3.4.3. Electrostatic and Steric Effects in Immobilized Enzyme Systems

When enzymes are immobilized in a charged matrix as a result of a change in the microenvironment of the enzyme, the apparent bulk pH optimum of the immobilized enzyme will shift from that of soluble enzyme. The charged matrix will repel or attract substrates, product, cofactors, and H^+ depending on the type and quantity of surface charge. For an enzyme immobilized onto a charged support, the shift in the pH-activity profile is given by

$$\Delta\text{pH} = \text{pH}_i - \text{pH}_e = 0.43\frac{zF\psi}{RT} \tag{3.61}$$

where pH_i and pH_e are internal and external pH values, respectively; z is the charge (valence) on the substrate; F is the Faraday constant (96,500 coulomb/eq. g); ψ is the electrostatic potential; and R is the gas constant. Expressions similar to eq. 3.61 apply to other nonreactive charged medium components. The intrinsic activity of the enzyme is altered by the local changes in pH and ionic constituents. Further alterations in the apparent kinetics are due to the repulsion or attraction of substrates or inhibitors.

The activity of an enzyme toward a high-molecular-weight substrate is usually reduced upon immobilization to a much greater extent than for a low-molecular-weight substrate. This is mainly because of steric hindrance by the support. Certain substrates, such as starch, have molecular weights comparable to those of enzymes and may therefore not be able to penetrate to the active sites of immobilized enzymes.

Immobilization also affects the thermal stability of enzymes. Thermal stability often increases upon immobilization due to the presence of thermal diffusion barriers and the constraints on protein unfolding. However, decreases in thermal stability have been noted in a few cases. The pH stability of enzymes usually increases upon immobilization, too.

3.5. LARGE-SCALE PRODUCTION OF ENZYMES

Among various enzymes produced at large scale are proteases (subtilisin, rennet), hydrolases (pectinase, lipase, lactase), isomerases (glucose isomerase), and oxidases (glucose oxidase). These enzymes are produced using overproducing strains of certain organisms. Separation and purification of an enzyme from an organism require disruption of cells, removal of cell debris and nucleic acids, precipitation of proteins, ultrafiltration of the desired enzyme, chromatographic separations (optional), crystallization, and drying. The process scheme varies depending on whether the enzyme is intracellular or extracellular. In some cases, it may be more advantageous to use inactive (dead or resting) cells with the desired enzyme activity in immobilized form. This approach eliminates costly enzyme separation and purification steps and is therefore economically more feasible. Details of protein separations are covered in Chapter 11.

The first step in the large-scale production of enzymes is to cultivate the organisms producing the desired enzyme. Enzyme production can be regulated and fermentation conditions can be optimized for overproduction of the enzyme. Proteases are produced by using overproducing strains of *Bacillus*, *Aspergillus*, *Rhizopus*, and *Mucor*; pectinases are produced by