Amino acids

Physical and Chemical Properties

Physical Properties

- **Solubility**: Most of the amino acids are soluble in water and insoluble in organic solvent.
- **Melting point**: Amino acids generally melt at high temperatures often above 200ºC.
- **Taste:** Amino acid can be sweet (Gly, Ala, val), tasteless (Leu) bitter (Arg, Ile). Monosodium glutamate (MSG; ajinomoto) is used as a flavoring agent in food industries.
- **Optical rotation:** Except glycine, all amino acids show optical rotation due to the presence of asymmetric carbon atoms. Some amino acids also have a second asymmetric carbon e.g. Ile and Arg.
- **Spectroscopic Properties:** All amino acids absorb in the infrared region, **only Phe, Tyr, and Trp absorb UV Absorbance at 280 nm.**

Acid base properties of amino acids

- Amino and carboxyl groups of amino acids, along with the ionizable R groups function as weak acids and bases.
- When an amino acid lacking an ionizable R group is dissolved in water at neutral pH, it exists in solution as the dipolar ion, or **zwitterions** (German word for "hybrid ion"), which can act as either an acid or a base.
- Substances having this dual (acid-base) nature (or negative and positive charge both) are **amphoteric** and are often called **ampholytes** (from "amphoteric electrolytes").
- A simple monoamino monocarboxylic α-amino acid, such as alanine, is a diprotic acid when fully protonated; it has two groups, **the —COOH group and the —**NH_3 group, that can yield protons:

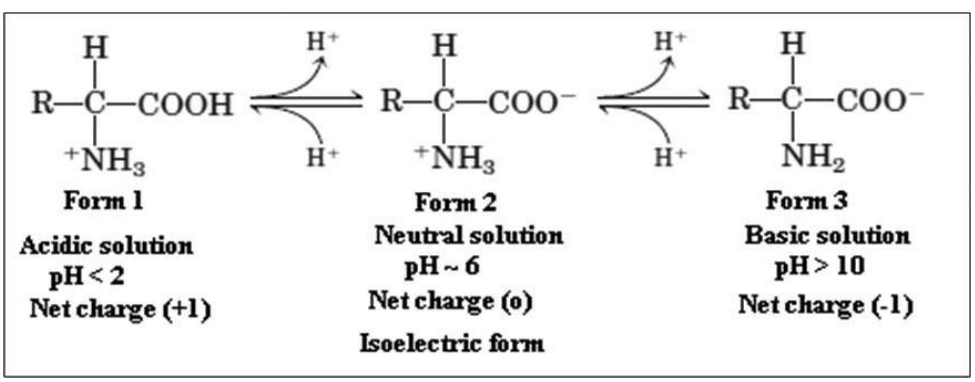


Figure 1: Acid-base behavior and zwitterion

Acid basic properties of amino acid can be studies using titration curve

Titration curve of Glycine

- The two ionizable groups of glycine, the carboxyl group and the amino group, are titrated with a strong base such as NaOH.
- **The titration curve is Biphasic due to two ionizable groups**
- At very low pH, the predominant ionic species of glycine is the fully protonated form, $^{+}H_3N—CH_2—COOH$.
- At the midpoint —COOH group of glycine loses its proton, equimolar concentrations of the proton-donor ($^{+}H_3N—CH_2—COOH$) and proton-acceptor ($^{+}H_3N—CH_2—COO^{-}$) species are present.
- At this point the pH is equal to the p*K*a of the protonated group being titrated. For glycine, the pH at the midpoint is 2.34, thus —COOH group has a p*K*a of 2.34.

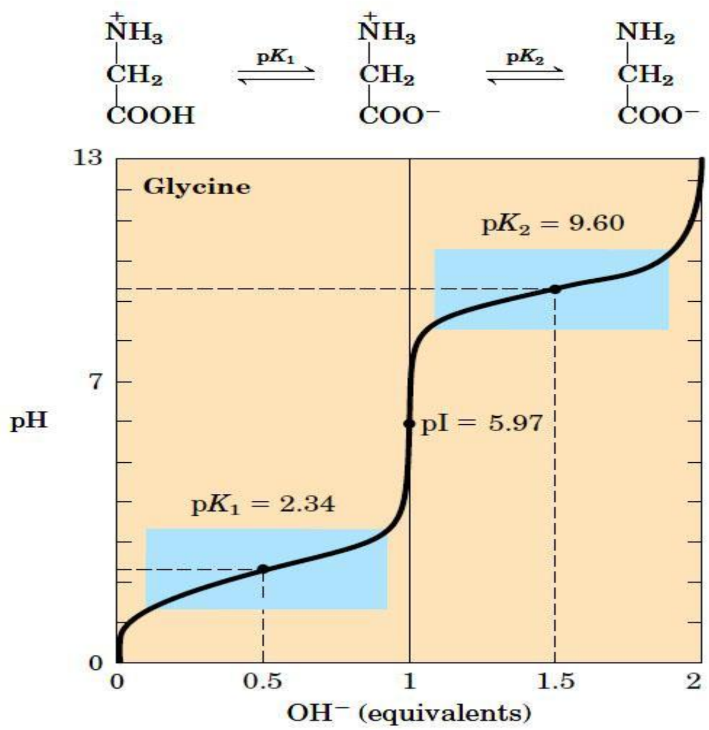


Figure 2: Titration curve of Glycine

- The p*K*a is a measure of the tendency of a group to give up a proton and it decreases tenfold as the p*K*a value increases by one unit.
- As the titration proceeds, there is another point of inflection at pH 5.97 in which, –COOH group of glycine completely loses its proton and it is present largely as the dipolar ion (zwitterion) $^{+}H_3N—CH_2—COO^{-}$.

- The second stage of the titration corresponds to the removal of a proton from the —NH_3^+ group. pH at the midpoint of this stage is 9.60, equal to the p*K*a (pK_2) for the —NH_3^+ group.
- The titration is essentially completed at a pH of about 12, at this point the predominant form of glycine is H_2N— CH_2—COO^-.
- From the titration curve of glycine we can derive several important pieces of information.
- First, it gives a quantitative measure of the p*K*a of each of the two ionizing groups: 2.34 for the —COOH group and 9.60 for the —$NH3^+$ group. This is helpful in calculating buffering range of amino acids.

Significance of amino acid titration curve

- **It provides the buffering range of amino acids**
  - Titration curve of glycine suggested *two* regions of buffering power. The first buffering zone is at 1 pH unit on either side of the first p*K*a of 2.34.
  - The other buffering zone is centered on pH 9.60. (Note that glycine is not a good buffer at the pH of intracellular fluid or blood, about 7.4.)
- **Titration Curves Predict the Electric Charge of Amino Acids**
  - **T**itration curve of an amino acid also explains the relationship between the net charge on amino acid and the pH of the solution.
  - The characteristic pH at which the net electric charge is zero is called the **isoelectric point** or **isoelectric pH**, designated **pI**. For glycine, the pI is simply the arithmetic mean of the two p*K*a values:

$$pI = 1/2\ (pK1 + pK2)$$

$$pI = 1/2\ (2.34 + 9.60) = 5.97$$

Chemical properties of Amino acids

- The carboxyl groups of amino acids undergo all the common reactions of this functional group.
- Reaction with ammonia and primary amines yields unsubstituted and substituted amides, respectively.
- Ester and acid chlorides are also readily formed. Esterification proceeds in the presence of the appropriate alcohol and a strong acid.
- Polymerization can occur by repetition of the reaction.
- Free amino groups may react with aldehydes to form Schiff bases and can be acylated with acid anhydrides and acid halides

- ❖ Amino acids can be readily detected and quantified by reaction with ninhydrin.
- ❖ Ninhydrin (or triketohydrindene hydrate), a strong oxidizing agent causes oxidative deamination of the α-amino group.
- ❖ The products of the reaction are the resulting aldehyde, ammonia, carbon dioxide, and hydrindantin, a reduced derivative of ninhydrin.

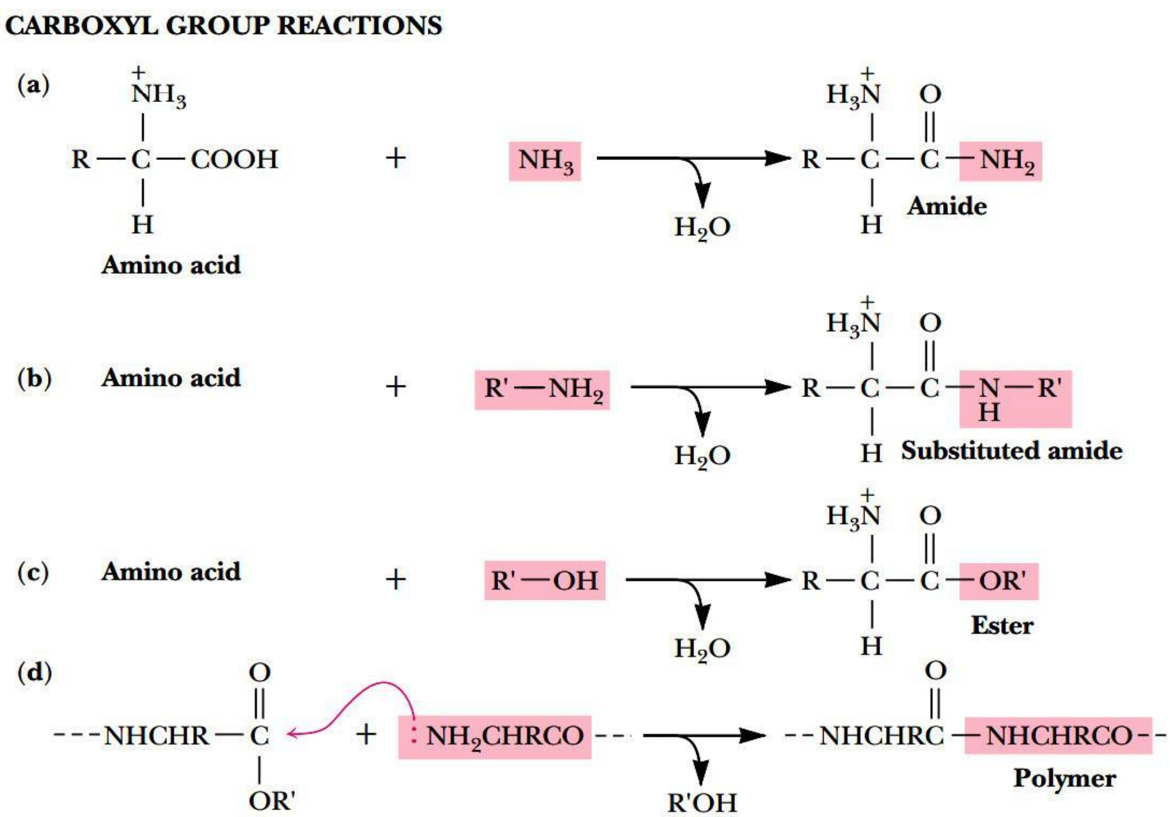


Figure 3: Some of the reactions of –COOH group

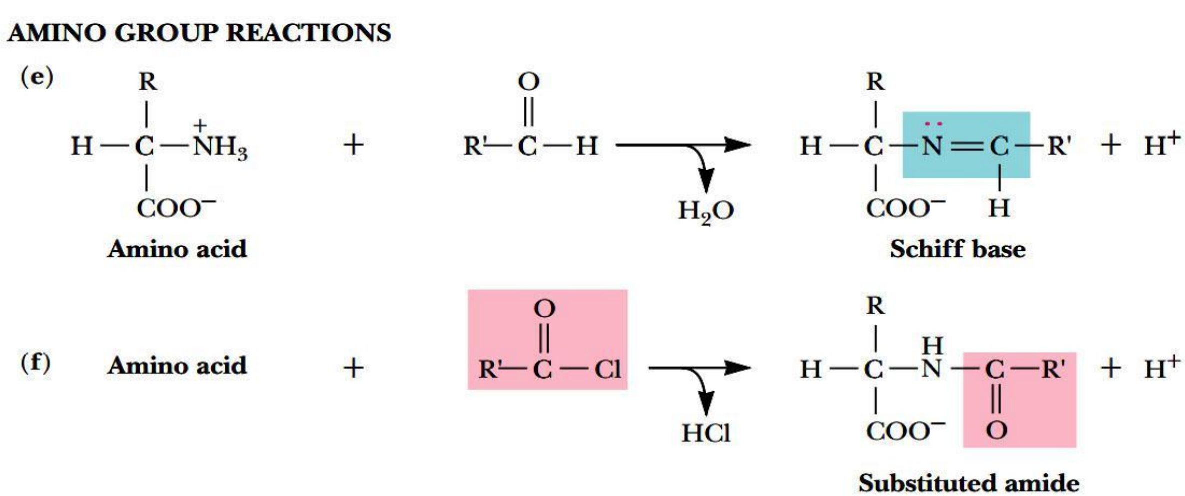
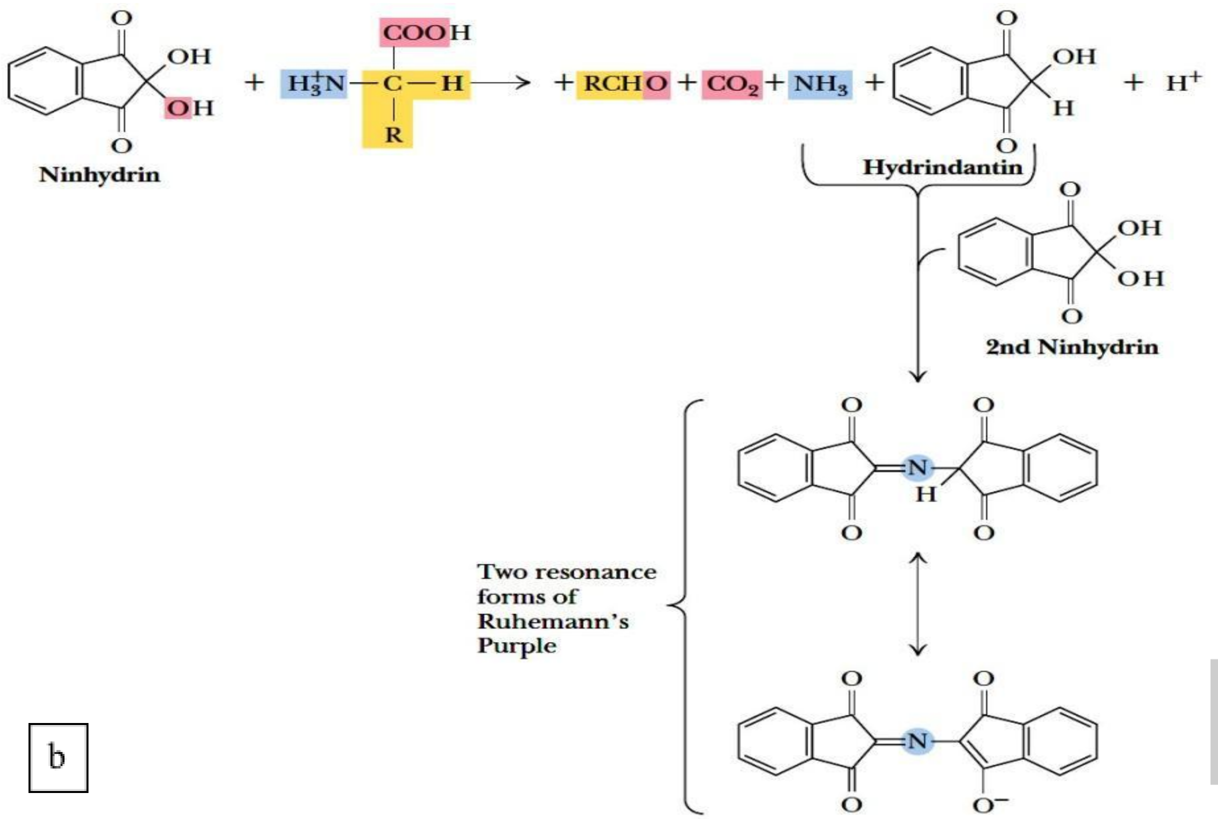


Figure 4: -NH3 group reactions

❖ The ammonia can react with the hydrindantin and another molecule of ninhydrin to yield a purple product (Ruhemann's Purple) that can be quantified spectrophotometrically at 570 nm.



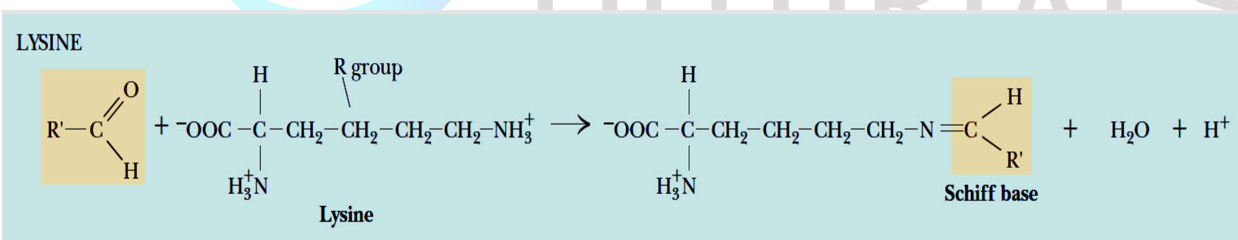


Figure 5: (a) Ninhydrin reaction (b) ε-NH2 group of lysine forms Schiff base with –COOH group

❖ α-Imino acids, such as proline and hydroxyproline, give bright yellow ninhydrin products with absorption maxima at 440 nm, allowing these to be distinguished from the α-amino acids.

❖ Because amino acids are one of the components of human skin secretions, the ninhydrin reaction was once used extensively by law enforcement and forensic personnel for fingerprint detection.

❖ More sensitive fluorescent reagents are now used routinely for this purpose.

- ❖ Cysteine residues in proteins react with one another to form disulfide species and also react with a number of reagents, including maleimides (typically N-ethylmaleimide).
- ❖ Cysteines also react effectively with iodoacetic acid to yield S-carboxymethyl cysteine derivatives.

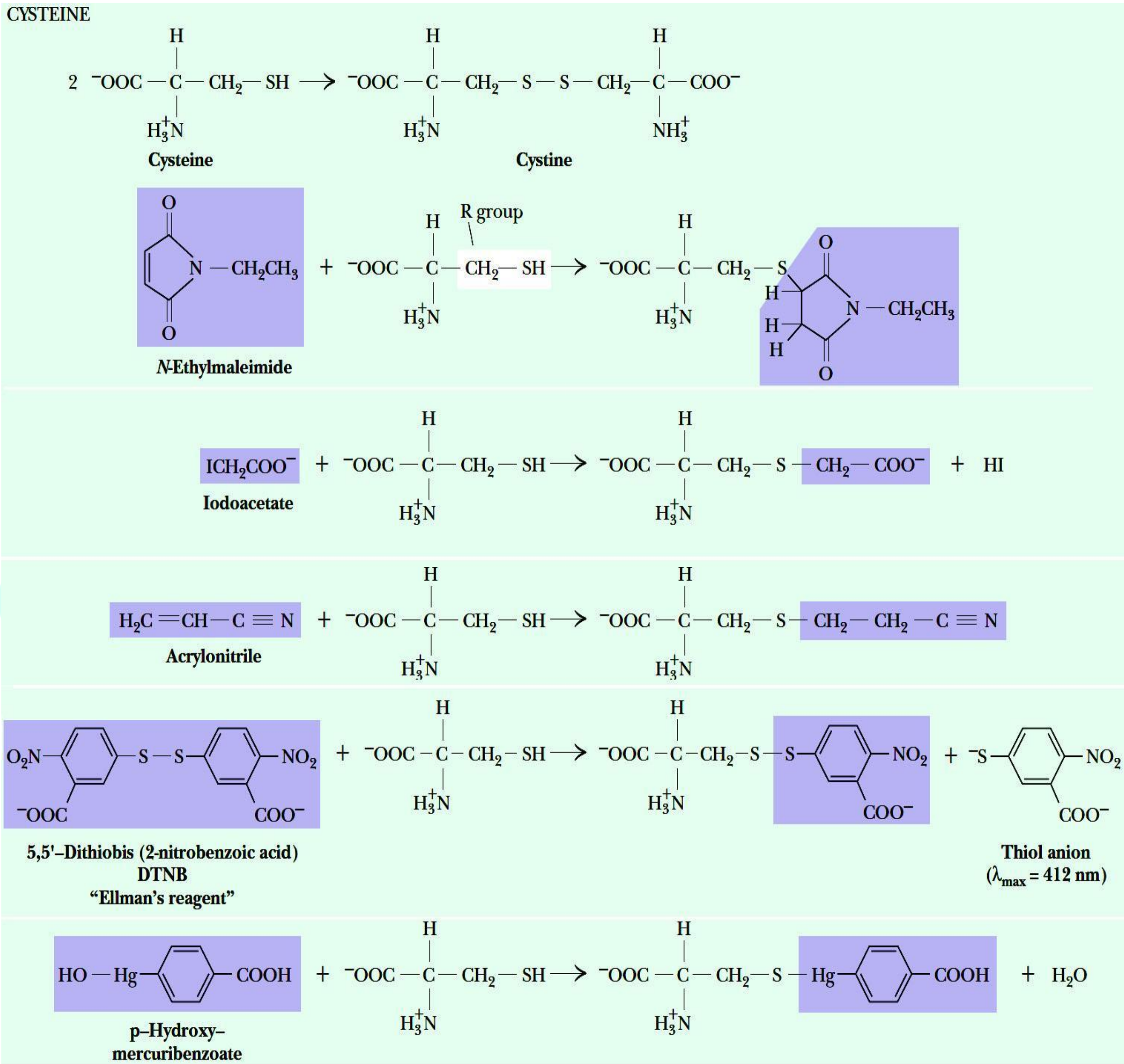


Figure 6: Some of the reactions of –SH group of Cysteine

References

- ❖ Nelson DL and Cox MM. *Lehninger Principles of Biochemistry*, *5th Edition* 2008, W.H. Freeman and Company, New-York
- ❖ Reginald H. Garrett, Charles M. Grisham –Biochemistry 2nd Edition 1998, Cengage Learning Canada

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