+ \leftrightarrow H_2O$$

The measure of acidity is the pH scale. pH = log $1/H^+$

The scale runs from 1 (extremely acidic) to 14 (extremely basic), with 7.0 being neutral.■

Polymers

Proteins (and the polysaccharides that I will deal with in later articles) are polymers.

Polymers are very large molecules in which smaller molecules are joined together into chains either linearly or with branches.

The individual units that join together are referred to as *monomers*. And so in proteins the monomer is an amino acid. If two monomers are joined together we get a *dimer* (for two amino acids this would be a dipeptide).

Three monomers joined together yield a *trimer*, four a *tetramer*, and so on. Short chains of perhaps 2 to 10 monomers are collectively referred to as *oligomers*. Larger chains are referred to as *polymers*.

● ***A BREWER'S BIOCHEMISTRY.***

In the May edition of *The Brewer International*, Professor Bamforth continues with Polysaccharides. In July he will discuss Lipids, followed by Nucleic acids and Metabolism in September and November respectively.