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## PHENOLIC GROUPS

Phenolic groups of tyrosine residues are important structural and functional components of most proteins. They are subject to a large number of chemical modification procedures. The phenolic hydroxyl group is susceptible to modification by many acylating and alkylating agents. The aromatic ring is susceptible to attack and substitution by a number of electrophilic reagents. Both types of reactions become more rapid at higher pH's, where the phenolic group is increasingly more ionized. In most cases, modification of phenolic groups is readily quantitated, because most modifications result in a distinctive change in absorbance.

*Reaction with N-Acetylimidazole.* The most useful procedure for modification of phenolic hydroxyl groups is that first described by Riordan et al. (1965) using N-acetylimidazole to effect their acetylation (see Section 5-1):

The protein in 0.05 M sodium borate, or 0.02 M veronal, buffer at pH 7.5 is treated with approximately a 60-fold molar excess of N-acetylimidazole. The solution after conclusion of the reaction is dialyzed or subjected to gel filtration to separate the protein from low-molecular-weight components of the solution. To avoid deacetylation of the phenolic groups, the pH of the solution should not exceed 8. The extent of reaction can be determined from the change in absorbance using  $\Delta\epsilon_{278} = 1160 M^{-1} \text{ cm}^{-1}$ , or by the hydroxamate procedure of Balls and Wood (1956). Acetylation of amino groups, which also frequently occurs upon such treatment, can be detected by determining the number of such groups remaining as compared to unmodified protein (see Section 4-1).

*Iodination* of phenolic groups has been one of the most useful protein modification techniques. The reaction conditions as well as the reagent itself are subject to great variation (see Section 9-1). The following procedure of Azari and Feeney (1961) for the iodination of chicken conalbumin with  $\text{KI}_3$  is suitable for extensive iodination of most proteins (for less extensive reaction, less reagent should be used):

The protein is dissolved in 0.1 M borate buffer (pH 9.5) and chilled to 1°-2°C. A chilled 0.05 M solution of iodine in 0.24 M KI is added, and the solution is incubated for about 15 minutes. The amount of iodine added is that required to convert all tyrosine to diiodotyrosine ( $\text{tyrosine} + 2 \text{I}_2 \rightarrow \text{diiodotyrosine} + 2 \text{I}^-$ ). The progress of the reaction can be followed from the decrease in intensity of the reddish-brown to yellow color of the reagent, and a few drops of 1.0 M  $\text{NaHSO}_3$  can be added to stop it at any time. The modification of tyrosine can be estimated by the spectrophotometric procedure of Edelhoch (1962). Total iodine incorporated can be determined by standard iodide analysis following alkali-metal fusion.

*Nitration with Tetranitromethane.* An important recently developed procedure for ring substitution of phenolic groups is that of Sokolovsky et al. (1966) for nitration with tetranitromethane:

The reaction is normally carried out in 0.05 M Tris buffer (pH 8.0) at room temperature. Aliquots of the reagent, 0.84 M in 95% ethanol, are added and the reaction allowed to proceed for about 30 minutes. The amount of reagent required will vary with different proteins and with extent of modification desired. A 64-fold excess of tetranitromethane over carboxypeptidase A gave modification of 6.7 of the 19 phenolic groups. Gel filtration or dialysis can be used to separate the protein from the reaction solution. The

nitrotyrosyl residues are stable and can be readily quantitated from their absorbance (see Section 9-2) or by amino acid analysis after acid hydrolysis. A useful further modification is the reduction of the nitro groups to amino groups (see Section 9-2).

## REFERENCES

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