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Immobilization of Amyloglucosidase Using Two Forms of Polyurethane Polymer

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ABSTRACT

Amyloglucosidase was covalently immobilized using two hydrophilic prepolymers: Hypol® FHP 2002 (creates foams) and Hypol® FHP 8190H (creates gels). The foamable prepolymer was superior as a support for enzyme immobilization. The percent activity immobilized in the polyurethane foams was 25±1.5%. Large substrates (>200,000 daltons in mol wt) were hydrolyzed as effectively as smaller ones by the immobilized enzyme. The K_m value of the foam-immobilized enzyme increased from 0.76 mg/mL (free) to 0.86 mg/mL (immobilized), whereas the V_{max} dropped from 90.9 (free) to 12.4 nmol glucose/min/mL (immobilized). The long-term (2 mo) storage stability of amyloglucosidase was enhanced by immobilization in foams (70% activity retained; free enzyme only retained 50%). Immobilization also improved the enzyme stability to various denaturing agents (sodium chloride, urea, and ethanol). The immobilized enzyme exhibited increased stability compared to the free enzyme at high temperatures (95°C). Both glycogen and starch could be utilized by the immobilized enzyme, indicating that this technique could prove useful for starch hydrolysis.

Index Entries: Amyloglucosidase; enzyme immobilization; polyurethane polymers; starch degradation.

INTRODUCTION

Starch serves as the major food reserve molecule for plants (*1*). Development of technology for starch utilization has recently become essential

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owing to economic considerations (e.g., increases in sugar prices). The glucose generated from starch hydrolysis can be used as fuels and/or sweeteners (*1*). Enzymatic, as opposed to chemical, methods for starch hydrolysis are preferred because specificity and because acid hydrolysis results in browning reactions (*2*).

Amyloglucosidase (also known as glucoamylase) (EC 3.2.1.3) is an extracellular enzyme that catalyzes the stepwise hydrolysis of α (1,4) linkages in starch and glycogen (*2*). The enzyme also shows a low rate of hydrolysis of α (1,6) linkages (*2*). Amyloglucosidase is used commercially in free form for batch starch hydrolysis (*2*). It is an inexpensive enzyme, hence an immobilized system must offer significant advantages in order for industrial utilization to occur. Many different techniques have been described for amyloglucosidase immobilization (*2–8*).

In the present study, we immobilized amyloglucosidase using two different hydrophilic polyurethane prepolymers (Hypol® FHP 2002 and 8190H). Apparently, the enzyme is covalently attached to the polymers during the process of polymerization (*9*). The properties of amyloglucosidase immobilized using this technique, and the inherent properties of the foams/gels that result, lead us to believe that the techniques described here could be used to create a system potentially superior to those previously proposed for amyloglucosidase immobilization.

MATERIALS AND METHODS

Chemicals

All biochemicals and coupling enzymes were purchased from Sigma Chemical Co., St. Louis, MO, J. T. Baker Chemical Co., Toronto, ON, or Boehringer Mannheim Corp., Montreal, PQ.

Polymer

The hydrophilic prepolymers, one that produced a foam (Hypol® FHP 2002) and one that produced a gel (Hypol® FHP 8190H), were graciously supplied by W. R. Grace and Co. (Lexington, MA). The foamable prepolymer is a water-activated derivative of toluene diisocyanate, whereas the gelable prepolymer is a water-actiated derivative of methylenediphenyl diisocyanate (W. R. Grace Co., product information). Enzyme immobilization apparently occurs when primary amino groups on the enzyme react with isocynanate groups on the foam or gel to produce amide linkages during the process of polymerization (W. R. Grace Co., product information) (*9*).

Immobilization of Amyloglucosidase

The standard amount of amyloglucosidase (Sigma) used for all the experiments, unless stated otherwise, was 1.3 U (where 1 U is defined as

the amount of enzyme that liberates 1.0 mg glucose from starch in 3 min at pH 4.5 at 55°C; Sigma). The standard buffer used for all experiments was 100 m*M* sodium acetate, pH 4.5. To prepare the foam-immobilized enzyme, amyloglucosidase (1.3 U) was dissolved in 2 mL of buffer in a petri dish. A 1.0 g aliquot of prepolymer was then added. The mixture was agitated vigorously to achieve a homogeneous distribution of enzyme within the prepolymer. Agitation was stopped when extensive polymerization was detected visually and by increased viscosity of the mixture. The resulting foams were allowed to cure at room temperature for at least 20 min before use. After polymerization was complete, the foams were washed with 6 mL of buffer and squeezed repeatedly to ensure complete absorption of the wash.

Gels were prepared by the same method, except that only 0.3 g prepolymer was added to the buffer and enzyme mixture and gels were washed with 5 mL of buffer after being allowed to cure for 20 min. Homogenization of the gels, when required, was conducted using a Polytron PT/10 tissue homogenizer for 3×5 seconds.

Amyloglucosidase Assay

Amyloglucosidase activity was determined by measuring the glucose liberated from various forms of starch and glycogen (Sigma) suspended in 100 m*M* sodium acetate buffer (pH 4.5). Standard reactions were carried out at 23°C using 4 mg/mL solutions of dialyzed glycogen or starch, unless otherwise indicated. To initiate a reaction, forms were squeezed to remove excess wash buffer and then placed in a petri dish containing 4 mL of the appropriate substrate solution. The foam was again squeezed to absorb the substrate into the foam. The gels had little capacity to absorb water, hence substrate solutions were just placed on top of them. To sample the reaction mixture, foams or gels were gently squeezed, and aliquots of reaction mixture (0.1 mL) were sampled and immediately mixed with 0.1 mL 100 m*M* Tris buffer (pH 9.5). The resulting pH change stopped any amyloglucosidase activity (if present). As needed, excess Tris buffer was added to dilute samples with high glucose concentrations before assay. Glucose was assayed enzymatically by monitoring the reduction of NAD^+ at 340 nm using hexokinase and glucose-6-phosphate dehydrogenase, as described by Lowry and Passonneau (*10*). Hexokinase (0.28 U) was added to a 1 mL solution of buffer (100 m*M* Tris, pH 8.0), NAD^+ (0.50 m*M*), $MgSO_4$ (5.0 m*M*) ATP (0.50 m*M*), glucose-6-phosphate dehydrogenase (0.10 U), and the test sample (0.100 mL).

Kinetic Parameters

The kinetic parameters for amyloglucosidase were determined using rabbit liver glycogen (Sigma) as the substrate. Glucose production after 10 min at room temperature was determined for reactions using 0.2, 0.5,

1.0, 2.0, 4.0, and 8.0 mg/mL glycogen for both the free and immobilized amyloglucosidases. K_m and V_{max} values were calculated from Hanes plots.

Optimum Temperature and Temperature Stability Studies

Free vs immobilized amyloglucosidases were tested for the ability to hydrolyze glycogen at different temperatures. Substrate solutions were preheated to the appropriate temperature, the enzyme added, and incubated for 30 min.

Free and immobilzied amyloglucosidase were tested for their ability to hydrolyze glycogen at high (95°C) temperatures over a 2-h period with sampling at 30-min intervals. Room temperature (23°C) samples served as controls.

Optimum pH

Foams/gels containing immobilized amyloglucosidase were preequiliberated by washing with 6 mL of 100 m*M* acetate buffer of the appropriate test pH. Free amyloglucosidase samples were prepared in 100 m*M* acetate buffer of the desired pH. Substrate solutions of 4 mg/mL glycogen in 100 m*M* acetate buffer at the appropriate pH were prepared and added to the foams or gels; aliquots were sampled for glucose production after 30 min at 23°C.

Long-Term Amyloglucosidase Storage

Free and foam-immobilized amyloglucosidase were stored in sealed containers at 4°C for up to 2 mo. Enzymes were removed from storage, equilibrated to room temperature, and then, tested for their activity by measuring glucose production after 30 min at 23°C. Foams were thoroughly washed before each use, and then fresh substrate was added. Between uses, foams were kept in buffer solutions containing 0.04% sodium azide.

Data Calculations

Data are reported as means $\pm$SEM, $n=3$. Where the SEM bars are not shown in figures, these values were within the dimensions of the symbol used.

RESULTS

Immobilization of Amyloglucosidase in Foams and Gels

The percentages of amyloglucosidase activity associated with the foams vs washes are given in Table 1 for the various immobilization con-

Table 1
Percentage of Amyloglucosidase Activity
Retained During Foam-Immobilization Under Various Conditions[a]

Immobilization conditions	Activity, %		
	Foam	Wash	Lost
Standard	25.1±1.5	70.4±3.8	4.5
0.65 U AG used	6.7±0.8	59.7±3.0	33.6
pH 8.0 buffer used	9.6±2.4	83.9±15.2	6.5
Polymerized at 5°C	14.4±0.7	43.5±7.1	42.1
Cured for 16 h	14.0±1.9	65.7±8.1	20.3

Data are the means±SEM, $n=3$. Activities are expressed relative to an equal amount of free enzyme given the same treatment. The standard conditions for preparation of the foam-immobilized enzyme are as follows: amyloglucosidase (1.3 U) was dissolved in 2 mL of 100 m*M* acetate buffer (pH 4.5) in a petri dish. A 1.0 g aliquot of prepolymer was then added, and the mixture was agitated vigorously. Agitation was stopped when extensive polymerization was detected visually and by increased viscosity of the mixture. The resulting foams were allowed to cure at room temperature for approximately 20 min before use.

Table 2
Effect of the Amount of Prepolymer and Homogenization
on the Activity of Gel-Immobilized Amyloglucosidase[a]

Amount of Gel prepolymer added, g	Homogenized	Activity (%)		
		Gel	Wash	Lost
0.2	Yes	3.6±0.3	27.7±1.4	68.7
0.3	Yes	4.0±0.3	17.3±1.7	78.7
0.3	No	1.3±0.2	5.4±1.1	93.3
0.4	Yes	1.5±0.6	12.8±7.5	85.7
0.6	Yes	2.2±0.7	12.4±3.4	85.4

[a]Data are the means±SEM, $n=3$. Gels were prepared as outlined in the Materials and Methods section. Percent activity was determined by comparison with the same amount of free amyloglucosidase (1.3 U) allowed to react for 30 min.

ditions. All calculations of activity were made relative to an equal amount of the free enzyme. The highest percentage immobilized (25±1.5%) was for foams in pH 4.5 buffer at 5 or 23°C, with curing for either 20 min or overnight (16 h). Thus, the standard conditions chosen for further use were immobilization in pH 4.5 buffer at 23°C, with a curing time of 20 min.

At optimum conditions, the average amount of amyloglucosidase activity eluted in the wash was 70.4±3.8%. Therefore, nearly all enzyme activity is accounted for in the wash and foam, with the total yield of activity (wash and foam) being 95.5±5.3%.

The effect of various treatments on the percentage of amyloglucosidase activity immobilized within gels is given in Table 2. Very little enzyme could be immobilized in the gel polymer, only 1–4% of the initial activity. Homogenization of gels to permit better substrate penetation did not im-

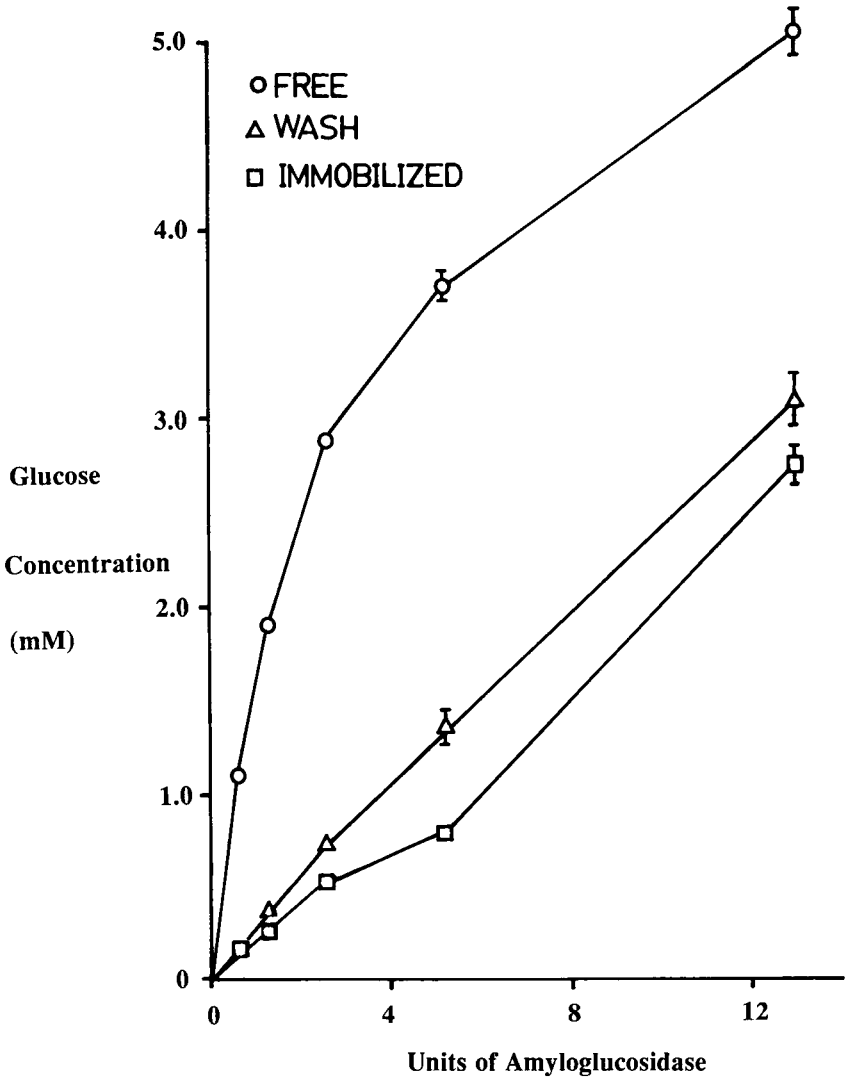

Fig. 1. Effect of attempting to immobilize increasing concentrations of amyloglucosidase on the resulting immobilized and wash activities (m*M* glucose produced). Free enzymes in identical amounts served as controls. Data are the means ± SEM, $n=3$. Where error bars are not shown, SEM values were within the dimensions of the symbols used.

prove this result. Enzyme released in the wash was also low. Therefore, the net result of gel immobilization is a high loss (>68%) of total enzyme activity.

Figure 1 shows the effect of foam-immobilizing increased concentrations (0.65–13 U) of amyloglucosidase. The percentage of enzyme activity immobilized appeared to remain fairly constant as the amount of enzyme

Table 3
Kinetic Parameters for Free and Immobilized Amyloglucosidase[a]

	K_m, mg/mL	V_{max}[b]	k_{cat}[c]
Free	0.76±.08	90.9±3.2	120
Immobilized			
Foam	0.86±.15	12.4±1.2	58
Gel	2.82±.14	4.4±0.8	39

[a]Data are the means±SEM, $n=6$ ($n=3$ for the gel samples). The substrate used was glycogen. $k_{cat}=V_{max}/K_m$. All k_{cat} values were adjusted for the percentage of enzyme activity immobilized.
[b]nmol glucose/min/mL.
[c]nmol glucose/min/mg.

added was increased. This indicated that high activities of enzyme can be loaded per gram of foam as experiments are scaled up.

Effect of Substrate Size on Free and Immobilized Amyloglucosidase

Glycogen was subjected to gel filtration (G-200) to determine whether small molecular weight glycogen fragments were, in fact, the substrate being utilized by the foam-immobilized amyloglucosidase. All glycogen molecules recovered after the gel filtration were larger than 200,000 daltons in molecular weight. Activity of the free enzyme was 1.90±.07 μmol glucose/mL for the "whole glycogen" vs 2.38±.10 μmol glucose/mL for the G-200 treated glycogen. The activity of the immobilized enzyme (0.26±.02 μmol glucose/mL) was identical for both forms of substrate (whole glycogen vs G-200 glycogen), indicating that small and large substrates can be used equally well by the immobilized enzyme. No diffusional limitation for the high molecular weight substrate (G-200 treated) by the foam-immobilization technique was indicated.

Kinetic Properties

Table 3 outlines the kinetic properties of the free, foam-immobilized, and gel-immobilized amyloglucosidases. Compared to the free enzyme, the K_m value for glycogen of the foam-immobilized enzyme increased 14%, whereas the gel-immobilized enzyme increased 3.7-fold. The V_{max} value of the foam-immobilized enzyme dropped to 14% of the free enzyme, whereas the gel-immobilized enzyme showed a V_{max} of only 5% of the control value. Both of these V_{max} decreases reflect the percentage of enzyme immobilized within each polymer. The k_{cat} ($=V_{max}/K_m$) value of the foam-immobilized enzyme was 48% of the value for the free enzyme, whereas the k_{cat} of the gel-immobilized enzyme was only 33% (taking into account the percent activity immobilized).

Table 4
Effect of Temperature on the Activity
of Free and Foam-Immobilized Amyloglucosidase[a]

Temperature, °C	Activity, nmol glucose produced/min/mL	
	Free	Immobilized
93	257±4 (104%)	15±1 (48%)
80	237±7 (96%)	20±0.2 (65%)
60	246±6 (100%)	31±2 (100%)
23	69±1 (38%)	10±0.1 (31%)
5	22±0.7 (9%)	6±0.1 (21%)

[a]Data are the means ± SEM, $n = 3$. The values in brackets are the relative activities (compared to 60°C) observed at the various temperatures. Substrate solutions were preheated to the desired temperature, and then enzyme was allowed to digest them for 30 min.

Optimum Temperature and Temperature Stability Studies

Table 4 shows the effects of temperature on the activity of free and foam-immobilized amyloglucosidase. The immobilized enzyme appears to be inferior to the free one at very high temperatures (80 and 93°C), with the free enzyme retaining virtually constant activity over the 60–93°C range.

Figure 2 shows the time course of glycogen hydrolysis at very high (about 95°C and moderate (23°C) temperatures for free and foam-immobilized amyloglucosidase. The free enzyme showed steadily decreasing activity at 23°C, whereas the immobilized enzyme showed increasing activity. The activity of both enzymes dropped precipitously at 95°C over the 2 h time course examined. However, the immobilized enzyme retained a slightly larger fraction of its initial activity, compared to the free enzyme after 60 min at 95°C. This indicated increased long-term enzyme stability for the immobilized enzyme.

Optimum pH

Figure 3 shows the effect that pH had on the relative activities of free, foam-, and gel-immobilized amyloglucosidase. The enzyme in all three cases showed a broad optimum between pH 3 and 5.5. The immobilized enzyme retained this broad optimum.

Time Course of Glycogen Degradation

Figures 4 and 5 show the time course of glycogen hydrolysis by free and foam-immobilized enzyme and the gel-immobilized enzyme, respectively. Both immobilized forms showed linear rates of glucose production, indicating constant activity over time. The free enzyme showed an

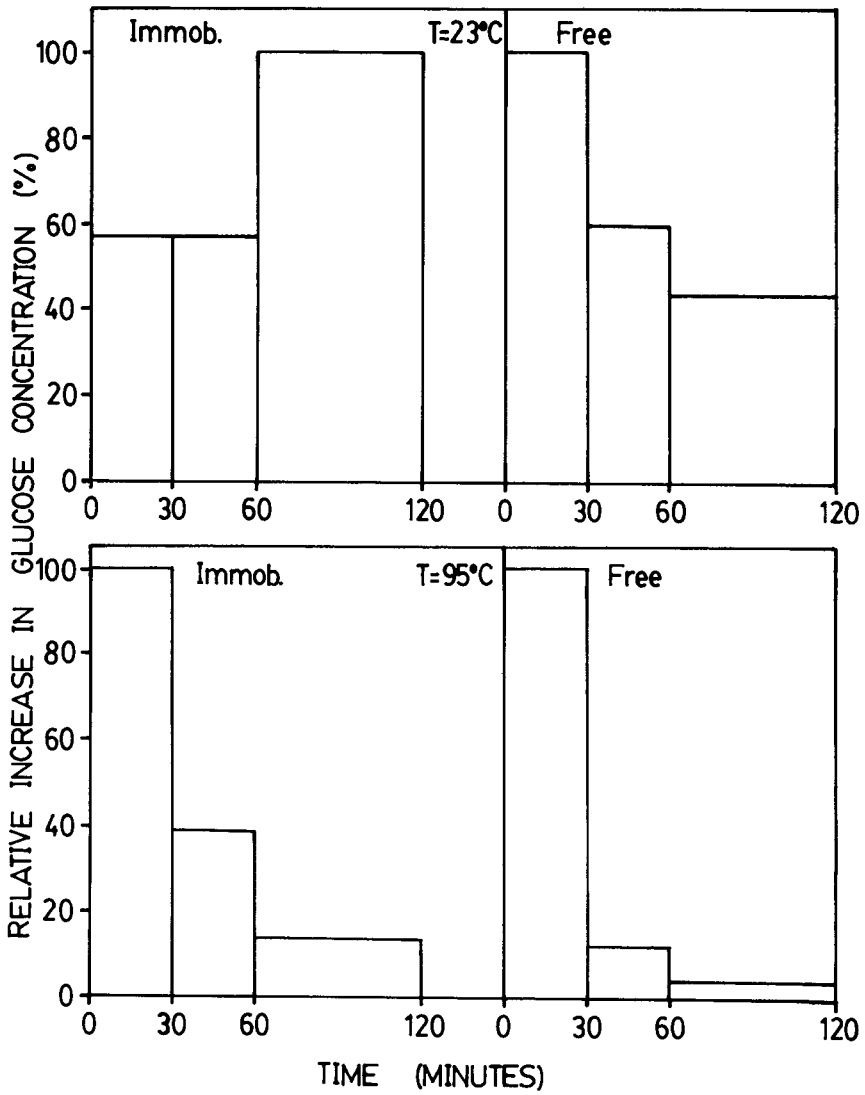

Fig. 2. Time course of glycogen hydrolysis at 23 and 95°C by free and foam-immobilized amyloglucosidase, using glycogen (4 mg/mL) as substrate. The increases in glucose concentrations over the time intervals sampled are expressed as percentages of the maximum increase observed. Data are the means ± SEM, $n = 3$. Where error bars are not shown, SEM values were within the dimensions of the symbols used.

initially higher rate of glucose production, which leveled off as the time course continued. This pattern indicated a high initial activity, which then declined steadily over the time course.

Long-Term Amyloglucosidase Storage Stability

The free amyloglucosidase lost 48% of its initial activity after 67 d. The foam-immobilized enzyme retained 70% of its activity after the same

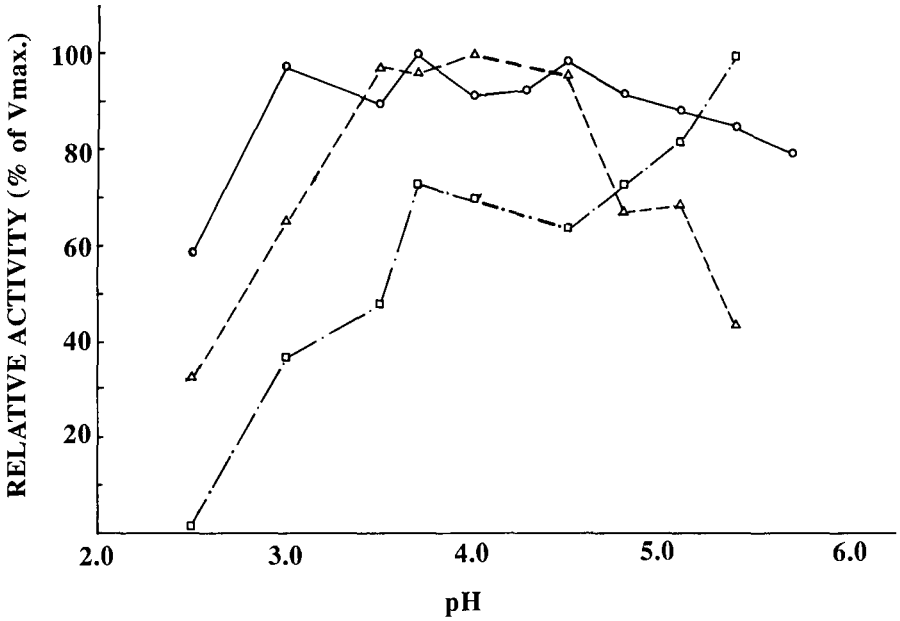

Fig. 3. Effect of pH on the relative activity of free (○) vs foam-immobilized (△) and gel-immobilized (□) amyloglucosidase at 23°C, using glycogen as substrate. Data are the means ± SEM, $n=3$.

period of storage. Hence, immobilization within the foam appeared to increase the stability of the enzyme for long-term storage.

Effects of Denaturing Agents on Amyloglucosidase Activity

The effects of various denaturing agents (5*M* sodium chloride, 4*M* urea, and 20% (v/v) ethanol) on free and foam-immobilized amyloglucosidase are outlined in Table 5. Immobilization appears to enhance amyloglucosidase stability to all three denaturants.

Testing of Different Substrates

Table 6 shows amyloglucosidase activity with alternative substrates. The free enzyme utilized glycogen, lintner starch, and potato starch equally. The immobilized enzyme showed slightly reduced activity with the starch substrates.

Diffusion Limitation of the Immobilized System

Two methods were used to determine the role that diffusion limitation played in the system under study. If enzyme:substrate diffusion is

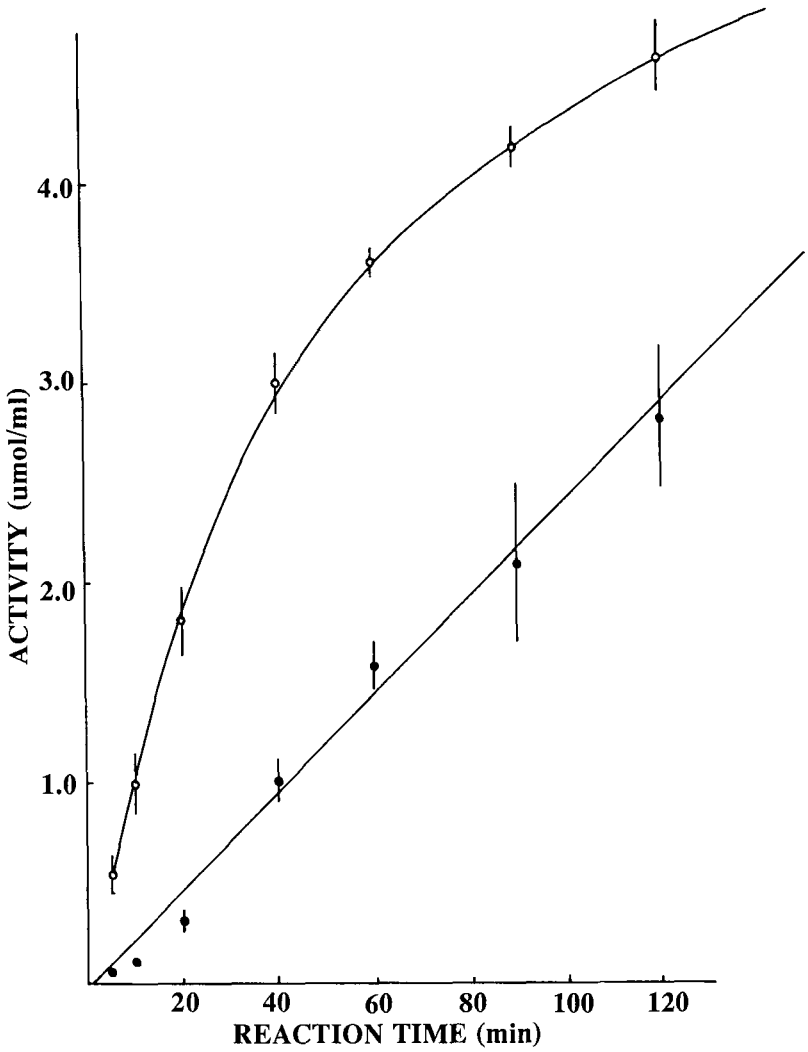

Fig. 4. Time course of glycogen hydrolysis by free (○) and foam-immobilized (●) amyloglucosidase at 23°C, using glycogen as substrate.

limiting in an immobilized enzyme system, one or both of the following should be observed: deviations in the Arrhenius relationship (relating temperature and reaction velocity), especially at high temperatures; and alterations in enzyme kinetic parameters (slope and shape of the Lineweaver-Burk plot) (*11*). In our experiments, no differences were observed for the free vs foam-immobilized enzymes, with respect to the Arrhenius plot, nor were there differences in either slopes or intercept characteristics of the Lineweaver-Burk plots (data not shown). Hence, it can be concluded that diffusional limitations play an insignificant role in the system presented (*11*).

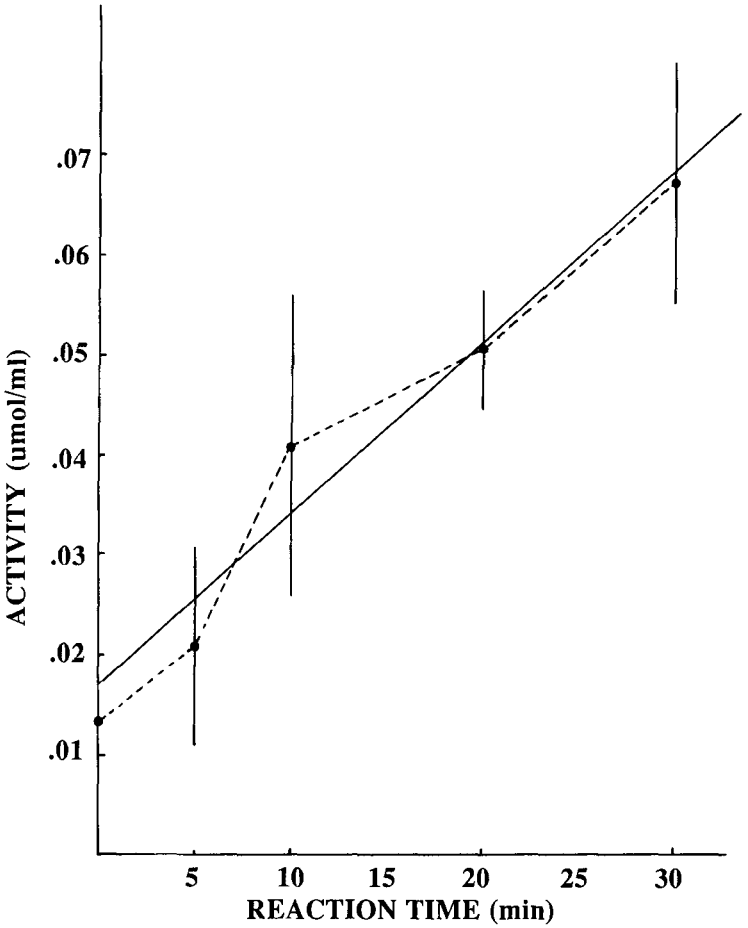

Fig. 5. Time course of glycogen hydrolysis by gel-immobilized amyloglucosidase at 23°C, using glycogen as substrate.

Table 5
Effect of Denaturing Agents on Free and Foam-Immobilized Amyloglucosidase[a]

	Activity, nmol glucose produced/min/mL	
Sample	Free	Immobilized
Control	56.7±1.0 (100%)	8.0±0.3 (100%)
5*M* NaCl	41.3±2.0 (73%)	7.3±0.3 (92%)
4*M* Urea	47.3±0.7 (84%)	8.7±0.3 (108%)
20% Ethanol	58.0±0.3 (102%)	9.3±0.3 (117%)

[a]Data are the means ± SEM, $n = 3$. Relative activities are given in brackets. Control values (using standard conditions) were set to 100% for comparison with the other samples. Reactions were conducted at 23°C for 30 min.

Table 6
Comparison of Glycogen, Lintner and Potato Starches
as Substrates for Free and Foam-Immobilized Amyloglucosidase[a]

Substrate	Activity, nmol glucose produced/min/mL	
	Free	Immobilized
Glycogen	44.7±0.5 (100%)	11.7±0.7 (100%)
Lintner Starch	46.7±0.5 (105%)	9.7±0.7 (83%)
Potato Starch	44.7±1.2 (100%)	8.0±0.7 (69%)

[a]Data are the means±SEM, $n=3$. The relative activities are given in brackets. Control values (glycogen) were set to 100%. All reactions were carried out at 23°C for 1 h.

DISCUSSION

In the starch processing industry, amyloglucosidase is typically used at 55–60°C at pH 4.5, with continuous hydrolysis for 2–4 d (*2*). For industrial use, immobilization permits a drastic reduction in the time required for reaction, because very high enzyme concentrations can be used and recovered (*2*). The shorter residence times decrease the cost of refining because side-reactions are reduced. The volume of immobilized amyloglucosidase plants is also typically smaller, along with capital costs as a result (*2*). Thus, development of new methods for amyloglucosidase immobilization appears to be quite important industrially.

The method presented here for immobilizing amyloglucosidase has several advantages over other methods that have been developed (*2–8*). The use of the commercially-available polyurethane polymers described here is fast, requires no special catalysts, and binds enzymes irreversibly (W. R. Grace Co., product information) (*9*). By contrast, amyloglucosidase immobilization on two copolymers of methacrylate, described by Nithiananda et. al. (*6*), required the production of the copolymer through a very involved process. The process, described by Svec et. al. (*8*), also required special preparation of the carrier and needed very long times for immobilization to occur (roughly 20 h).

Entrapment or encapsulation techniques appear to be inappropriate for amyloglucosidase immobilization because they impede access of high molecular weight substrates (such as starch) to the enzyme (*12*). Immobilization by adsorption (e.g., DEAE-cellulose) can create problems because the attachment is not permanent and, therefore, enzyme can be released when conditions vary (*12*). Covalent bonds, in contrast, are so strong that loss of enzyme by breakage of these bonds is almost impossible (*12*).

Our foam-immobilization methods overcome many of the potential problems of amyloglucosidase immobilization

1. Covalent linkages result in permanent enzyme immobilization.
2. No diffusion limitations were observed in this system.

3. Less product inhibition is seen for the immobilized vs free systems (Fig. 2).
4. Enzyme storage stability is enhanced by immobilization.
5. Glucose production at very high temperatures is enhanced by immobilization (Fig. 2).
6. Low K_m and broad pH profile parameters are largely conserved.

Our standard method for foam-immobilization resulted in 25% of activity bound to the foam. This is substantially better than previous results for amyloglucosidase immobilization: Nithianandam et. al. found 19% of the amyloglucosidase activity was immobilized (*6*), whereas Cabral et. al. found only 12% activity retention (*7*).

Previous work by our lab (*9,13,14*) has shown that a number of enzymes can be successfully immobilized using the foamable prepolymer. Immobilized foam systems containing cellulase, β-glucosidase, and/or glucose isomerase all offer significant advantages for the hydrolysis of cellulose substrates. With these systems and the present work, we have consistently found no problems concerning diffusional limitations in dealing with high molecular weight substrates. Blue Dextran (mol wt 2,000,000 daltons) readily penetrated the foam (*9*), and immobilized enzyme in the present study showed no difference in activity using G-200 treated or untreated glycogen. Kinetic data (Lineweaver-Burk plots) and Arrhenius plots have previously established (*9*), confirmed by results from the present studies, that the diffusional limitations imposed by the foams were of no significance.

The changes in kinetic parameters, noted for the foam-immobilized enzyme, indicated that it was a slightly less effective catalyst (a 14% rise in the K_m. However, immobilization by our method produced much smaller changes in the enzyme K_m than have been reported previously. Nithianandam et al. found that the K_m value of immobilized enzyme rose 10-fold (*6*). Nehete et. al. found that the K_m increase for their best immobilized preparation was 1.7-fold (*4*). Use of immobilized enzymes with low K_m values is considered advantageous, even if V_{max} values are reduced, because higher starch conversion rates can be achieved (*8*). The drop in V_{max} value observed (to 14% of the free value) can be largely attributed to the percentage of enzyme actually immobilized (25%).

The pH and temperature stabilities of the foam-immobilized amyloglucosidase are virtually equal to those of the free enzyme, particularly over the range of temperature and pH values that can be used industrially. The foam-immobilized enzyme appears to function at or near optimum levels at these temperature (60°C) and pH values (approximately 4.5 (Table 4) and Fig. 3). In contrast, Tomar and Prabhu found that immobilization of amyloglucosidase, using DEAE-cellulose activated with cyanuric chloride, narrowed the pH optimum from a broad range (3.8–5.2) to a narrow acidic one (3.6) (*5*).

Overall, polyurethane foam-immobilized amyloglucosidase offers several advantages over the free form. The immobilized enzyme is more re-

silient to long-term storage and the effects of denaturing agents. The reuse of enzyme allows for reduced production costs. The ease of enzyme/product separation eliminates the need for removal of enzyme activity by heat denaturation, for example (*2*).

The foams themselves possess several advantageous physical properties. They are flexible and nonreactive once polymerized and offer homogeneous distribution of the immobilized enzyme (W. R. Grace Co., product information) (*9*). They can also be made into a variety of sizes and shapes. Therefore, they are amenable for use in column (after being frozen in liquid nitrogen and ground to a powder) or batch processes (*9*). The low activity and unfavorable kinetic parameters (increased K_m and low V_{max}) for immobilization of amyloglucosidase in the polyurethane gels make this method appear to be of little practical use for starch hydrolysis.

The main thrust of future research will involve introducing additional enzymes to be coimmobilized in foam with amyloglucosidase. Pullulanase, which acts to hydrolyze the α (1,6) branchpoints of starch or glycogen, could be added to enhance rates of glucose production (*2*). Glucose isomerase could also be added to allow a one-step producton of high fructose syrups from starch substrates. Further developments of the immobilization system outlined in this paper could serve to improve the hydrolysis of starch for industrial purposes.

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