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Scanning Electron Microscopic Observation of Porous Glass Fibers with Immobilized Glucoamylase

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ABSTRACT

Porous glass fibers with silane-glutaraldehyde immobilized glucoamylase have been examined by Scanning Electron Microscopy (SEM). Partial multilayer coating ("sheeting") on the fibers' surfaces has been observed even on gold uncoated samples by using a high resolution SEM. This "sheeting" is attributed to the fiber chemical activation treatment prior to enzyme loading. A 40% reduction of free pore area as a consequence of enzyme attachment is also observed.

Index Entries: Scanning electron microscopy; Enzyme covalent attachment; Immobilized glucoamylase observation; Porous glass fibers; Problems associated to silane–glutaraldehyde coupling.

INTRODUCTION

Glucoamylase is used on a large scale for the saccharification of liquified starch to a syrup of high glucose content (92–97%). Potential immobilized glucoamylase industrial use has been extensively reviewed (*1,2*).

A great variety of carriers and binding agents has been tested and reported in the literature (*3,4*). Covalent attachment is one of the most

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employed methods of immobilization. Glutaraldehyde is the most common binding chemical used specially with silica or glass supports (*5*).

Chemical activation and enzyme attachment usually take place in shaking beakers as a batch process (*6*). However, some serious attempts have been carried out for continuous operation (including chemical activation and enzyme loading) as a pilot plant (*7*) or laboratory scale (*8,9*) trying to facilitate future scale up.

However, microscopic enzyme attachment phenomena are often overlooked, especially for engineering considerations.

Recently, porous glass fibers—a new support offering high specific surface areas, good mechanical properties, and low pressure drop—were successfully tested for glucoamylase immobilization (*9,10*).

In the present work, successive steps of silane–glutaraldehyde coupling method for glucoamylase immobilization in porous glass fibers are observed by SEM.

MATERIALS AND METHODS

Materials

Porous glass fibers (23 μm diameter, 349 Å pore size, 0.41 cm^3/g pore volume, and 61 m^2/g surface area) were supplied by PPG Industries Inc. (Fiber Glass Research Center, Pittsburgh, PA). 349 Å pore size was chosen because of optimal enzyme activities (*6,9*).

Glucoamylase (1,4 α-D-glucan glucohydrolase EC 3.2.1.3) from *Aspergillus niger* was a gift from Finnsugar Biochemicals Inc. (Spezyme GA 200).

Glass fibers were washed with a 10% nitric acid solution, rinsed with distilled water, and dried at 75°C. These fibers (0.060 g) were packed in microreactors of 0.5 mL bed volume (2.95 cm length × 0.5 cm id).

Immobilization Method

Reactors were treated with a 3%-aminopropyltriethoxysilane solution at pH 7 (25 mL for each reactor) recirculating at 0.9 mL/min (with a multichannel Ismatec peristaltic pump) for 3.5 h at 75°C and washed at the same rate with 100 mL of distilled water. Glutaraldehyde solution (2.5% w/v) in 0.1*M* phosphate buffer at pH 7 was fed (10 mL per reactor) and left in contact at reduced pressure (1 h) and atmospheric pressure (1 h). Each reactor was washed with 300 mL of distilled water to remove excess glutaraldehyde and, finally, with 100 mL of acetate buffer (pH = 4.5). Then, one reactor was stored under refrigeration.

The other two reactors were used for enzyme immobilization. The initial glucoamylase-to-glass ratio was 150 mg protein/g of glass fiber.

Glucoamylase solution (18.5 mL) in 0.06*M* acetate buffer (pH = 4.5) was recycled through each reactor (0.9 mL/min) at 30°C. Enzyme loading took 2 h. Nonattached glucoamylase was removed by washing at the same rate with 40 mL of 0.06*M* acetate buffer (pH = 4.5), 5 mL of 6*M* urea, 50 mL of 1*M* NaCl in the acetate buffer containing 1% w/v maltose and, finally, 125 mL of 1% maltose in the acetate buffer. Both reactors were fed with a 20% w/v maltose in 0.06*M* acetate buffer (pH = 4.5) and stored under refrigeration until microscopic examination. High maltose concentration increased glucoamylase stability for a longer time (*10*).

Scanning Electron Microscopy

Fibers were picked out of reactors with a fine forceps. Treated fibers were fixed in 2.0% glutaraldehyde in 0.01*M* phosphate buffer at pH 7.0 (NaH_2PO_4–Na_2HPO_4) for 2 h. Samples were rinsed twice in phosphate buffer, dehydrated in an ethanol graded series (30, 50, 75, 95% once each and 100% three times) 15 min per exchange, and critical point dried in a Polaron E3000 Critical Point Dryer. Treated and untreated fibers for low magnification work were mounted onto aluminum specimen stubs with silver paint and gold coated in a Polaron 5100 Cool Sputter Coating Unit. Samples for high-resolution work (S-900) were affixed to stubs with carbon paint and viewed uncoated. Specimens were observed in either a Hitachi S-530 at 10 kV (60 Å resolution), Hitachi S-800 at 5–6 kV (20 Å resolution), or Hitachi S-900 at 1 kV (8 Å resolution) scanning electron microscope. Images were recorded on Kodak Tri-X Pan 4 × 5 film.

RESULTS AND DISCUSSION

Different aspects and details of porous glass fibers used as support for glucoamylase immobilization are shown in Fig. 1. The external porous surface (Fig. 1b) in contact with the bulk solution during enzyme loading presents an extremely high porosity with uniformly distributed pores (325 Å mean pore diameter). This observed external porosity has continuity inside of the fibers, as can be appreciated in a fiber cross-section (Figs. 1c,d), which reveals a spongy internal structure. Pores look about the same whether viewed from the end-on (a cross-section) or directly at the surface. The observed high uniformity of the external and internal structures of porous glass fibers make them an excellent support for biocatalyst attachment processes.

When fibers with attached glucoamylase are observed, some kind of film like a "sheet" appears covering part of the porous surface. This film is nonuniformly distributed, covering extensive fiber areas (Figs. 2a,b). These micrographs show partially detached "sheets," probably because

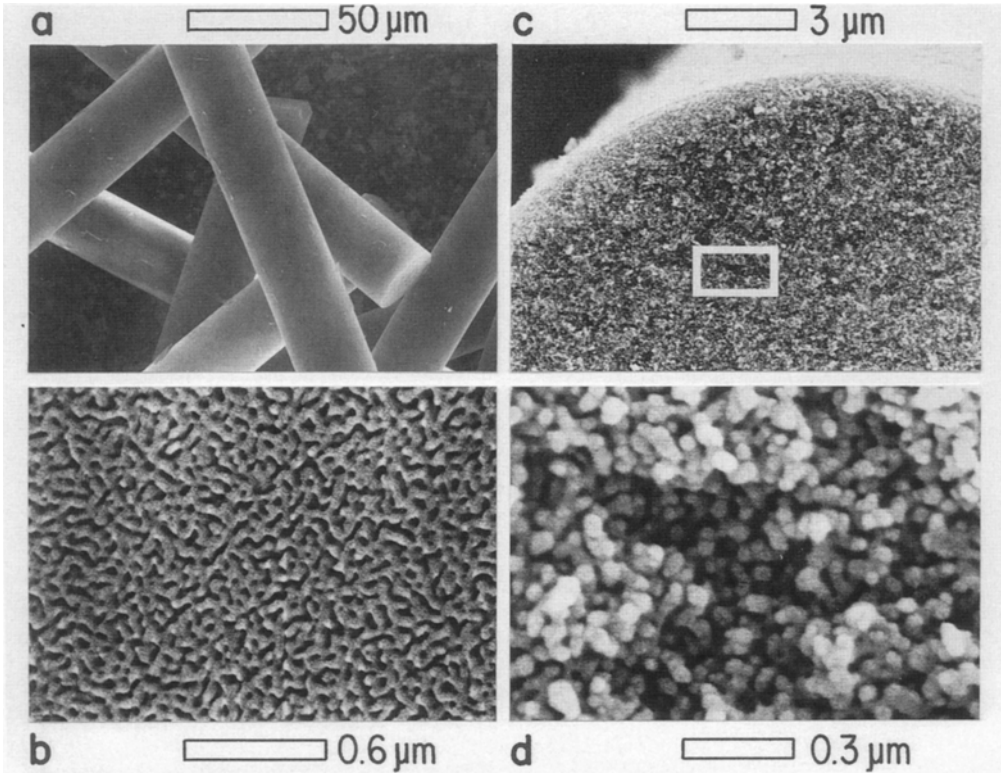

Fig. 1. Micrographs of raw porous glass fibers: (a) general view, (b) detail of lateral porous surface, (c) cross-section, and (d) detail of cross-section.

of physical manipulation of samples. Better details of one of these fiber surfaces with immobilized enzyme are shown in Figs. 2c,d. At first, the "sheeting" could be attributed to the attached protein and/or a faulty gold coating. However, the same "sheeting" has been observed, by using a high-resolution SEM, in chemically-activated fiber samples containing no enzyme and neither gold coated nor glutaraldehyde fixed (Figs. 3a,b,c). Thus, "sheets" could not be caused by sample manipulation before its SEM observation, neither to attached enzyme as deduced when comparing these fibers (Figs. 3a,b,c) and those with immobilized glucoamylase (Fig. 3d). So, it is concluded that these "sheetings" are caused by the glass activation treatment with silane–glutaraldehyde. Furthermore, the conditions used in this step may be the cause of multilayer "sheeting" (Fig. 3) as indicated by Lynn (*11*). The presence of "sheeting" causes a gradient distribution of chemical groups susceptible to enzyme attachment. Then, when glucoamylase solution is presented to the activated fibers, enzymes should be randomly distributed over the "sheets" and the fiber pores' surfaces (Fig. 3d).

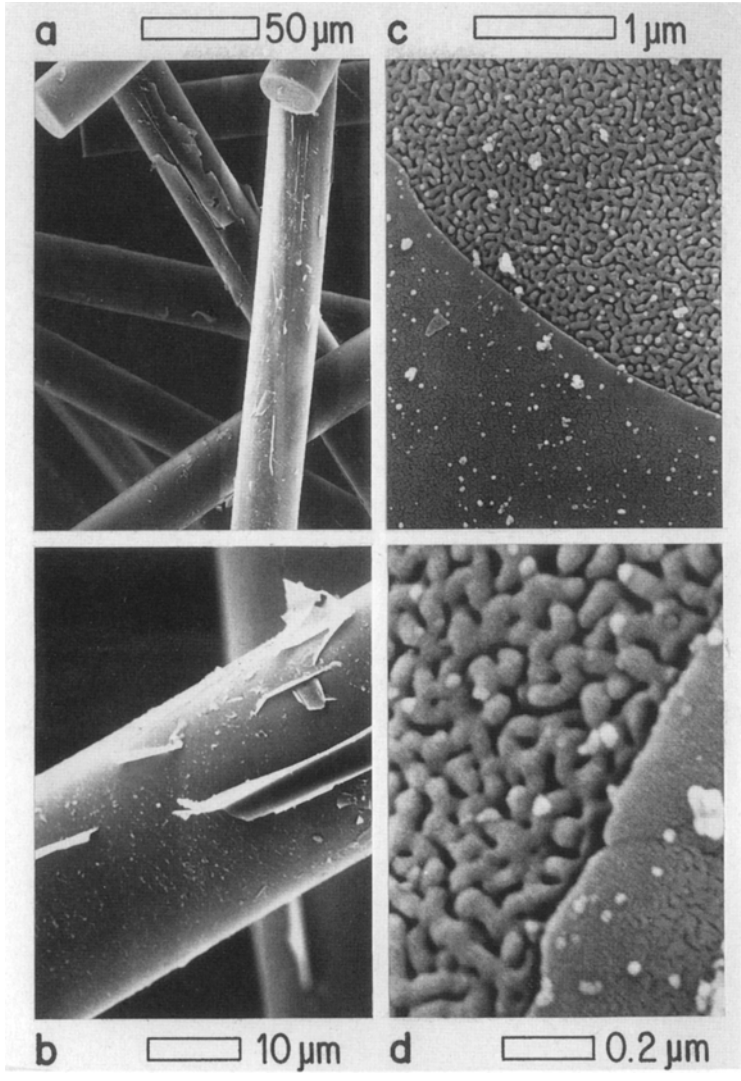

Fig. 2. Porous glass fibers with immobilized glucoamylase showing general aspects of "sheeting" (a and b) and specific details of the boundary area (c and d).

Attached glucoamylase changes the effective free pore radius (from 325 Å to 250 Å) by blocking part of the pore mouths (Figs. 4a,b), causing about 40% reduction in free cross-sectional pore area. This could be one of the main reasons for enzyme-loading interruption during immobilization before enzyme concentration in the immobilizing solution is depleted, as previously described (*8,10*).

All the above reinforce the idea that both facts, "sheeting," and decrease in free pore radius must be considered in engineering assumptions and approaches.

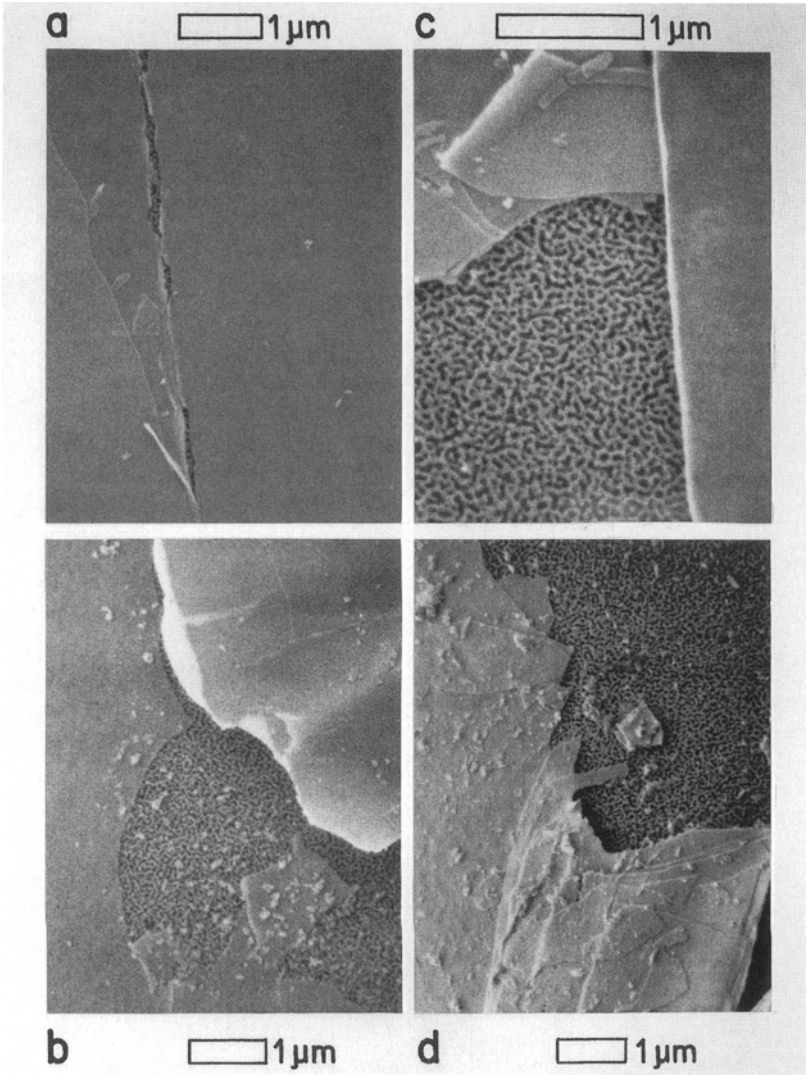

Fig. 3. Comparison between chemically activated glass fibers (a, b, and c) and those with immobilized glycoamylase (d). Samples were noncoated with gold and observed under high resolution SEM at 1 kV.

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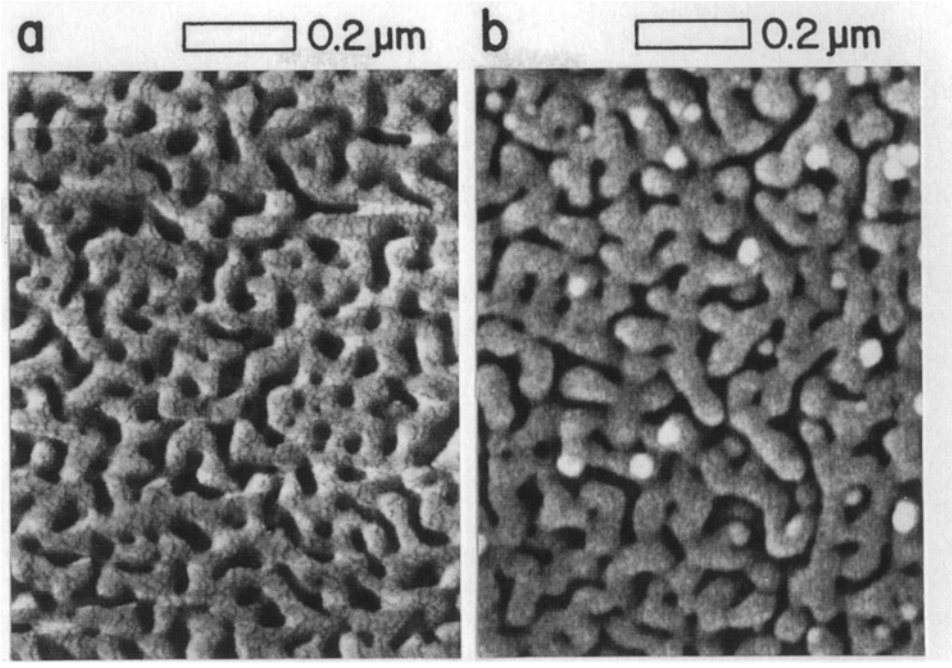

Fig. 4. Micrographs of porous glass fibers surfaces showing differences between those with nonattached enzyme (a) and those with attached glucoamylase (b).

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