of activity by the product provides strong support for the crystallographic hypothesis of the activity of lysozyme.

Selective Photo-oxidation of Amino Acids in Proteins

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The photoactivation of oxygen, mediated by sensitizers, produces a reagent capable of modifying the methionyl, cysteinyl, histidyl, tyrosyl and tryptophyl side chains in proteins (Spikes & Straight, 1967). The photo-oxidative attack can be directed towards specific residues by careful control of the reaction conditions. This can be achieved by several approaches.

First, a high selectivity can be obtained by a suitable choice of the sensitizer. For example, in the presence of haematoporphyrin, methionine is specifically photo-oxidized to the sulphoxide both in neutral aqueous solution and in acetic acid solution (Jori, Galiazzo & Scoffone, 1969). On the other hand, proflavine can be made to act selectively on tryptophan by irradiation in aqueous formic acid (Benassi, Scoffone, Galiazzo & Jori, 1967; Galiazzo, Jori & Scoffone, 1968); the competing photo-oxidation of methionine can be overcome by chemical reduction of the sulphoxide with thiols (Jori, Galiazzo, Marzotto & Scoffone, 1968a,b). Finally, Crystal Violet sensitizes the selective photo-oxidation of cysteine to cysteic acid over the complete pH range and in acetic acid solution (Jori, Galiazzo & Scoffone, 1969b).

Secondly, the photo-oxidation can be rendered more selective by taking advantage of the shielding effect exerted by the protein conformation on some of the susceptible residues. A method for differentiating the exposed from the buried residues by means of dye-sensitized photo-oxidation was developed by Ray & Koshland (1962). Their procedure was refined by us in order to detect the presence of partially exposed residues (Jori, Galiazzo & Scoffone, 1970b). The method involves the induction of a multi-stage unfolding of protein molecules by the stepwise addition of a suitable denaturant: in this way a gradually increasing number of residues become susceptible of photo-oxidation, first the fully exposed ones, then the partially buried ones and finally the deeply buried ones. This approach was applied to the elucidation of the degree of exposure of the methionyl residues in lysozyme (Jori *et al.* 1969a; Galiazzo, Tamburro & Jori, 1970) and in ribonuclease A.

Finally, the photoreaction can be confined to predetermined regions of a protein molecule by using a protein-bound sensitizer. Owing to the

short lifetime of the intermediate photoexcited species, only those photo-oxidizable residues that are close to the sensitizer are modified. In this connexion, one can utilize the enhanced reactivity displayed by functional groups in the active site of many enzymes to label them in a specific fashion. Both some natural coenzymes, such as the porphyrins or pyridoxal phosphate, and many group-specific reagents, containing nitro or keto groups, are efficient sensitizers for the photo-oxidation of amino acids (Jori *et al.* 1970b). The validity of this procedure was successfully tested with ferricytochrome *c* (Jori, Galiazzo, Gennari & Scoffone, 1970a), as well as with 41-DNP-ribonuclease A (Scoffone, Galiazzo & Jori, 1970) and *S*-DNP-papain. Therefore this novel strategy appears to provide a valuable tool for mapping the topology of proteins in solution.

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Pulse Radiolysis: An Oxidative-Reductive Probe in General Biochemical and Enzyme Studies

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Pulse radiolysis, the high-energy analogue of flash photolysis, provides a versatile high-speed technique for the study of electron transfer and other redox processes in general biochemical and enzymic systems. As in flash photolysis, changes in the optical absorption properties of a system are