Chem Factsheet

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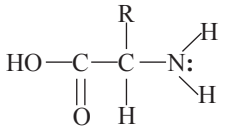
Amino Acids

Amino acids are bifunctional compounds because their molecules contain two distinct functional group – the primary amine group ($-NH_2$) and the carboxylic acid group (-COOH). In naturally occurring amino acids (so-called alpha [α] amino acids) the NH_2 and COOH groups are bonded to the same C atom (C2). Hence the general structure of an α-amino acid is:



The R group varies from one amino acid to another. In nature, 22 different R groups can occur resulting in 22 different naturally occurring amino acids. Some examples are shown in the following table.

R	Common Name of Amino Acid	IUPAC Name of Amino Acid
-H	Glycine	2-Aminoethanoic acid
$-CH_3$	Alanine	2-Aminopropanoic acid
$-CH(CH_3)_2$	Valine	2-Amino-3-methylbutanoic acid
$-CH_2COOH$	Aspartic acid	2-Aminobutanedioic acid
$-(CH_2)_3CH_2NH_2$	Lysine	2,6-Diaminohexanoic acid

Note: Each and every IUPAC name contains the element "2-amino-" because the NH_2 group is always bonded to the C atom next to a COOH group (the C1 atom).

Classification

There are various classifications of amino acids, all of which depend on grouping according to similar properties of the R groups. One simple but very useful classification is (a) neutral (b) acidic and (c) basic.

In neutral amino acids the R group does not contain any additional NH_2 or COOH groups. e.g. alanine (see table).

In acidic amino acids the R group contains an additional COOH group. e.g. aspartic acid (see table). Such amino acids have an enhanced ability to react with a base (e.g. aqueous NaOH) – see later.

In basic amino acids the R group contains an additional NH_2 group. e.g. lysine (see table). Such amino acids have an enhanced ability to react with an acid (e.g. aqueous HCl) – see later.

Preparation

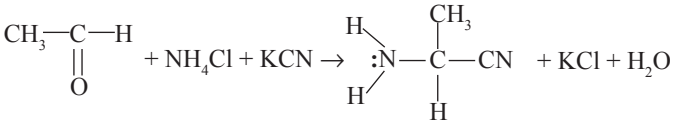
Amino acids are best obtained by acid-catalysed hydrolysis of naturally occurring proteins (polypeptides) followed by separation and purification – see later.

Living organisms can synthesize amino acids from simpler substances obtained in an animal's diet or a plant's growth medium. Biotechnology employs various bacteria to supply appropriate enzymes for the synthesis of amino acids. Not all organisms can synthesize all amino acids. For example, humans are able to synthesize only 13 of the 22 standard amino acids. This leads to the dietary requirement whereby it is necessary for humans to eat foods containing the other 9 so-called "essential" amino acids.

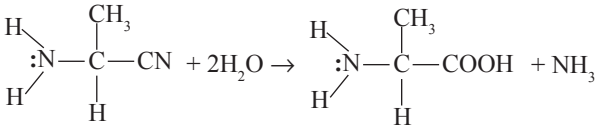
Essential Amino Acids	Non-Essential Amino Acids
Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine	Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Glutamic acid, Glutamine, Glycine, Ornithine, Proline, Selenocysteine, Serine, Tyrosine

Amino acids can be synthesised in the laboratory using traditional chemical reactions but this is rather "academic" because they are much more readily available from bio sources.

For example, the Strecker synthesis (named after Adolf Strecker, German chemist, 1822-1871) can be used to make many amino acids. For example, ethanal is reacted with ammonium chloride and potassium cyanide to form 2-aminopropanenitrile. In effect, this is the nucleophilic addition of hydrogen cyanide to the aldehyde.



The nitrile is then hydrolysed by refluxing with sulphuric acid catalyst to form 2-aminopropanoic acid (alanine).



Notes :

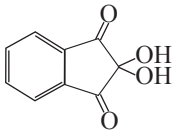
(a) The CH_3 group in the original aldehyde can be varied to produce different amino acids.

(b) The acid catalyst used in stage 2 will absorb the basic NH_3 product.

(c) The alanine is produced as a racemic mixture, with zero optical activity (see later). This is because there is a 50:50 chance of the nucleophile (CN^-) attacking the planar >C=O group of the original aldehyde above or below the plane.

Testing for amino acids

A substance can be shown to be an amino acid by reacting it with ninhydrin.



When warmed, a mixture of amino acid and ninhydrin produces a purple colour.

This is often used to detect the position of colourless amino acid spots during chromatography and electrophoresis – see later.

Acid-Base properties

A carboxylic acid group (-COOH) is weakly acidic because it has the ability to donate a proton from the O-H group

$-COOH + H_2O \rightleftharpoons -COO^- + H_3O^+$

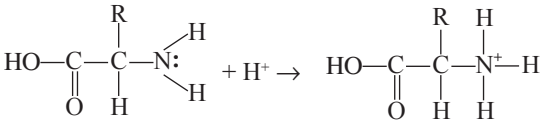
A primary amine group ($-NH_2$) is weakly basic because it has the ability to accept a proton via a coordinate bond with the lone pair of the N atom.

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

Hence, since they have both of these groups, amino acids are able to act as either acids or bases. They are said to be amphoteric or amphiprotic.

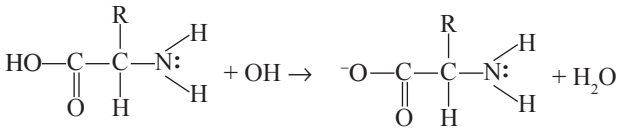
(a) Amino acids in solution at low pH

When dissolved in for example 0.1 $moldm^{-3}$ HCl(aq), amino acids show their basic property whereby the $-NH_2$ group(s) present are converted to $-NH_3^+$ by accepting protons.



(b) Amino acids in solution at high pH

When dissolved in for example 0.1 $moldm^{-3}$ NaOH(aq), amino acids show their acidic property whereby the –COOH group(s) present are converted to $-COO^-$ by releasing protons.



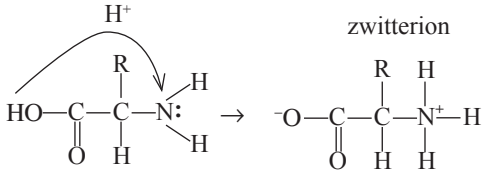
Notes :

(i) Be sure to look for additional NH_2 or COOH groups in the R side chain. These will also be converted according the above reactions.

(ii) These acid-base properties mean that amino acids can act as buffers in biological systems in that they can absorb slight excesses of acid or base and so maintain a more or less constant pH – see later.

(c) Amino acids in crystalline solid or in solution at their isoelectric pH

As shown in the following diagram, when in the solid state or dissolved in a solution which has pH equal to the isoelectric pH of the particular amino acid, a proton (H^+) transfers internally from the –COOH group to the $-NH_2$ group to form a particle with separate positive and negative charges. This particle has net zero charge and is called a zwitterion.



Notes:

(a) The isoelectric pH of an amino acid is *defined* as the pH at which the amino acid exists as its zwitterion.

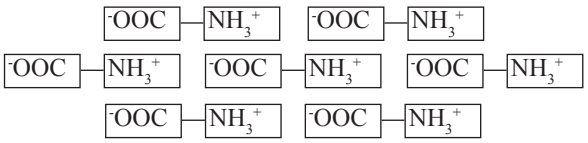
(b) Zwitterions are named after the German word "Zwitter" which means "hybrid" – they are both anionic (-ve) and cationic (+ve).

Each amino acid has a characteristic isoelectric pH which can be used to identify the amino acid by electrophoresis – see later. Some values are shown in the following table. Note that a basic group in the R group raises the isoelectric pH considerably, whereas an acidic group lowers it.

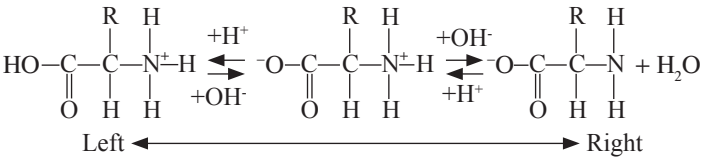
R	Amino Acid	Isoelectric pH
$-CH_3$	Alanine	6.00
$-CH_2COOH$	Aspartic acid	2.77
$-(CH_2)_3CH_2NH_2$	Lysine	9.74

Amino acids are generally water soluble but, if the pH is adjusted to the isoelectric pH, the solubility reaches a minimum and the amino acid will often precipitate. This has applications in isolating and purifying amino acids.

Pure amino acids are all white, crystalline solids at room temperature and pressure. The fact that even the smallest molecules (e.g. glycine) form solids rather than liquids or gases, is accounted for by the fact that the amino acid exists in the zwitterion form in the solid. This means there are unusually strong electrostatic forces of attraction between positive $-NH_3^+$ and negative $-COO^-$ groups in neighbouring molecules which maintain the lattice structure.



Buffer Action



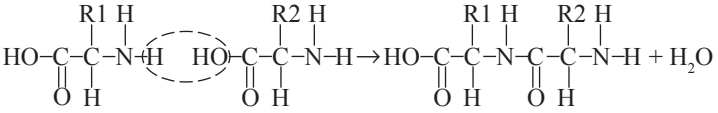
If small amounts of acid (H^+) are added, the equilibria will shift to the left resulting in the absorption of most of the H^+ ions. The pH will therefore remain approximately constant.

If small amounts of base (OH^-) are added, the equilibria will shift to the right resulting in the absorption of most of the OH^- ions. The pH will therefore remain approximately constant.

Polymerisation

Under the control of the genetic code, thousands of amino acids are linked by repeated condensation reactions to form polypeptide chains, the primary structures of proteins. The number, type and sequence of the amino acids in the chain are very precisely controlled.

Linkage occurs by elimination of a molecule of water (hence "condensation") between the $-NH_2$ group of one amino acid and the –COOH group of another.

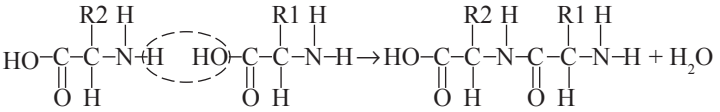


The linking group (-NH-CO-) is an amide type grouping but is more commonly referred to as a peptide link.

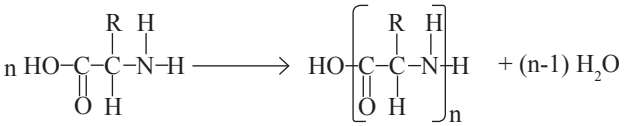
Notes:

(a) Two amino acids link to form a "dipeptide", three to form a "tripeptide" etc.

(b) Two possible dipeptides can be formed from any two amino acids because the sequence can be AA1-AA2 or AA2-AA1. Hence,



Repeated condensations can occur because the dipeptide has a free $-NH_2$ group and a free –COOH group.



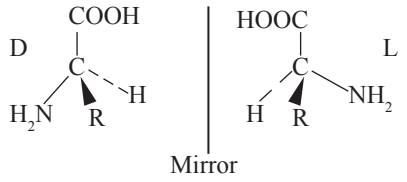
Amino acids Polypeptide (R varies along the chain)

Hence, polypeptide chains result composed of thousands of amino acid residues in a very strict sequence which is, in part, responsible for controlling the function of the derived protein.

Note: Amino acids are being used to develop biodegradable polymers which have applications, amongst others, as environmentally friendly packaging, in medicine for drug delivery and for the construction of prosthetic implants. This is possible because the peptide bond is hydrolysable. -NH-CO- + H_2O → -NH_2 + -COOH

Optical Activity

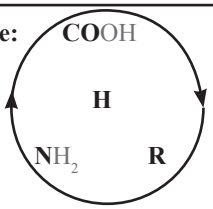
Amino acids show stereoisomerism. With the exception of glycine, all α-amino acids can exist as either of two optical isomers (also called enantiomers). These are non-superimposable mirror images of each other.



Note: Glycine (R=H) does not have optical isomers because the central C atom is not bonded to four different groups. It is said to be non-chiral. This means the mirror images of glycine *are* superimposable. All other α-amino acids do have a chiral C because they are bonded to H, COOH, NH_2 and R groups.

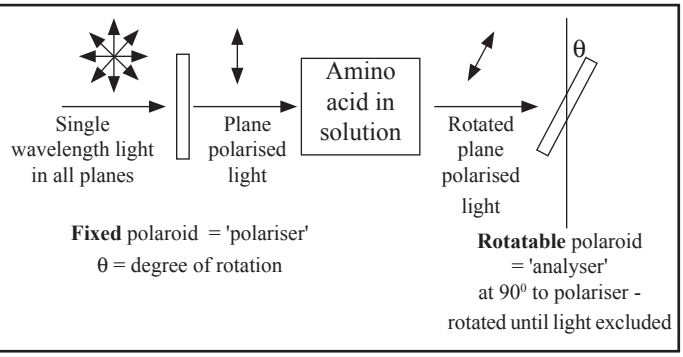
The two optical isomers are called the D and the L isomer. Only the L type occurs naturally, but if the amino acid is synthesized artificially, a 50:50 mixture of isomers is produced. This is called a *racemic mixture*.

Note:



Looking down from the H atom, if the COOH, R and NH2 read '**CORN**' clockwise then the isomer is an L type. Otherwise it is the D Type

The two optical isomers are identical in their chemical properties and all their physical properties except their effects on plane-polarized light. For equivalent concentrations at a fixed temperature, one isomer rotates the plane of the plane-polarized light clockwise (dextrorotary) while the other rotates it equally but anti-clockwise (laevorotary). This is measured using a polarimeter.



Measured under standard conditions (concentration, temperature, path length in cell, wavelength) each amino acid produces a *specific rotation* value. This can be used to identify amino acids.

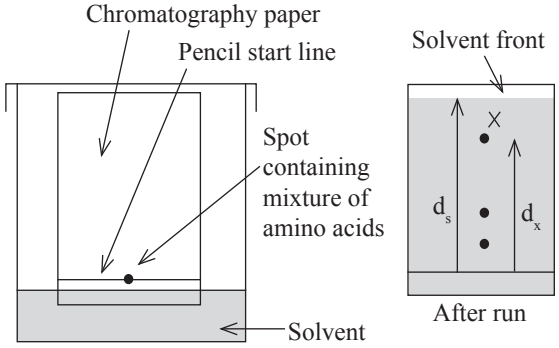
Note: A racemic mixture will show zero rotation because the equal but opposite rotations of the 2 isomers will cancel.

Chromatography

Amino acid mixtures, especially those derived from acid hydrolysis of proteins or polypeptides, can be separated and identified by various chromatography methods.

Paper chromatography of amino acids

1. Sample applied in concentrated spot using fine pipette.
2. Spot must remain above solvent surface
3. Lid applied to saturate inside of tank with solvent
4. Solvent allowed to run up paper by capillary action
5. Solvent front should not run off top of the paper
6. Spots not visible until 'developed'. Ninhydrin shows up amino acids.
7. R_f value can identify a component by comparison with know data but varies with temperature - better to compare directly with authentic sample run alongside the sample.



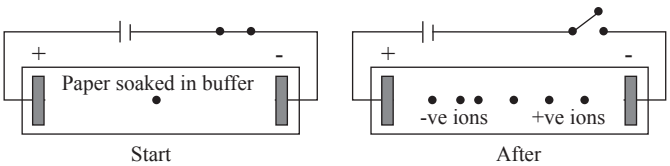
$$R_f \text{ of component X} = \frac{\text{Distance moved by component X}}{\text{Distance moved by solvent}} = \frac{d_x}{d_s}$$

In this technique, separation is achieved as a result of different amino acids having different affinities for a stationary phase [SP] (e.g. paper in paper chromatography as shown above) relative to a mobile phase [MP] (e.g. the solvent). Higher affinity for the SP than the MP leads to lower R_f values, and vice versa. Different amino acids can be identified by comparison with tabulated R_f and confirmed by running the unknown amino acids against known amino acids.

Electrophoresis

Electrophoresis is a technique for the separation and identification of charged particles according to the mass and charge of those particles. Since amino acids can be caused to be charged by pH adjustment (see earlier), electrophoresis is applicable to amino acid determination.

In simple terms, a mixture of amino acids is applied to the centre of a piece of paper soaked in a buffer of known pH and electrodes are applied to the ends of the paper (see diagram).



Positive ions (those with $-NH_3^+$ group – more likely at lower pH – see earlier) move towards the negative electrode and negative ions (those with $-COO^-$ group – more likely at higher pH – see earlier) move towards the positive electrode. For a fixed charge, heavier ions move more slowly and lighter ions move more rapidly, resulting in separation.

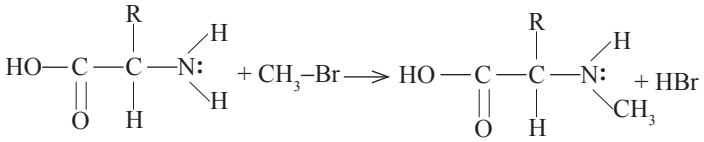
Notes:
(a) Ions with multiple charges because of $-NH_3^+$ or $-COO^-$ groups in the R group move faster.
(b) No movement indicates the presence of the zwitterion (see earlier) at the operating pH. Moreover, this pH gives the isoelectric pH for the amino acid concerned.

Having discussed the characteristic properties of amino acids, don't forget that the COOH and NH_2 groups can show their individual characteristic properties. These are summarised below.

Notes: Be care to consider whether the R group also reacts!

Alkyation of the NH_2 group(s)

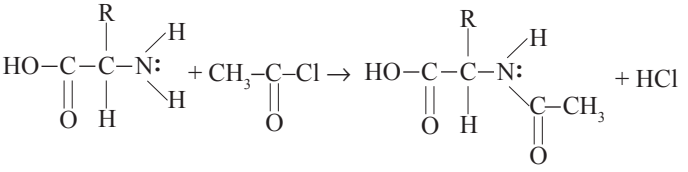
The primary amine group acts as a nucleophile and substitutes for the halogen in a haloalkane (e.g. bromomethane, CH_3Br) to form a secondary amine.



If the proportion of haloalkane is increased, multiple substitutions can occur giving a mixture of tertiary and quaternary amines [AA-$N(CH_3)_2$ and [AA-$N(CH_3)_3]^+$ where AA represents the rest of the amino acid].

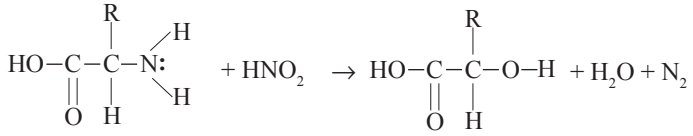
Acylation of the NH_2 group(s)

An acyl chloride (e.g. ethanoyl chloride, CH_3COCl) undergoes a nucleophilic addition-elimination reaction with the amine group to produce the related amide.



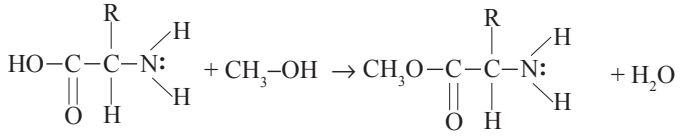
Diazotisation of the NH_2 group(s)

When reacted with a mixture of potassium nitrite and hydrochloric acid [≡ HNO_2] at low temperature, the NH_2 group is converted to a diazonium group ($-N_2^+$) which immediately hydrolyses to form a secondary alcohol group (except glycine → primary alcohol since R = H).



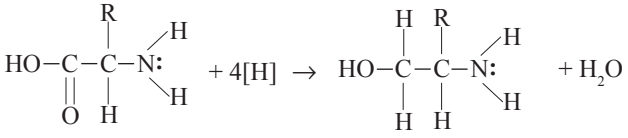
Esterification the COOH group(s)

In the presence of a concentrated sulphuric acid and an alcohol (e.g. methanol, CH_3OH), the COOH is esterified to form an ester group.



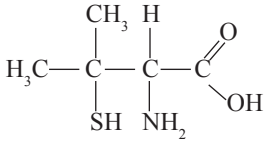
Reduction the COOH group(s)

Using sodium borohydride ($NaBH_4$) as a reductant (a source of H^-), the COOH group is reduced to a primary alcohol group.



Practice Questions

1. This question looks at the properties and chemistry of some α-amino acids. The general formula of an α-amino acid is $RCH(NH_2)COOH$.
   (a) In the α-amino acid alanine, $CH_3CH(NH_2)COOH$, R is CH_3. The isoelectric pH of alanine is at pH 6.0.
      (i) What is meant by the term isoelectric pH?
      (ii) Draw the structures of the ions formed by alanine at pH 6.0, pH 11.5 and at pH 1.5.
      (iii) Different R groups in α-amino acids result in different isoelectric points. Suggest the functional group, in the R group, that results in the isoelectric point being lower than pH 3 and higher than pH 10.
   (b) The α-amino acid serine, where R is CH_2OH, readily forms a condensation polymer containing peptide links. Draw a section of poly(serine), showing **two** repeat units. Display the peptide linkage.
   (c) Apart from glycine, where R is H, all α-amino acids show optical isomerism.
      (i) Why does glycine not show optical isomerism?
      (ii) Draw 3-D diagrams for the two optical isomers of the α-amino acid cysteine, where R is CH_2SH.
2. Penicillamine is an α-amino acid that is used as a drug to treat rheumatoid arthritis. The structure of penicillamine is shown below.

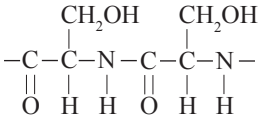


   (a) Explain why penicillamine is described as an α-*amino acid.*
   (b) Penicillamine exists as a zwitterion in aqueous solution.
      (i) Draw the structure of this zwitterion.
      (ii) Dilute aqueous acid is added to an aqueous solution of penicillamine. Draw the structure of the ion that forms.

Answers

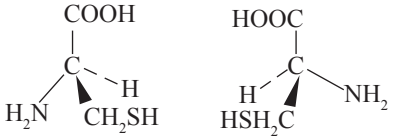
1. (a) (i) The pH value at which an amino acid exists as the zwitterion, $CH_3CH(NH_3^+)COO^-$.
   (ii) pH 6.0 : $CH_3CH(NH_3^+)COO^-$; pH 11.5 : $CH_3CH(NH_2)COO^-$; pH 1.5 : $CH_3CH(NH_3^+)COOH$.
   (iii) Need basic group in R to increase the isoelectric pH or an acidic group to decrease it. Hence, for pH 3 $HOOCCH_2CH(NH_2)COOH$ and for pH 10 $H_2NCH_2CH(NH_2)COOH$.

   (b)



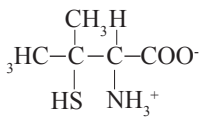
   (c) (i) Glycine does not contain a chiral C atom. The central C is bonded to 2H atoms, a COOH group and a NH_2 group. Hence, it is not bonded to four different groups which is required for chirality.

   (ii)

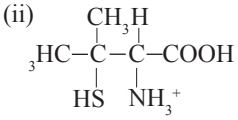


2. (a) It contains both a NH_2 (amino) and an acid (COOH) bonded to the same C (α) atom.

   (b) (i)



   (ii)



Acknowledgements: *This Factsheet was researched and written by Mike Hughes.*