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INVERTASE IMMOBILIZATION BY ADSORPTION ON POLYMER MICROSPHERES STUDIED BY RADIOIODINATION TECHNIQUE

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ABSTRACT

In this paper, we report the study of the diffusion behavior of invertase onto polystyrene-divinylbenzene (PS-DVB) microspheres. To detect the surface concentration of protein adsorbed on the microspheres, the chloramine-T method was used to label invertase and has been applied to the study of protein sorption properties on the microspheres. The sorption isotherms and the diffusion coefficient (D_f) were computed from experimental results and the concentration of bound invertase was determined in terms of the Fick´s law. The Hill equation was applied to the data and the binding capacity of the microspheres were estimated. The results of adsorption show that the radiolabelling invertase method are efficacious at the protein surface concentration detection and can be used to investigate the enzyme immobilization by sorption properties of polymer microspheres.

1. INTRODUCTION

The separation and purity of products resulting from biotechnology processes are taking on greater commercial importance. For this reason, the topic of bioactive molecules immobilized on a great variety of supports with various polymer matrices is of increasing current interest. Immobilization of enzymes renders them recyclable, reusable, and easily separable from the products of their catalytic activity.

The styrene-divinylbenzene copolymers have been used widely for proteins immobilization by adsorption due to the non-covalent superficial forces such as ionic interactions between the biocatalysts and the polymer carrier [1].

Adsorption tends to be less disruptive to the protein than chemical means of attachment because the binding is mainly by hydrogen bonds, multiple salt linkages, and van der Waal's forces [2].

Invertase (β-fructofuranosidase, EC3.2.1.26) is a yeast-derived enzyme that splits sucrose into glucose and fructose as shown in Figure 1. Invertase is mainly used in the food (confectionery) industry where fructose is preferred over sucrose because it is sweeter and does not crystallize as easily. The invertase adsorption to polymer microspheres is a mild process, and suitable for obtaining carriers for high fructose syrup (HFS) production [3].

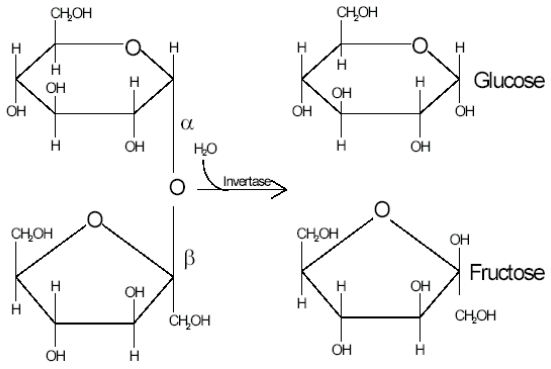

Figure 1. Reaction of glucose hydrolysis by invertase.

Enzyme diffusion followed immobilization by interfacial forces in the carrier plays a central role responsible by the interaction of the protein with the polymer carrier altering the protein structure or same their bioactivity. Thus, it is an appealing usually random diffusion may be unrestricted or corralled subject for studies about invertase immobilization by adsorption technique.

Measurements of the diffusion coefficients of protein through polymer matrix may be important in developing immobilized enzyme bioreactors. The knowledgement of the effective diffusion coefficients, D_f, of reacting compounds is of crucial importance for the quantitative analysis of bioprocesses using immobilized biocatalysts.

The objective of this work was to study the protein diffusion onto polysterene-divynylbenzene (PS-DVB) copolymers using invertase labeled with ^{125}I by the chloramine-T method.

2. MATERIALS AND METHODS

2.1. Materials

These were obtained from the following suppliers: polystyrene crosslinked with 4% divinylbenzene microspheres (Dowex®, Sigma-Aldrich Co), invertase from Saccharomyces cerevisiae (Sigma-Aldrich Co, 250 $IU.mg^{-1}$ of solid), ^{125}I (Amersham International).

2.2. Methods

2.2.1. ^{125}I invertase labeling

Invertase was labeled with ^{125}I by the chloramine-T procedure as described by Greewood et al [4] to a specific activity of 30 μCi.mg^{-1}. Thus, 2 μg of purified protein was mixed with 1 mCi Na^{125}I and 5μL chloramines-T (1 mg.mL^{-1}) in a final volume of 100 μL in 100 mM phosphate buffer (pH 7.4). After mixing briefly at room temperature 5 μL of sodium metabisulfite was added (1 mg.mL^{-1}). The whole mixture was then filtered on a Sephadex G-250 column, and labeled protein was isolated and concentrated from the void volume.

2.2.2. ^{125}I-Invertase adsorption

The appropriate poly(tetrafluoroethylene) (Teflon®) tubes containing the copolymer microspheres (PS-DVB) were injected with 50 mM cold sodium acetate (pH 5.0) buffer to displace the air and then thermally equilibrated at room temperature (25 ^{o}C). Any air bubles, which would adhere to the samples, were removed by allowing the samples to cross the air-buffer interface several times. Aliquots (4 mL) of the labeled invertase solution were then introduced into the tubes. After the protein solution remained in contact with the samples for different time intervals, the adsorption was terminated by dilution of the labeled protein into the tubes with sodium acetate, in order to avoid contact of the sample with the protein solution-air interface where some denaturation can take place. The samples were further rinsed gently until the radioactivity of the surface remained constant. The amount of adsorbed proteins was determined by gamma radiation counting, using a Beckman Gamma 4000.

To investigate the labeled of invertase activity, the initial rate of the sucrose hydrolysis solution (50 g.L^{-1} in 0.025 mol.dm^{-3} sodium acetate buffer, pH 5.0) was carried out for 3-6 h at 25 ^{o}C under constant stirring. For monitoring the hydrolysis, 1.0 mL samples were taken every 1 h and transferred to a polarimeter (Jasco Model DIP 370) for the measurement of the fructose contents.

3. RESULTS AND DISCUSSION

Chloramine-T (CAT) is the reagent of choice for releasing radioactive iodine from their corresponding salts in radiolabeling procedures involving halogenation of biomolecules. However, CAT is a strong oxidizing agent and can cause significant damage to proteins due to direct interaction with either peptide bonds or other functional groups. Since invertase is a multisubunit molecule whose subunits are held together by intermolecular forces [5], it seems logical to propose that oxidation during iodination may be destructive to these bonds and that the protein is fragmented.

The labelling efficiency for invertase as a function of activity is shown in Figure 2. The labeling efficience and invertase activity were 70% and 90% respectively, with a substitution level of 1.35 atoms of iodine/molecule of invertase.

The invertase activity indicates that iodine substitution no leads significant loss in biological function of enzyme using the CAT method at efficiency labeling no greater than 80%.

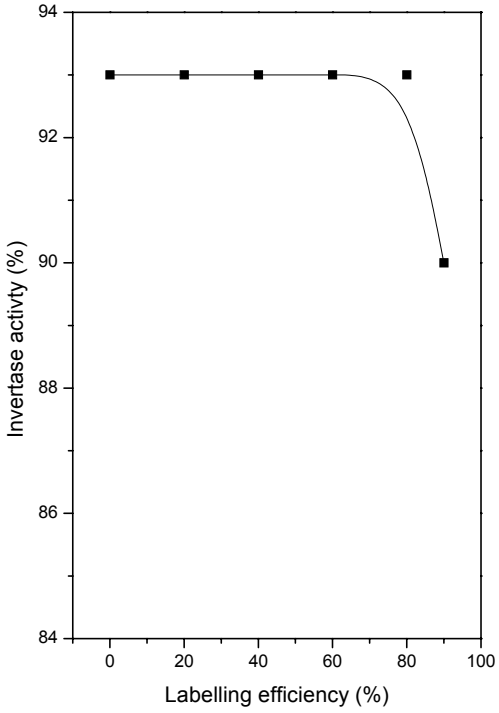

Figure 2. The effect of iodine substitution level on the invertase activity (pH 5.0 and 25 ^{o}C).

Immobilization of enzymes by adsorption on solid support has been pursued since the 1950s and is the method used the most in industrial biocatalysis [6]. The procedure consists of combining the biocatalyst and a support with adsorption properties, under suitable conditions such as pH, ionic strength for an incubation period, followed by collecting the immobilized material and extensive washing to remove nonbound biological component. Two of the most attractive properties are the simplicity of the method and the retention of activity during immobilization. Furthermore, the adsorption method is a very economical procedure for the immobilization of enzymes. The main disadvantage of this method is the weak binding of enzymes. However, desorption is turned to an advantage if the regeneration of the support is built into operational regimen to allow rapid expulsion of exhausted biocatalyst and replacement with fresh enzymes.

The ability to control solute diffusion through PS-DVB microspheres is the basis for various applications of such materials in bioengineering. The protein diffusion onto copolymer microspheres may be described by Flick's law, which correlates the solute's flux with its chemical potential gradient in the system. Thus, the structure and pore size of the PS-DVB, the water content and the nature and size of the solutes are all taken into account by the diffusion coefficient of solute.

Diffusion in PS-DVB microspheres may be regarded as diffusion in porous solid matrix, where pores are connected with each other and filled with solvent. The pore size distribution is a critical parameter because it influences the diffusion of molecules.

Invertase diffuses into a given concentration gradient defined by the Fick´s law [7]:

$$j = -D\left(\frac{\partial C}{\partial x}\right) \tag{1}$$

where J is the mass flux describing the mass transfer through microspheres, D is the diffusion coefficient and dC/dx is the gradient of concentration.

In the experiments the protein concentrations inside and outside of the microsphere are actually fixed and finite, thus:

$$\frac{\Delta C}{\Delta x} = \frac{\left(C_i - C_o\right)}{r} \tag{2}$$

and

$$j_{i\to 0} = \frac{1}{A}\frac{dn}{dt} = -D\frac{C_i - C_o}{r} \tag{3}$$

where r is the radius of the microspheres, n is the number of moles of protein, C_i and C_o are the concentrations of invertase inside and outside, respectively and A is a constant.

Under the above conditions:

$$\frac{dn}{dt}\alpha\,\frac{dC_i}{dt}\alpha - \frac{dC_o}{dt} \tag{4}$$

and

$$\frac{dC_o}{dt} = k\left(C_i - C_o\right) \tag{5}$$

where k is a constant.

The average number (ν) of invertase molecules bound to the microspheres may be estimated by:

$$\nu = \frac{C_i{}^o - C_i}{C_p} \tag{6}$$

The values of ν at different concentrations of C_i from equation (6) were estimated and then the binding isotherm was plotted in Figure 3.

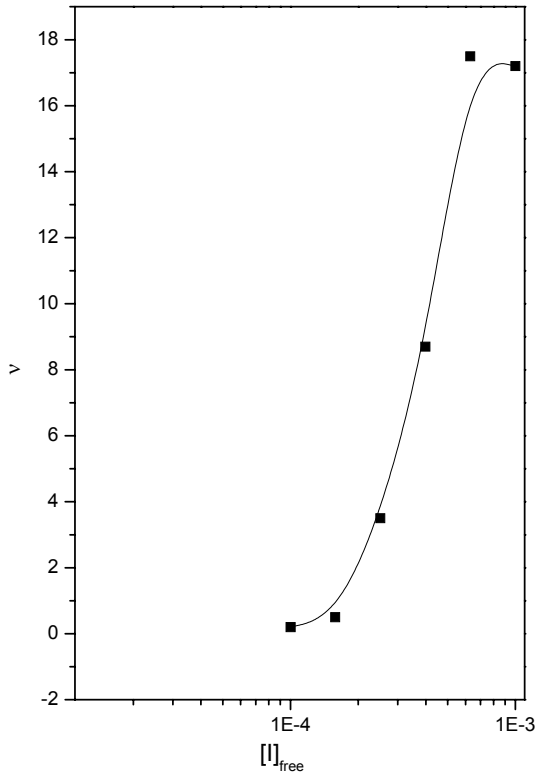

Figure 3. Binding isotherm for invertase to PS-DVB microspheres at pH 5.0 and 25 ^{o}C. $[I]_{free}$ is concentration of free invertase.

The Scatchard plot shown in Figure 4 is nonlinear which means that the PS-DVB and invertase interaction is positively cooperative.

The diffusion rate of invertase molecules into PS microspheres may be expressed by Wilson´s equation [8]:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{4}{\beta^2} e^{\left(-\beta_n^2 \left(\frac{D_f t}{r^2}\right)\right)} \tag{7}$$

where M_t and M_∞ are amounts of invertase taken up by a microsphere of radius r at time t and at equilibrium respectively, D_f is the diffusion coefficient of invertase in the microspheres ($cm^2.s^{-1}$) and βn are positive (non-zero) roots of $J_o(\beta_n)=0$.

The rate of invertase uptake (M_t/M_∞) and the sorption value of M_t were investigated and diffusion coefficient from the inverse Hill´s model were computed and compared.

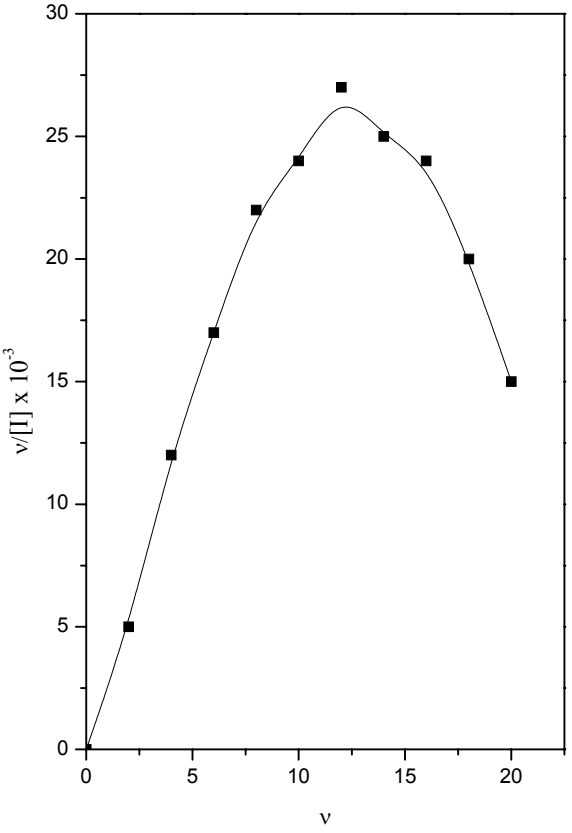

Figure 4. Scatchard plot for invertase interaction with PS-DVB at pH 5.0 and 25 °C.

In Figure 5, the influence of the microspheres size in the diffusion coefficient (D_f) is presented. It is evident that the microsphere size influences the diffusion coefficient. In fact, the diffusion is better at higher sizes. The large surface area exposed to invertase molecules may explain this phenomenon.

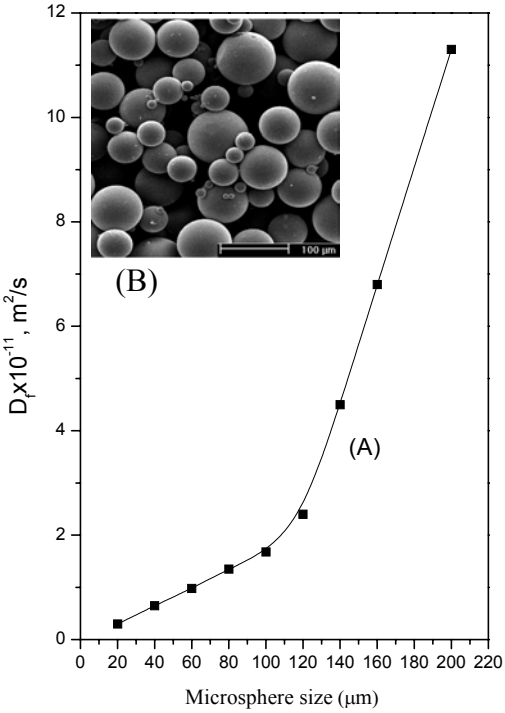

Figure 5. Diffusion dependence on microsphere size and invertase diffusion coefficient (A) and scanning electron micrograph of the PS-DVB microspheres (B).

4. CONCLUSIONS

Based on the above studies, it would appear that chloramine-T method has a great potential for use in radiolabeling technique of invertase. Radioiodination of invertase is practical for the detection of enzyme immobilized onto polystyrene-divinylbenzene microspheres. The invertase after radioiodination not changes their catalytic activity significatively. The sorption isotherms of PS-DVB microspheres indicated that invertase immobilization is dependent on the size of PS-DVB microspheres. The surface area may explain the changes in the invertase uptake rates. Invertase diffuse freely in the pores of the PS-DVB microspheres and the effective diffusion coefficient is dependent on concentration of protein.

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