# FOR INDUSTRIAL REACTORS

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The main difference between a free and an immobilized enzyme is that, once immobilized, the enzyme is no longer completely surrounded by an aqueous environment. One can suspend the immobilized enzyme in a solution of substrate, activators, or other components at a particular pH and ionic strength; but one does not have the assurance that the conditions in the medium immediately surrounding the enzyme are the same as those in the external solution. Indeed they may be quite different. This phenomenum can be the result of the charges or the physico-chemical properties of the supporting matrix or may result from diffusional limitations. This latter aspect will be discussed later.

Consider the simple case of proteins adsorbed onto glass which has a negative surface charge. This system will usually exhibit an apparent pH optimum higher than that observed with the free enzyme. In other words, the negative charges in the immediate neighborhood of the enzyme must be neutralized before the pH in this area is raised to that of the solution. Similarly, any charged locations can affect the apparent pH optimum shifting it up or down as the case may be. Goldstein and his group at the Weizmann Institute purposely prepared immobilized enzyme systems containing large numbers of charged groups. They copolymerized chymotrypsin with polyornithine and the resultant copolymer, containing between 34 and 820 positive groups per molecule shifted the apparent pH optimum from 8.3 to as low as 7.5. Similarly, the ethylene maleic anhydride copolymer of chymotrypsin containing 290 carboxyl groups per molecule raised the apparent pH to 9.5 (1).

In addition to charge, other properties of the carrier may influence the observed reactivity of the immobilized enzyme. An excellent case in point is the study by Brockman,et.al(2). They adsorbed pancreatic lipase to solid glass beads which had been siliconized creating a surface with a strong hydrophobic character. They found that the catalytic

These types of effects are major ones and can be summed up in the term "microenvironmental effect", a term coined by Katchalski (3). This means that, with the exception of hollow fiber, dialysis, gel entrapped, or ultra-filtration systems, one can never say with certainty just what actual conditions exist in the neighborhood of the enzyme regardless of the conditions set in the external solution. In most of these cases, the changes in the properties observed are apparent changes and not changes in the actual enzymatic properties. Other apparent changes which can result from the microenvironmental conditions are changes in the observed Michaelis constant and the effect of product, substrate, inhibitor, or activator concentrations. In addition, these would result in changes in the observed rate of the reaction.

The immobilization process, particularly if the enzyme is entrapped in a gel, copolymerized, or adsorbed or covalently coupled within pores of a matrix, can impart diffusional problems which must be considered. In order for the substrate to be acted upon, it must diffuse from the external solution into the rather static liquid layer that surrounds the particle and then into the pore where the solution is almost stagnant and where the enzyme is located. The product must diffuse in the reverse direction. These mass transfer effects can create problems in assay and in the use of the immobilized enzyme system. In many cases, the kinetic expressions are considerably altered. This matter, primarily with respect to industrial applications, will be treated in detail in a subsequent chapter in this text. Certain representative laboratory studies or theoretical approaches to describing the kinetics of particular systems are included in the references (10-24) to this chapter.

Diffusional effects also result in apparent changes in the measured enzymatic properties. Im-

explanation is that the immobilization occurred through side chains required for catalytic activity. Even under the best conditions of immobilization, it is unusual to immobilize more than 80% of the available enzyme in an active form. A good example to illustrate this point is the data of Bernfeld, et.al. (4) which is listed in Table 1. Since a crystalline, uniformly labeled protein was used, radioactivity measurements are indicative of total protein. These data show that 55% of the radioactivity but only 10% of the enzymatic activity was recovered in the immobilized enzyme preparation.

TABLE 1

IMMOBILIZATION OF $^{14}C$-LABELED ALDOLASE ON POLYACRYLAMIDE

| Source | % Recovery Activity | % Recovery Radioactivity |
|---|---|---|
| Insoluble enzyme | 10.4 | 55.0 |
| Aqueous phase after polymerization | 33.1 | 44.2 |
| Liquid phase after first wash | 1.0 | 0.7 |
| Liquid phase after second wash | 0 | 0.3 |
| Liquid phase after third wash | 0 | 0 |
| TOTAL RECOVERY | 44.5 | 100.2 |

It is dangerous to assume that all unrecovered enzyme is immobilized and active. For this reason, many of the reported effects of immobilization on V and the value of the catalytic constant (k') are invalid since any change in the effective enzyme concentration will affect these values. Unless an independent method of determining the active enzyme concentration, other than simple assay is employed, these reports should be minimized.

One of the primary causes of thermal deactivation of the enzyme is the disruption of the rela-

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