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Immobilization of Protein on Ferromagnetic Dacron

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ABSTRACT

Ferromagnetic Dacron (polyethyleneterephthalate) is proposed as a matrix to immobilize proteins covalently. Dacron in powder was magnetized by reacting ferrous (Fe^{+2}) and ferric (Fe^{+3}) ions with its hydrazide form at pH 8.3. Ferromagnetic hydrazide Dacron was converted to ferromagnetic azide Dacron and amyloglucosidase (E.C. 3.2.1.3) was covalently bound through the latter group. The catalytic property of the enzyme was preserved (8% of the specific activity estimated for the soluble enzyme) and all the magnetic amyloglucosidase Dacron derivative was recovered by using a magnetic field. No activity was detected in the supernatant.

Index Entries: Ferromagnetic Dacron; polyethyleneterephthalate; immobilized amyloglucosidase.

INTRODUCTION

Dacron (polyethyleneterephtalate) has been proposed as an attractive matrix to immobilize proteins (enzymes). Weetall (*1*) first reported a four-step procedure: (a) silanization of Dacron with aminopropyltrithoxysilane; (b) conversion of Dacron-alkylaminosilane to an arylamine derivative using nitrobenzoylchloride; (c) conversion of the arylamine derivative to arylazide

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using sodium nitrite; and (d) fixation of L-asparaginase. Goldstein (*2*) also used Dacron, proposing a four-step method: (a) partial alkaline hydrolysis of the polymer; (b) controlled oxidation of the hydroxylic to aldehyde groups using dipiridilcromic oxide; (c) a reaction-type Passerini was carried out between the carboxylic and aldehyde groups using 1,6-diisocyanohexane; and (d) immobilization of trypsin to the activated polymer.

In our lab, Dacron has been used to immobilize proteins employing a procedure consisting in three steps: partial hydrazinolysis of Dacron; conversion of hydrazide groups to azide groups using sodium nitrite; and immobilization of several enzymes on the activated polymer (*3–5*). When films of Dacron were used, this procedure yielded a derivative in the form of fine powder, which presented disadvantages when used in columns. As an alternative, plates of Dacron were proposed to overcome this limitation (*6*). Some reports have proposed magnetization of the matrix as an easy and cheap method of separation of water insoluble derivatives (*7–11*). These matrices can be conjugated to magnetite (Fe_3O_4) and be readily recovered by magnetic force without loss of enzymic activity. Here, magnetization of Dacron powder is proposed. Amyloglucosidase (E.C. 3.2.1.3) immobilization on this ferromagnetic modified polymer is presented as a model. Other proteins can be used, and this conjugate concept can be applied in many fields, such as immunology, clinical biochemistry, and the like.

MATERIAL AND METHODS

Dacron was produced by Rhodia do Brasil SA. Amyloglucosidase (12.92 μmole of glucose released/min/mg of protein) was purchased from Sigma Chemical Co. Soluble starch, $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ were acquired from Reagen SA. All other reagents were of analytical grade (Merck).

Films of Dacron (4 g) were cut in strips and incubated in methanol (100 mL) containing hydrazine hydrate (10 mL) at 40°C for 48 h with stirring. Afterward, the hydrazide-Dacron (powder) was washed twice with methanol and 90% v/v methanol: H_2O, successively.

Hydrazide-Dacron (2 g) was stirred in deionized water (100 mL) and an aqueous solution (10 mL) containing $FeCl_3 \cdot 6H_2O$ (300 mg/mL) and $FeCL_2 \cdot 4H_2O$ (121 mg/mL) was added dropwise. Under vigorous stirring, the mixture was adjusted to pH 8.3 by the addition of 28% v/v ammonium solution and then incubated at 60°C for 10 min. The resulting magnetic hydrazide-Dacron was converted to magnetic azide-Dacron by incubating the material in 0.6*N* HCl (16 mL) containing 5% w/v sodium nitrite (2 mL) at 25°C for 210 min with stirring. Then, the magnetic azide-Dacron was washed with deionized water (twice), 1*M* NaCl (twice), and deionized

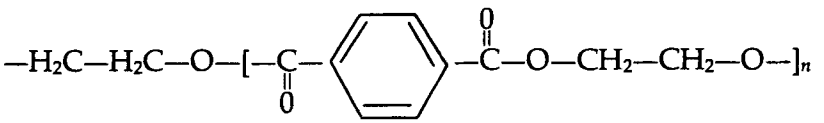
water (twice). Amyloglucosidase (15 mL of a preparation containing 1.0 mg/mL prepared in 0.025*M* citrate-phosphate buffer, pH 5.0) was incubated with the magnetic azide-Dacron (3 g) for 3 h at 4°C with mild stirring. Finally, the magnetic powder of amyloglucosidase-Dacron was washed with 0.025*M* citrate-phosphate buffer, pH 5.0 (100 mL), 1*M* NaCl (100 mL), and the buffer solution (100 mL) again.

Amyloglucosidase activity was established by incubating either the soluble (0.2 mL of the preparation described above) or the magnetic amyloglucosidase-Dacron powder (2 g) in 1% w/v soluble starch (15 mL) prepared in 0.025*M* citrate-phosphate buffer, pH 5.0, at 40°C. The determination of the activity for the magnetic amyloglucosidase-Dacron derivative required stirring. Aliquots (1 mL) were withdrawn at appropriate time intervals. Those obtained from the magnetic immobilized enzyme incubation were submitted to a magnetic field (6000 Oe) for about 30 s to precipitate and recover the derivative. The supernatant and the aliquots from the soluble enzyme incubation were added to 3,5-dinitrosalicylic acid (1 mL), prepared according to Bernfeld (*12*). The reducing power in these mixtures were determined at 540 nm (*12*), and the glucose content calculated by using a standard curve previously established. The magnetic amyloglucosidase-Dacron derivative was washed with 0.025*M* citrate-phosphate buffer, pH 5.0, and finally suspended in this buffer.

Protein content was determined according to the method of Lowry et al. modified (*13*).

RESULTS AND DISCUSSION

Dacron is a polyester with the following structure:



where n can assume a value of about 15,000.

The procedure proposed to immobilize proteins consisted of three steps (*3–6*): partial hydrazinolysis of Dacron; conversion of hydrazide groups to azide groups using sodium nitrite; and immobilization of several enzymes on the activated polymer. In this work, magnetic hydrazide-Dacron was obtained by using the methodology described above and readily separated in 0, 5–1, 0 min. The incubation time of the Dacron (film) with hydrazine hydrate was reduced from 72 to 48 h in order to obtain larger particles (powder). This made recovery by magnetic force more efficient.

Table 1
Specific Activity of Immobilized Amyloglucosidase on Different Preparations of Dacron

Preparation, Dacron	Specific activity, I.U. mg protein	Retention, %	Ref.
Powder	0.005	66	3
Plates	1.695	31	6
Magnetized powder	1.044	8	—

The reaction of ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions at pH 8.3 in the presence of hydrazide-Dacron yielded a magnetic powder that was capable of being converted to magnetic azide-Dacron. Protein (amyloglucosidase) was covalently bound to this magnetic azide-Dacron at a concentration of approx 6.56 mg/g of support. The enzyme maintained its biological activity (catalysis). No alteration in magnetic characteristics could be detected, even after the immobilization of the protein (amyloglucosidase).

Tamaura et al. (7) reported a chemical modification of lipase with ferromagnetic modifier that was prepared by reacting Fe^{2+} and Fe^{3+} with polyethylene glycol having two carboxyl groups. It was proposed that the reaction yields magnetite (Fe_3O_4), which is irreversibly adsorbed on the hydrazide-Dacron powder. This derivative is easily recovered using a magnetic field.

The specific activity (SA) of the magnetic amyloglucosidase-Dacron was approx 8% of that observed for the soluble enzyme (Table 1). A retention of 66% and 31% was found for the same derivatives in powder form (without magnetization) and plates, respectively (6). This low SA retention can be attributed to a protein overload immobilization (6.56 mg of protein/g of support). The unmagnetized Dacron powder derivative presented a lower coupled protein value (1.8 mg/g). Furthermore, this enzyme acted on a substrate with a high mol wt (starch). All amyloglucosidase activity measured in the magnetic derivative was recovered after the use of the magnetic field and no activity was detected at the supernatant. Figure 1 depicts a typical glucose release vs time relationship by the action of the amyloglucosidase magnetic derivative on starch.

According to these results, one can conclude that magnetic modified Dacron is a suitable matrix to immobilize proteins covalently so that a magnetic field replaces centrifugation or filtration to recover the water insoluble derivative from the incubation mixture. In our lab, antigen has been immobilized on this support and ELISA procedure has been carried out successfully.

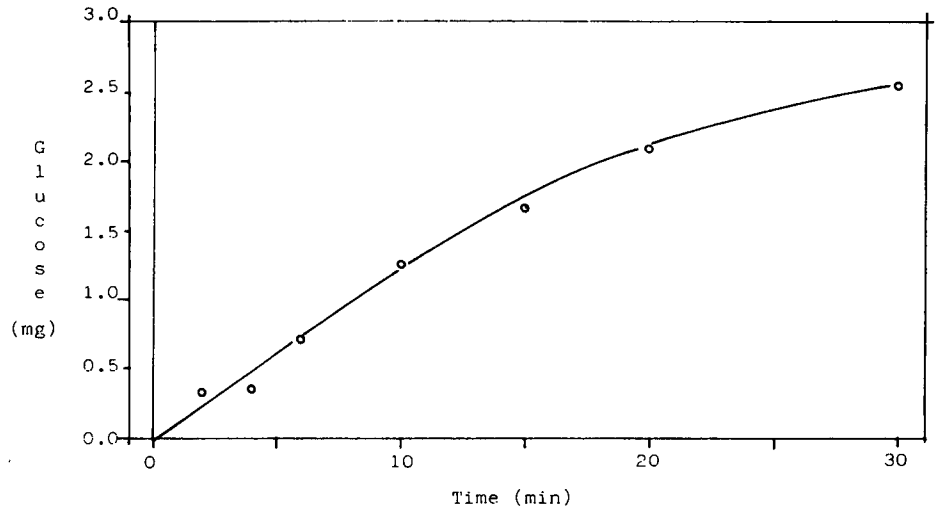


Fig. 1. Glucose release vs time by the action of magnetic amyloglucosidase Dacron derivative on starch. Magnetic amyloglucosidase-Dacron powder (2 g of the derivative) was incubated with stirring in 1% w/v soluble starch (15 mL), prepared in 0.025*M* citrate-phosphate buffer, pH 5.0, at 40°C. This enzymatic derivative was removed from the incubation mixture by magnetic field. Aliquots (1 mL) were withdrawn at appropriate time intervals and the contents of glucose were established according to Bernfeld (*12*).

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