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Immobilization of Proteins on Plates of Dacron

E. A. OLIVEIRA, M. P. C. SILVA, Z. M. B. FIGUEIREDO, AND L. B. CARVALHO, JR.*

Laboratorio de Enzimologia, Departamento de Bioquimica, Centro de Ciencias Biologicas, Universidade Federal de Pernambuco, Cidade Universitaria, 50.000, Recife, PE, Brazil

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ABSTRACT

Dacron (polyethylenetherephthalate) in the form of plates if proposed as a maxtrix to immobilized proteins. A three-step procedure is used to activate this support and to fix the protein on it. Amyloglucosidase was used as a model to test this method, and it showed advantages compared to the powder form of Dacron.

Index Entries: Dacron; polyethylenetherephthalate; immobilization; amyloglucosidase.

INTRODUCTION

Dacron (polyethylenetherephthalate) was first used as a water insoluble support to immobilize proteins by Weetall (*1*), using a four-step procedure.

1. Silanization of Dacron with aminopropyltriethoxysilane;
2. Conversion of Dacron-alkylaminosilane to an arylamine derivative using nitrobenzoylchloride;
3. Conversion of the arylamine derivative to arylazide using sodium nitrite; and
4. Fixation of L-asparaginase.

*Author to whom all correspondence and reprint requests should be addressed.

Goodman (2) also used Dacron, proposing a four-step method:

1. Partial alkaline hydrolysis of the polymer;
2. Controled oxidation of the hydroxylic to aldehyde groups using dipiridilcromic oxide;
3. A reaction type Passerini was carried out between the carboxylic and aldehyde groups using 1,6-diisocyanohexane; and
4. Immobilization of trypsin to the activated polymer.

In our lab, a simple method (3–5) was proposed to immobilize proteins on Dacron consisting in three steps: partial hydrazinolysis of Dacron; conversion of hydrazide to azide groups using sodium nitrite; and immobilization of several enzymes on the activated polymer. However, this procedure yielded a derivative in the form of powder and it was a disadvantage to use it in reactors. Here, we present an alternative in which activated Dacron is presented in the form of plates, and amyloglucosidase is used as a model to test the use of this derivative, and a comparison between the properties of the soluble, powder, and plate derivatives of the enzyme is shown.

MATERIAL AND METHODS

Dacron was produced by Rhodia do Brasil SA. Amyloglucosidase (10.9 μmol of glucose released/min/mg of protein) and soluble starch were purchased from Sigma Chemical Co and Reagen SA, respectively. All other reagents were analytical grade obtained from Merck.

Plates of Dacron (10 g) measuring about 5.0×5.0×1.0 mm were incubated in methanol (30 mL) containing hydrazine hydrate (3 mL) at 40°C for 17 h under stirring. Afterward, the hydrazide-Dacron was washed twice with methanol and 90% v/v methanol, successively. Hydrazide-Dacron was converted to azide-Dacron by incubating the material in 0.6 *N* HCl (24 mL) containing sodium nitrite (3 mL) at 25°C for 4 h under stirring. Then, azide-Dacron was washed with deionized water (twice), 0.1 *M* NaCl (twice), and deionized water (twice). Amyloglucosidase (25 mL of a preparation containing 10 $mg.mL^{-1}$ prepared in 0.025 *M* citrate-phosphate buffer pH 5.0) was incubated with the plates of azide-Dacron for 24 h at 4°C under mild stirring. Finally, the plates of amyloglucosidase-Dacron derivative were washed according to Crook et al. (*6*) and stored in buffer at 4°C until use.

Amyloglucosidase activity was established by incubating either the soluble (0.1 mL of the preparation described above) or the water insoluble preparation (2 g) in 1% w/v starch (15 mL) prepared in 0.025 *M* citrate-phosphate buffer pH 5.0 at 40°C. The determination of the activity for the amyloglucosidase-Dacron derivative required stirring. Aliquots (1 mL) were withdrawn at appropriate time intervals and added to 3,5-dinitro-

salicylic acid (2 mL), prepared according to Bernfeld (*7*). The reducing power in these mixtures were determined at 540 nm (*7*) and the glucose content calculated by using a standard curve previously established. Initial velocities were calculated by plotting glucose released vs time. To calculate Michaelis constants, the concentration of starch, amylose, and maltose ranged from 0.025 to 1.000% w/v, 0.14 to 5.87 μM, and 0.25 to 10.00 mM, respectively. These values were statistically calculated from the data using a regression analysis based on a Taylor series, and the comparison between the values obtained was statistically processed according to Student's *t*-test (*8*). All data processing was carried out in a DEC-10 computer (Digital Corp.) by using appropriate programs written in Basic language.

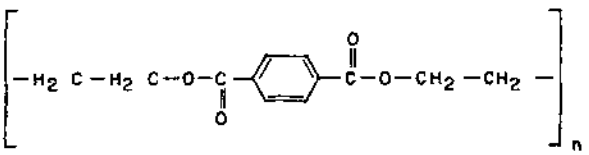
The average of DP (degree of polymerization) for the employed amylose was established as follows: amylose (800 mg) was dissolved in deionized water (100 mL) at 4°C and 0.37 *M* sodium m-periodate (20 mL) was added. This mixture was divided in two aliquots: ethyleneglycol (1 mL) was added to one aliquot and after 30 min the pH was measured; the second aliquot was stored at 4°C for 24 h, then ethyleneglycol (1 mL) was added and after 30 min the pH was also measured, then 0.001 *M* NaOH was added to the second aliquot until the pH reached that established for the first aliquot, and this volume indicates the moles of formic acid released by the oxidation of the amylose by the sodium m-periodate. DP is easily calculated as follows:

$$\text{Moles of formic acid released} = \text{vol of NaOH} \times 10^{-6} \text{ moles}$$
$$\text{Moles of glucose} = \text{weight of amylose}/162 \text{ moles}$$
$$\text{Number of chains} = \text{moles of formic acid released}/3$$
$$\text{DP} = \text{moles of glucose}/\text{number of chains}$$

A volume of 0.8 mL of 0.001 *M* NaOH was found for the employed amylose (duplicate) and using the value of 0.8 g a DP of 9.460 was calculated. Therefore, an average molecular weight of about 1,700,000 can be estimated for this amylose.

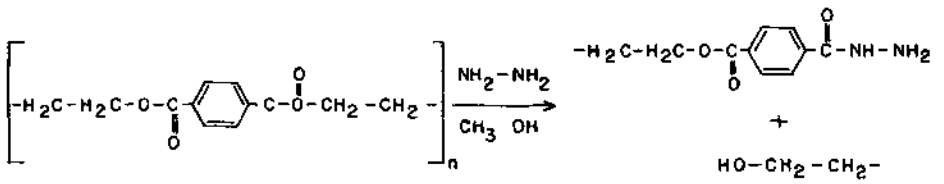
RESULTS AND DISCUSSION

Dacron, used in this work, is a polyester with the following structure.

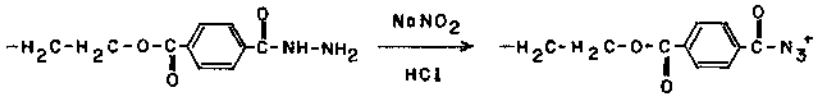


where *n* can assume a value of about 15,000. The steps used to immobilize amyloglucosidase can be summarized as follows.

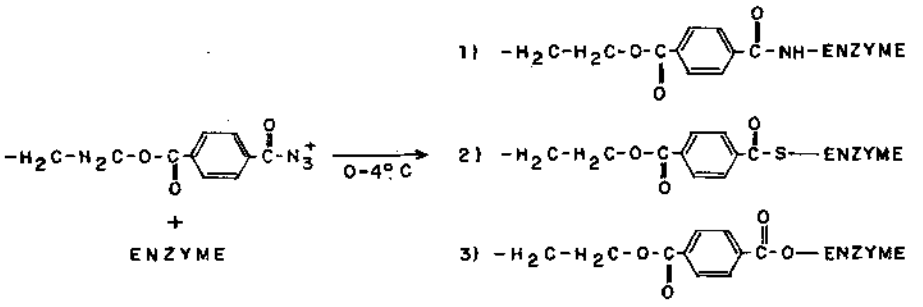
First Step—Hydrazynolysis



Second Step—Conversion of Hydrazide Groups to Azide



Third Step—Immobilization of Protein



Films of Dacron were previously used as matrices to immobilize enzymes (3–5), so that powder was obtained after the first step. Attempts proceeded in order to reduce the time of incubation with hydrazine hydrate, but unsatisfactory protein retention was observed. Then, plates of Dacron were used and the incubation time was also reduced (17 h). It is worthwhile to notice that powder of hydrazide-Dacron is still obtained in this procedure, but the treatment is not enough to reduce all plate in powder. Consequently, part of the hydrazide-Dacron is kept linked to the untreated Dacron through the other end of the molecule. Amyloglucosidase was used as a model to test this method because of its industrial importance.

Table 1
Michaelis Constants of Soluble and Immobilized Amyloglucosidase on Powder and Plates of Dacron Acting on Starch, Amylose, and Maltose

Enzymatic preparation	Km values[a]		
	Starch, g/L^{-1}	Amylose, μM	Maltose, mM
Soluble	2.20±0.05	2.60±0.05	1.70±0.42
Immobilized on powder	15.80±0.29	203.30±0.43	7.78±1.37
Immobilized on plates	6.60±0.20	2.20±0.17	1.49±0.69
Significance between:			
Soluble and powder	$p<0.001$	$p<0.001$	$p<0.05$
Soluble and plates	$p<0.01$	ns[b]	ns
Powder and plates	$p<0.01$	$p<0.05$	$p<0.001$

[a]Km±standard deviation.
[b]ns—not significant.

The specific activity of the plates of amyloglucosidase-Dacron derivative was 31% of that observed for the soluble enzyme. A retention of 66% was found for the same derivative but in powder form (*3*).

Comparing Michaelis constants found for amyloglucosidase-dacron derivative either in powder or in plate with that calculated for the native enzyme (Table 1) is evident that the enzymatic preparation using plate showed better performance acting on different substrates (starch, amylose, and maltose) than that employing powder. Diffusional limitations are supposed to play less importance when the enzyme is covalently fixed on the surface of the plate, mainly if the substrate has a large molecular weight (starch and amylose).

A half-life of 52 d was found for the plate of amyloglucosidase-Dacron derivative (Fig. 1) used intermitently and stored in 0.025 *M* citrate-phosphate buffer pH 5.0 at 4°C.

Dacron becomes liquid at temperatures between 60°C and 150°C without having its chemical structure altered. Therefore, by increasing the temperature, any shape can be established to the Dacron and its application be extended. For instance, tubes of Dacron can be produced and used as vascular implants containing biologically-active proteins inside, with the advantage that this material is well known to induce little rejection.

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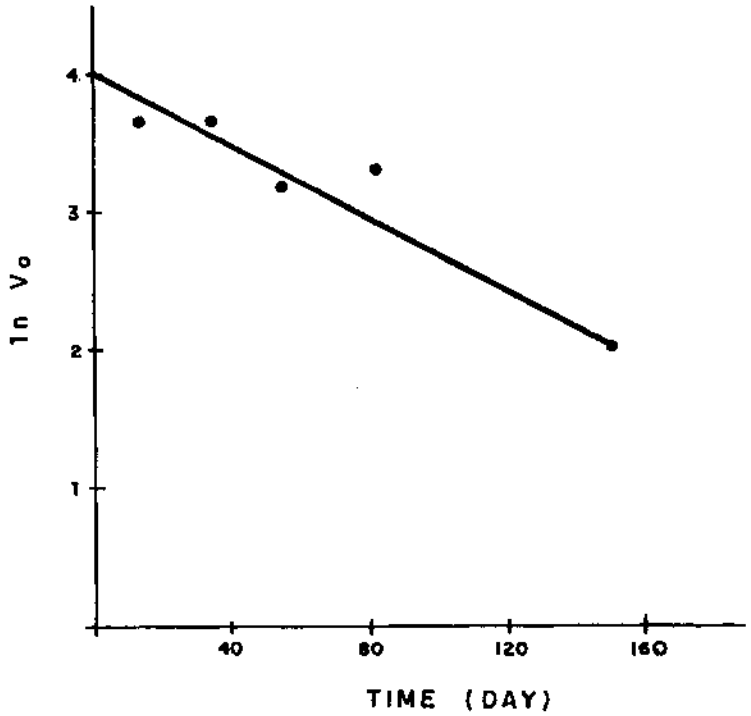

Fig. 1. A Half-life of the plate of amyloglucosidase-Dacron derivative.

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