must be taken into account during examination of these reactions, since, besides the general danger of denaturation, the complexity of a protein molecule, where many reacting groups form a unique environment, may bring about considerable alterations in the chemical reactivity of these groups, which often does not closely resemble that shown by the same groups in simpler compounds.

Ox liver glutamate dehydrogenase is one enzyme that has recently been the object of chemical modifications. Although these investigations are still at a very preliminary stage, the studies on the active and regulatory sites of this enzyme that we and other investigators are carrying out indicate that both amino and aromatic groups are involved in the reactivity of the enzyme sites. Fisher & Cross (1965) have suggested, on the basis of solvent-perturbation difference spectra, that tryptophan is present at the glutamate-binding site, and these findings are consistent with those of Brocklehurst *et al.* (1970). Colman & Frieden (1966a,b) have shown that enzymic activity is lost and binding constants of allosteric modifiers are altered by acetylation of several lysyl residues. The use of dinitrophenylation (di Prisco, 1967) has led to the finding that this enzyme possesses two separate or perhaps partially overlapping regulatory sites, one for GTP and the other for ADP. The reactivity of the regulatory sites, as well as that of the catalytic ones, which can be selectively protected by the presence of the specific ligand, is lost when, in each site, a set of six lysyl and three tyrosyl residues per molecule of active oligomer, composed of six, probably identical (Appella & Tomkins, 1966), polypeptide chains of molecular weight 53 000 (Eisenberg & Tomkins, 1968) has been dinitrophenylated (G. di Prisco, unpublished work). In agreement with these findings, Anderson, Anderson & Churchich (1966) have shown inhibition by pyridoxal phosphate, which, after reduction and total hydrolysis, was found combined in a Schiff base form with a lysyl residue involved in the reactivity of the catalytic site. The essential role of lysyl and tyrosyl residues in the catalytic and allosteric properties of the enzyme has been confirmed by different techniques, involving reaction with some other group-specific reagents (Brocklehurst *et al.* 1970).

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The Reactivity of Group-Specific Chromophoric Reagents

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The chemical modification of reactive side chains in enzymes can be used to obtain information about conformation, 'essential' groups and the role of such side chains in subunit interactions. In addition, if the reagent has environmentally sensitive optical properties, it can be used to explore the nature of specific sites.

Two group-specific reagents will be described.

2,4,6-Trinitrobenzenesulphonic acid has been used to characterize the reactivities of amino groups in several enzymes (Freedman & Radda, 1969). When the extent of reaction is compared with changes in activity and allosteric response, particular amino groups can be associated with these enzymic functions. 2,4,6-Trinitrobenzenesulphonic acid can also be employed to detect conformational changes (e.g. between the oxidized and reduced forms of cytochrome c).

7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (Ghosh & Whitehouse, 1968) reacts rapidly with thiol groups to give a fluorescent product. The uses of this reagent for the study of glyceraldehyde 3-phosphate dehydrogenase and muscle phosphorylase will be discussed.

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X-Ray Analysis of an Unusual Reaction in Lysozyme

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The iodination of lysozyme in acid media results in a product that is enzymically inactive. The reaction has been carried out in the crystalline state. X-ray analysis of the product by high-resolution difference Fourier methods show that glutamate-35 and tryptophan-108 have undergone an unusual reaction caused by environmental factors. The loss

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