Immobilized Bilayer Glucose Isomerase in Porous Trimethylamine Polystyrene Based on Molecular Deposition

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Bilayer glucose isomerase was immobilized in porous trimethyamine polystyrene (TMPS) beads by molecular deposition based on electrostatic attraction, multiplying the activity of the immobilized enzyme per unit mass by addition of enzyme in the support.

Enzyme immobilization has been the subject of attention for many years, and methods including absorbtion covalent and cross linkage and entrappment have been developed.[1–4] In order to increase the catalytic efficiency of immobilized enzyme, it is necessary to develop a new immobilization method which increases the activity of the immobilized enzyme per unit mass.

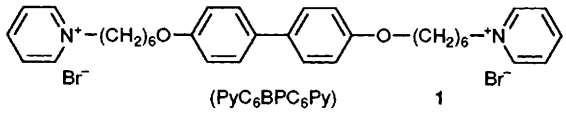
There are a number of reports of the preparation and properties of self-assembled films based on protein and enzyme,[5–7] but all experience difficulties in maintaining the enzyme activity. Decher[8] studied multilayer ultrathin film formation by alternative deposition of bipolar amphophilic cationic and anionic layers. This technique has not yet been used in enzyme immobilization.

Here glucose isomerase was immobilized in porous TMPS beads by alternative deposition with bipyridinum salts. The enzyme activity was maintained after immobilization. We refer to this method as molecular deposition due to its similarity with MD film method.

Beads of porous TMPS, mean pore diameter 46 nm, were provided by Jilin University. Glucose isomerase (D-xylose ketol isomerase, EC 5.3.1.5) from *Streptomyces* was purchased from Leaders Biotech. Co. (USA), with molecular mass about 160 000, molecular diameter 10 nm and isoelectric point at pH 4.7. The glucose isomerase activity was assayed following Tomas's cysteine-carbazole method for fructose.[9] The protein content of the immobilized enzyme was determined by hydrolysis, the amino acid was measured using a Hitachi 835-50 amino acid analyser. The bipyridinum salt **1** was synthesized and characterised by elemental analysis, ^{1}H NMR and FTIR.

To immobilize the enzyme, beads of porous TMPS were first dipped into glucose isomerase solution with pH 7.0 and activity 370 IU cm^{-3} for 3 h. Because this carrier contained positively charged quaternary ammonium groups and negatively charged glucose isomerase under these pH conditions, the first layer of enzyme was absorbed in the carrier through cationic and anionic electrostatic attraction. At the same time, the surface charge of the carrier was reversed because of the enzyme being absorbed in it. After rinsing with de-ionised water, immobilized glucose isomerase was then immersed in saturated bipyridinum salt solution, at pH 7.0, for 2 h. In this way the surface of the first layer of enzyme was covered with a positively charged compound. The second layer of enzyme was deposited in the carrier by repeating the first step. The bipyridinum salt contains the 4,4′-biphenylene group which would only allow the salt to form intermolecular bonds with the enzyme due to its rigidity. The ideal model for construction of immobilized bilayer enzyme is shown Fig. 1.

This molecular deposition technique is a new method for the formation of well-defined ultrathin films based on salt formation between cationic and anionic compounds. A multilayer film can be built through alternative deposition of either bipolar cationic compound/anionic polyelectrolyte or bipolar anionic compound/cationic polyelectrolyte.[10,11]



(PyC$_6$BPC$_6$Py) **1**

Because enzyme can be easily induced to be negatively charged when the pH value of the solution is above its isoelectric point, the first layer of enzyme could be immobilized in the positively charged TMPS based on cationic and anionic electrostatic attraction. Then with a similar process to molecular deposition, it is envisaged to immobilize multilayer enzyme by substituting enzyme for anionic polyelectrolyte.

Enzyme loading, activity and Michaelis constants (K_m) of glucose isomerase are given in Table 1. The activity and specific activity of the first layer of enzyme in TMPS were 1034 IU g^{-1} dry gel and 30 IU mg^{-1} protein, respectively. After the second layer of enzyme was deposited in the carrier, the total activity and the specific activity of the immobilized bilayer enzyme were 2238 IU g^{-1} dry gel and 29 IU mg^{-1} protein, respectively. The total activity of the immobilized bilayer enzyme was about two times that of the immobilized monolayer enzyme and the specific activity was not changed after the second layer of enzyme was immobilized in the carrier. These results indicated the bipyridinum salt did not

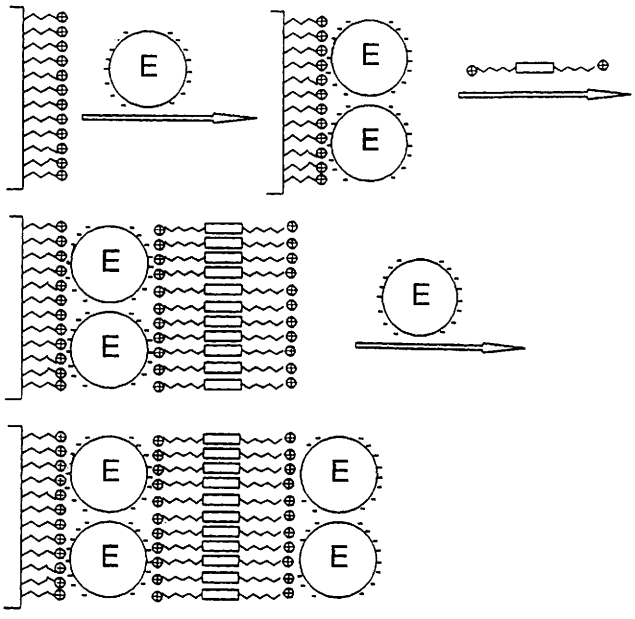


Fig. 1 The ideal model for construction of immobilized bilayer glucose isomerase based on alternative deposition of bipolar pyridine salt and glucose isomerase

Table 1 Properties of soluble, immobilized mono- and bi-layer glucose isomerase

Enzyme	Activity/ IU g^{-1} dry gel	Protein loading/ mg g^{-1} dry gel	Specific activity IU mg^{-1} protein	K_m/dm^3 mol^{-1}
Soluble	370[a]	6.6[b]	56	0.18
Monolayer	1043	34.5	30	0.30
Bilayer	2238	70.3	29	0.33

[a] Activity of soluble enzyme in IU cm^{-3}. [b] Protein content of soluble enzyme in mg cm^{-3}.

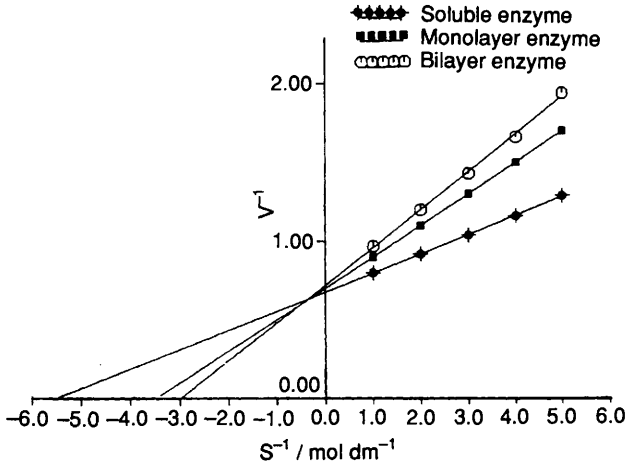


Fig. 2 Lineweaver–Burk plots of soluble, immobilized monolayer and bilayer glucose isomerase

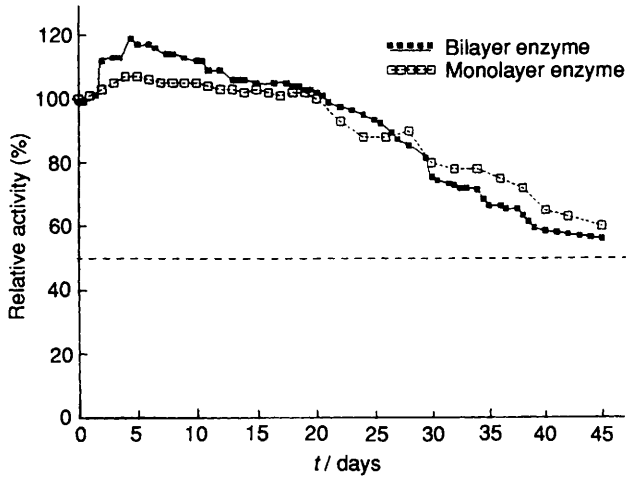


Fig. 3 Operation stability of immobilized bilayer and monolayer glucose isomerase in packed-bed reactors

alter the activity of the enzyme or affect the first layer of enzyme already absorbed in the support. Since the carrier pore diameter was 46 nm and isomerase diameter 10 nm, only two layers of enzyme could be deposited in the carrier due to the space limitations. If there was enough space in the porous bead, more than two layers of the enzyme could be immobilized in the carrier. This indicated this method could easily introduce multilayer enzyme and high activity, and it would have obvious advantages over other enzyme immobilization methods.

Lineweaver–Burk plots of soluble enzyme and immobilized enzyme are shown in Fig. 2.

In order to investigate the stability of this kind of immobilized bilayer enzyme, it was put in a packed-bed reactor to isomerize glucose. The reactor was filled with 0.8 g immobilized enzyme. The reaction temperature was maintained at 60 °C and the substrate solution containing glucose flowed through the reactor continuously at 12 $cm^3 h^{-1}$ (Fig. 3). The half-life was more than 45 days, similar to that of immobilized monolayer enzyme. This result indicated that the interaction between the enzyme and the bipolar cation was not strong enough to stably immobilise enzyme in the support. The enzymic activity hence decreased gradually due to the enzyme being removed from the carrier when continuous reaction in a packed-bed reactor was carried out.

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