IMIDAZOLE GROUPS

Imidazole groups of histidine residues are involved in the catalytic function of many proteins. They are weakly basic groups (pK ~ 6.5) and subject to a large number of modification reactions. They react readily with most acylating, alkylating, and many electrophilic reagents. Acylation of imidazole groups is seldom observed since most acylimidazoles rapidly hydrolyze in aqueous solutions. Dye-sensitized photochemical oxidation of imidazole groups has been the most generally useful procedure for their modification and for determining their important to various properties of proteins (see Section 8-8).

Photochemical oxidation in the presence of a sensitizing dye can be done under mild conditions and is frequently quite specific in its modification of imidazole groups. The dyes methylene blue or rose bengal appear to be suited for the modification of imidazole groups. Typical procedures employ 0.01 % dye (either methylene blue or rose bengal) at approximately neutral pH and at, or slightly below, room temperature. The solution, containing 0.5 to 10 mg/ml protein, is stirred rapidly to hasten exchange of oxygen with the atmosphere and is placed 8 to 15 cm in front of a relatively bright visible light source (i.e., a 150- to 300-watt spotlight, slide projector, etc.), and irradiated for a period of from a few seconds to a few minutes. The sample should be kept in the dark before and after this irradiation. The exact conditions to effect the desired loss of histidine will usually have to be determined by several trial experiments. Careful control of the reaction conditions is required to get reproducible results. A control containing protein and dye should be kept in the dark and run simultaneously. The dye can usually be removed by gel filtration, or more slowly by dialysis. Methylene blue can be absorbed on charcoal or Dowex 1-X10 (chloride form) and removed. For further information, the procedures of Ray (1967), Westhead (1965), or Ray and Koshland (1962) are recommended.

The reader is encouraged to investigate the possible usefulness of ethoxyformic anhydride (Section 5-1), halocetates (Section 6-1), iodine (Section 9-1), and diazonium reagents (Section 9-3), as histidine reagents which are sometimes useful.

REFERENCES