5. Hydrolysis of Proteins

Proteins are hydrolyzed to amino acids by treatment with acid, alkali, or enzymes. Each method has certain disadvantages as shown in Table 1. The most commonly used methods for total hydrolysis are described below.

Method for acid hydrolysis. A sample (50–100 mg) of air-dried or lyophilized protein is weighed into a tube, and 6 M HCl (1 mL for 5 mg of protein) is added. The tube is evacuated using a vacuum desiccator (8), sealed, and placed in a circulating air oven at 110°C with good temperature control (7). After hydrolysis for the appropriate period of time (24, 48, or 71 h), it is centrifuged gently. Then the tubes are cracked open and the HCl is removed as quickly as possible using a stream of N_2. The HCl can alternatively be neutralized by adding solid $Ba(OH)_2$ (up to pH 7) and removing white $BaSO_4$ by filtration or centrifugation. The clear hydrolysate may be frozen in an acetone–solid CO_2 bath, placed in a vacuum desiccator over NaOH or KOH, and lyophilized. However, clear hydrolysates can also be stored in the refrigerator for several days.

For more detailed discussion on hydrolysis of proteins for amino acid analysis one may consult Light and Smith (9), Moore and Stein (7), Savoy et al. (10), or Perham (11).

Method for sulfur-containing amino acids. Moore (12) determined cysteine and cystine as cysteic acid by performic acid oxidation. However, methionine can also be determined as methionine *S,S*-dioxide.

Performic acid is prepared by adding H_2O_2 (1 mL, 30%) to formic acid (9 mL, 88%) and allowing the mixture to stand at room temperature for 1 h. It is then cooled to 0°C. Performic acid (2 mL) is added to the protein (containing about 0.1 mg cystine) in a Pyrex tube, which is then allowed to stand at 0°C for 4 h for soluble proteins or overnight for proteins that do not dissolve. Then HBr (0.30 mL, 48%) is added with swirling, the mixture is evaporated to dryness at 40°C using a rotary evaporator, and the protein is hydrolyzed in vacuo with HCl (3 mL, 6 M) at 110°C for 18 h. The hydrolysate is treated as mentioned above, before analysis. A rapid method of protein hydrolysis by microwave irradiation has been described (12a). That article describes a design for a reusable Teflon–Pyrex tube for fast inert gas flushing under microwave irradiation. Results have been compared with those of conventional heating methods in terms of destruction or degradation of certain labile amino acids and their recoveries depending upon hydrolysis time by microwave irradiation.

Table 1 Disadvantages of Methods of Hydrolysis of Proteins

Method of hydrolysis	Disadvantages
1. Acid	
8 N H_2SO_4 at 110°C for 18 h	1. Tryptophan is destroyed; Ser and Thr are partially destroyed. 2. Presence of carbohydrates leads to formation of a black material, humin.
6 M HCl at 110°C for 18 h	1. Trp, Asn, Gln destroyed; Ser, Thr, Tyr partially lost. 2. Cys and Met are either partially destroyed or oxidized to cysteic acid and Met-*S,S*-dioxide, respectively.
2. Alkali	
$Ba(OH)_2$	1. Partial or complete destruction of Arg, Cys, Ser, Thr. 2. Causes racemization and some deamination.
NaOH (5) or LiOH (6)	LiOH is reported to be best (6) for tryptophan determination.
3. Enzymes pepsin, trypsin, papain, chymotrypsin	1. Each enzyme is generaly specific for a particular peptide bond. 2. May produce hydrolysis of enzymes, which would interfere with the amino acid analysis.