

9.9.4.6 Racemization

All amino acid residues except glycine are subject to racemization at an alkaline pH, resulting in the formation of the D-enantiomers of the residue. Racemization is inevitably associated with conformational changes and thereby loss of function. The racemization of proteins has been described in several reports. The initial step of the reaction is abstraction of the β -hydrogen by hydroxide ions. By uptake of a proton, this will result in either the L- or D-amino acid residue. The carbanion formed may also undergo β -elimination. At pH 5–12, Asn, Asp, Gln, and Glu may modify via a succinimidyl intermediate, resulting in both the D- and L-derivatives. The indicators of racemization include change of protein structure and loss of biological activity. The change in optical rotation correlates with the rate of racemization. The amino acid residues undergo racemization at different rates. Preventive actions against racemization are listed in Table 9.9.

9.9.4.7 Cysteinyl Residues

The reactive site of the cysteinyl residue is the thiol group, which is deprotonated at an alkaline pH (pK_a around 8.5). The residue under the oxidizing conditions (and neutral to alkaline pH) is able to react with a similar residue under the formation of a disulfide bond. Many proteins are stabilized by intramolecular disulfide bonds (e.g., insulin, growth hormone, and insulin-like growth factor [IGF]-1), but intermolecular bonds may also result from the reaction under the formation of aggregates. In order to avoid unintended disulfide bond formation and cleavage, the redox potential of the solution must be monitored and controlled. In practice, aqueous buffers contain micromolar amounts of dissolved oxygen, assuring a redox potential of 200–600 mV, which is sufficient to maintain the intramolecular disulfide bonds. Proteins with free cysteines may prefer slightly reducing conditions, which can be obtained by the addition of micromolar amounts of reducing agent (e.g., cysteine and DTT). The number of proteins containing both –SH groups and disulfide bonds are relatively small (e.g., albumin and β -lactoglobulin). In many cases, the disulfide bond stabilization

TABLE 9.9

Preventive Actions Against Racemization

Factor	Comment
pH	High pH will favor abstraction of the β -hydrogen under formation of a carbanion. pH is kept below 10 and use of NaOH in concentration above 0.1 M is avoided when adjusting pH.
Temperature	The temperature is kept low.
Time	The reaction is a function of time.
Conductivity	No data are available.
Redox potential	No data are available.
Co-solvents	No data are available.

Source: *Handbook of Biogenic Therapeutic Proteins: Regulatory, Manufacturing, Testing and Intellectual Property Issues*, Taylor & Francis Group, Boca Raton, FL, 2005.