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Biospecific Reversible Immobilization

A Method for Introducing Labile Structures into Analytical Systems

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Abstract

Immobilized lectins placed in continuous flow systems were used for biospecific reversible immobilization of labile biochemical structures, e.g., enzymes such as ascorbic acid oxidase and acetylcholine esterase, and cells, such as red blood cells and lymphocytes. The species thus immobilized were applied in continuous flow analytical processes.

Index Entries: Biospecific reversible immobilization; reversible immobilization, of labile structures, biospecific reversible immobilization of; lectin, immobilization of human lymphocytes with; human lymphocytes; immobilization with lectin; lymphocytes, human, lectin-immobilized; concanavalin A; lentil; cells, immobilized.

Introduction

The necessity of a constant response from immobilized enzyme preparations used in analysis has led to routine use of large quantities of catalytically active enzyme molecules in the immobilization process so that the final product, still containing many enzyme molecules active *per se*, is characterized by diffusion limitations. The result is that only a fraction of the catalytic potential is utilized, with the superfluous enzyme molecules then being kept as a buffer for use on denaturation of

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substrate-exposed enzyme molecules (*1*). The use of catalytic capacity in excess offers some obvious advantages:

1. High operational stability.
2. Insensitivity to changes in pH, temperature, and so on.
3. Reproducible operational characteristics from one preparation to another.

These advantages have led to a general acceptance of the concept of high operational stability. This in turn has resulted in a natural restriction of the choice of enzymes used, i.e., to the use chiefly of those that are rather stable and available in large amounts at low price.

There are situations when it may be advantageous to operate with systems designed for low operational stability. Such properties of a system can be achieved by immobilizing only a small amount of enzyme to the support. In analytical applications using such systems, either the whole immobilized preparation must be changed at regular time intervals or only the enzyme.

Experimental Procedure and Discussion

Analytical systems for preparations with low operational stability widen the field of potential applications. Some of these are given below:

1. Labile enzymes may be used.
2. Strong inhibitors may be quantified.
3. Cells may be used for bioassays.

There are examples on commercially available instruments based on exchangeable enzyme preparations (*2*) for analysis of strong inhibitors. However, for the other points listed above, a reversible immobilization of the biologically active substance would also be possible (*3*).

Examples of continuous flow systems as well as of conventional analytical devices for dipping into the sample to be analyzed are described in the literature. In the present paper, however only flow systems will be discussed. The concept of biospecific reversible immobilization is illustrated in Fig. 1.

Several potential receptor–ligand pairs are available, but so far antigen–antibody and lectin–carbohydrate are those most commonly used. In these cases, the antibody or the lectin is bound to the solid p hase and the enzyme is introduced as a pulse in the continuous flow. To assure binding, the molecules may sometimes be modified by coupling suitable ligands to their surfaces. (*4*).

The amount of receptor present on the support can be very large and thereby give the system a high operational stability. This can be done since receptor molecules are charged with enzymes only to a certain degree, varying with the amount of free enzyme added. This makes it possible to operate with an extremely high excess of binding positions—a situation similar to that of the apoenzyme electrode in which the immobilized apoenzyme was inactive and catalytic activity was gained only upon binding of the cofactor (*5*).

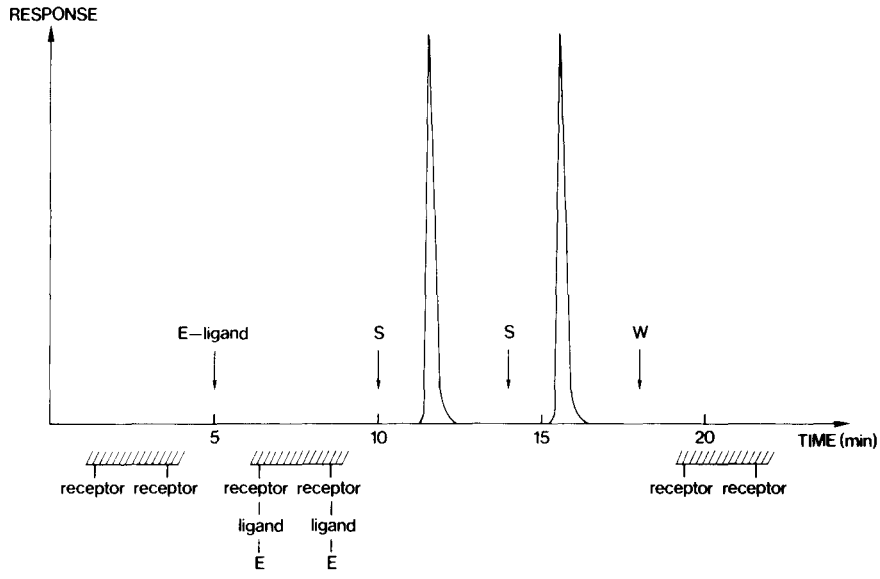

Fig. 1. Schematic presentation of an assay cycle. The arrows indicate "E-ligand": addition of enzyme to be immobilized via receptor–ligand interaction; "S" addition of a pulse of substrate for the immobilized enzyme; "W" wash with a pulse of, e.g., 0.2*M* glycine–HCl, pH 2.2.

Lectin–glycoprotein interactions have proved a very useful reactant pair in these studies since several of the most frequently used enzymes are glycoproteins. Thus, in model studies, glucose oxidase, peroxidase (*6*), and invertase were immobilized with this method (*7*). Acetylcholine esterase for quantitation of an inhibitor was also bound via lectin interaction (*8*), as was ascorbic acid oxidase (*7*). In the two last mentioned cases, only very small amounts of enzyme were used (1–5 U). Ascorbic acid oxidase was stable for 1–2 days under the conditions used. The enzyme was therefore washed off after 1 day and new enzyme was added the following day.

When glucose oxidase was immobilized for the thermometrical analysis of glucose, it soon turned out that the low amount of oxygen present in the solution to be analyzed restricted the concentration range that could be analyzed (*6*). However, when glucose oxidase and red blood cells were co-immobilized to a lectin-containing support, a much better result was obtained in that a broader concentration range could be analyzed since the hemoglobin in the RBC preparation operated as oxygen reservoirs that were depleted when the oxygen pressure dropped as a result of enzyme activity. In the period between the pulses of sample, the hemoglobin was recharged so that the system operated in a reproducible manner (*6*).

In attempts to apply modern bioanalytical procedures to bioassays, interest has been focused on the use of whole cells in combination with a transducer. Several reports on the use of microbial cells are now available (*9*, *10*).

The assay procedure *per se* prevents further use of the cell preparation since the metabolic status of the cell is markedly changed. The studies reported so far have all dealt with exchangeable immobilized preparations.

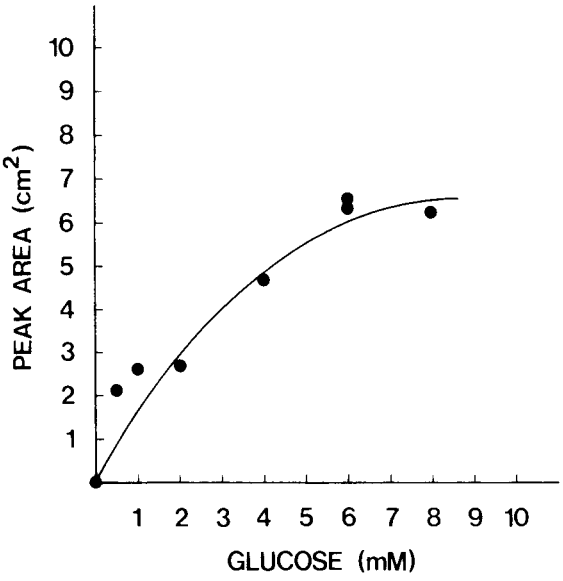

Fig. 2. Calibration curve obtained at 37°C for varying concentrations of glucose when using immobilized human lymphocytes. The buffer used was Tris-buffered Hank's solution, pH 7.4. Flow rate 0.5 mL/min.

Since lectin–carbohydrate interactions are very mild, we tried to use those for immobilization of mammalian cells for use in analysis. Lymphocytes, prepared by Ficoll-paque centrifugation (*11*) of blood from healthy persons, were immobilized on lectin–Sepharose. In this case, concanavalin A from *Canavalia ensiformis* or lentil from *Lens culinaris* were used. The immobilized cells responded to pulses of varying concentrations of glucose (Fig. 2)., indicating that at least a major part of their metabolism was intact. Further studies on the use of immobilized mammalian cells in bioassays are now in progress.

Acknowledgments

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