

Binding groups on the protein molecule are usually side groups (R) or the amino or carboxyl groups of the polypeptide chain.

The cross-linking of enzyme molecules with each other using agents such as glutaraldehyde, *bis*-diazobenzidine, and 2,2-disulfonic acid is another method of enzyme immobilization. Cross-linking can be achieved in several different ways: enzymes can be cross-linked with glutaraldehyde to form an insoluble aggregate, adsorbed enzymes may be cross-linked, or cross-linking may take place following the impregnation of porous support material with enzyme solution. Cross-linking may cause significant changes in the active site of enzymes, and also severe diffusion limitations may result.

The most suitable support material and immobilization method vary depending on the enzyme and particular application. Two major criteria used in the selection of support material are (1) the binding capacity of the support material, which is a function of charge density, functional groups, porosity, and hydrophobicity of the support surface, and (2) stability and retention of enzymatic activity, which is a function of functional groups on support material and microenvironmental conditions. If immobilization causes some conformational changes on the enzyme, or if reactive groups on the active site of the enzyme are involved in binding, a loss in enzyme activity can take place upon immobilization. Usually, immobilization results in a loss in enzyme activity and stability. However, in some cases, immobilization may cause an increase in enzyme activity and stability due to more favorable microenvironmental conditions. Because enzymes often have more than one functional site that can bind the surface, an immobilized enzyme preparation may be very heterogeneous. Even when binding does not alter enzyme structure, some enzyme can be bound with the active site oriented away from the substrate solution and toward the support surface, decreasing the access of the substrate to the enzyme. Retention of activity varies with the method used. Table 3.4 summarizes the retention of activity of aminoacylase immobilized by different methods.

TABLE 3.4 Effect of Immobilization Methods on the Retention of Enzymatic Activity of Aminoacylase

Support	Method	Observed activity (units)	Enzyme activity immobilized (%)
Polyacrylamide	Entrapment	526	52.6
Nylon	Encapsulation	360	36.0
DEAE-cellulose	Ionic binding	668	55.2
DEAE-Sephadex A-50	Ionic binding	680	56.2
CM-Sephadex C-50	Ionic binding	0	0
Iodoacetyl cellulose	Covalent binding	472	39.0
CNBr-activated Sephadex	Covalent binding	12	1.0
AE-cellulose	Cross-linked with glutaraldehyde	8	0.6

With permission, from D. I. C. Wang et al., *Fermentation and Enzyme Technology*, John Wiley & Sons, New York, 1979.