グルコアミラーゼのキトサンビーズへの固定化とデンプンのグルコースへの変換への固定化酵素の利用

誌名	佐賀大学農学部彙報
ISSN	05812801
著者	光, 富勝 大宝, 明 Mukerjee Dhar, G.
巻/号	74号
掲載ページ	p. 59-68
発行年月	1993年3月

Immobilization of Glucoamylases on Chitosan Beads and Application to the Conversion of Starch to Glucose

Gouri MUKERJEE DHAR, Masaru MITSUTOMI, Akira OHTAKARA
(Laboratory of Food Chemistry)

Received October 29, 1992

Summary

Glucoamylase from *Rhizopus niveus* was immobilized on chitosan beads BCW 3510 crosslinked with aromatic compound and that from *Aspergillus* sp. K-27 on chitosan beads, BCW 2510 and BCW 3010 crosslinked with aliphatic compounds, in addition to BCW 3510, with or without glutaraldehyde (GA) treatment. The amounts of these enzymes immobilized on BCW 3510 increased as the degree of deacetylation of chitosan beads increased. The enzyme immobilized on GA-untreated BCW 2510 and 3010 leaked into 1.0M Nacl and easily lost their activities, but these enzymes on BCW 3510 did not leak into 1.0M NaCl even without GA treatment. The leakage of the enzymes immobilized on BCW 2510 and 3010 was prevented by GA treatment. Glucoamylase of *R. niveus* immobilized on BCW 3510 stored about 67% of the initial activity after 9 months and retained its activity capable of hydrolyzing starch in column reactor after continuous use for 20 days.

Key words : chitosan beads, glucoamylase, immobilization, reactor

Introduction

Glucoamylase is an enzyme which can be used industrially for the conversion of starch to glucose. This enzyme has been immobilized on chitin with or without the aid of cross-linking reagents. Stanley *et al.*[1] reported the immobilization of glucoamylase on chitin cross-linked with GA and its utilization in column reactor. Glucoamylase from *Aspergillus awamori* var. *kawachi*[2] was reported to be immobilized onto chitin without the aid of a cross-linking reagent, the retained activity being more than 90%. Continuous production of high glucose syrup from starch[3] was effected by utilizing the immobilized glucoamylase and the enzyme activity in the column reactor was retained for 20 days. Bon *et al.*[4] immobilized glucoamylase from *Aspergillus niger* on various inexpensive supports including chitin which were activated by different reagents. In this study, chitin activated by hexamethylenediamine was found to be a very effective support for glucoamylase. They have further studied the optimization of chitin as a support for the immobilization of glucoamylase[5], a comparison of chitin with ceramics as supports[6] and characterization of the enzyme immobilized on chitin[7]. Chitosan, the deacetylated product of chitin, can also be used as a carrier for the immobilization of enzymes. Partially *n*-succinylated derivatives of chitosan and glycol chitosan[8] have been reported to be novel supports for

glucoamylase from *Rhizopus niveus*. The immobilized glucoamylase was found to retain 85.5% of its activity over 30 times of batch reactions.

In our previous reports[9) 10)], chitosan beads was effectively used for the immobilization of an acidic enzyme, α-galactosidase from *Pycnoporus cinnabarinus*. Therefore, we investigated the utilization of chitosan beads as carriers for the immobilization of a basic protein, glucoamylase from *Rhizopus niveus*, as well as an acidic one, glucoamylase from *Aspergillus* sp. K-27. This paper describes the preparation of immobilized glucoamylases, the optimal condition for immobilization, the stability of immobilized enzymes, and application to the conversion of starch to glucose in a column reactor.

Materials and Methods

Materials

Purified glucoamylase of *Rhizopus niveus* was purchased from Seikagaku Kogyo Co. Ltd. and crude glucoamylase of *Aspergillus* sp. K-27 was a gift from Daikin Kogyo Co. Ltd. α-Amylase from *Bacillus subtilis* was purchased from Seikagaku Kogyo Co. Ltd. Chitopearls, porous chitosan beads, were supplied by Fuji Spinning Co. Ltd. Chitopearl BCW 2510 was crosslinked with quarternary amine compound while chitopearl BCW 3010 and 3510 were crosslinked with bifunctional reagents, hexamethylene diisocyanate and 4, 4'-diphenyl methane isocyanate, respectively. Glucose, maltose, soluble starch, potato starch, and GA were purchased from Katayama Chemical Industrial Co. Ltd. Glucose C test kit was purchased from Wako Pure Chemical Industries Co. Ltd.

Immobilization of glucoamylase

The following two procedures were used for the preparation of immobilized glucoamylase.

Procedure 1: Glucoamylase (about 80 units) was added to 2g (wet weight) of chitosan beads in 4 ml of 0.025 M acetate buffer (pH 4.5). The mixture was shaken at 30°C for 1 hr. The beads were collected by filtration, washed with deionized water and stored in 0.05 M acetate buffer at 4°C.

Procedure 2: 2 g (wet weight) of chitosan beads were suspended in 4 ml of 0.025 M acetate buffer (pH 4.5) containing 2.5% GA. The mixture was shaken at 30°C for 1 hr. The beads were filtered and washed with deionized water to remove excess GA. The chitosan beads were then mixed with glucoamylase and immobilization was done as described above.

Assay of glucoamylase activity

The reaction mixture consisted of 1 ml of 0.05 M acetate buffer (pH 4.5), 1 ml of 1% soluble starch, and 20 mg of immobilized glucoamylase. The mixture was incubated for 15 min at 37°C in a reciprocal shaker. To 1 ml of alkaline copper reagent, 0.1 ml of the reaction mixture was added and the amount of reducing sugars was determined by the method of Somogyi-Nelson. One unit of glucoamylase activity was defined as the amount

of enzyme which produced 1 μmole of reducing sugar per min, with glucose as a reference compound.

Assay of maltase activity

The reaction mixture consisted of 1 ml of 0.1 M acetate buffer (pH 4.5), 1 ml of 0.05 M maltose, and 20 mg of immobilized glucoamylase. The mixture was incubated for 15 min at 37°C in a reciprocal shaker. The amount of glucose was determined enzymatically with Glucose C test kit. One unit of maltase activity was defined as the amount of enzyme which produced 1 μmole of glucose per min.

Hydrolysis of soluble starch in batch reactor

R. niveus glucoamylase (40 units) was immobilized on 1 g of autoclaved chitosan bead BCW 3510 according to procedure 1. One hundred fifty mililitres of soluble starch (1 to 10%) in 0.025 M acetate buffer (pH 4.5) in a reactor was sterilized and the immobilized glucoamylase was added. The reactor was incubated in water bath at 37°C and stirred continuously with magnetic stirrer. Samples were withdrawn aseptically after 1/2, 1, 2, 4, 6 and 24 hr of incubation and the amount of reducing sugar was determined by the method of Somogyi-Nelson.

Conversion of starch to glucose in column reactor

R. niveus glucoamylase (800 units) was aseptically mixed with 20 g of autoclaved chitosan beads BCW 3510 and the preparation of immobilized glucoamylase was done according to procedure 1. The immobilized enzyme was poured into a glass jacket column (16 × 168 mm) and the jacket was maintained at 50°C throughout the experiment. Fifty grams of potato starch was suspended in 1 litre of 0.05 M acetate buffer (pH 4.5), slowly heated in a water bath and liquefied by the addition of α-amylase (3,000 units). After the solution was left standing overnight at room temperature, the precipitate formed was removed by centrifugation and the supernatant was sterilized. The solution was passed through the column at the flow rate of 4.3 ml per hr.

Results

Immobilization of glucoamylases on different types of chitosan beads

Glucoamylases from *R. niveus* and *Aspergillus* sp. K-27 were immobilized on chitosan beads BCW 2510, 3010, and 3510, with or without GA treatment. Their activity yields with soluble starch and maltose are presented in Table 1. *R. niveus* glucoamylase was immobilized only on BCW 3510 giving high activity yields with maltose as a substrate and the activity yields with soluble starch were about 50% of those with maltose. The activity yield was higher in GA-treated beads than in GA-untreated ones. On the other hand, *Aspergillus* sp. K-27 glucoamylase was immobilized on all of the three types of chitosan beads. The activity yields with soluble starch as a substrate ranged from 32% to 45%, but their values were low as compared with the activity yields with maltose. GA treatment

Table 1 Immobilization of glucoamylases on different types of chitosan beads

Chitosan beads	*R. niveus* glucoamylase				*Asp.* sp. K-27 glucoamylase			
	Maltose		Soluble starch		Maltose		Soluble starch	
	Immobilized activity (units)	Activity yield (%)	Immobilized activity (units)	Activity yield (%)	Immobilized activity (units)	Activity yield (%)	Immobilized activity (units)	Activity yield (%)
BCW 3510	58.3	72.0	33.4	41.8	24.7	50.8	26.8	33.5
BCW 3510+GA	78.1	96.4	40.0	50.0	32.7	67.3	30.2	37.8
BCW 3010	5.7	7.0	3.5	4.4	39.3	80.9	29.4	36.8
BCW 3010+GA	10.0	12.3	5.2	6.5	29.4	60.5	25.7	32.1
BCW 2510	2.5	3.1	2.1	2.6	44.5	91.6	35.7	44.6
BCW 2510+GA	3.6	4.4	2.0	2.5	27.9	57.4	28.1	35.1

In the assay of maltase activity, 81.0 units of *R. niveus* glucoamylase and 48.6 units of *Aspergillus* sp. K-27 glucoamylase with maltose as substrate were added and in that of glucoamylase activity, 80.0 units of both glucoamylases were added. The activity yield was expressed as the percentage of immobilized activity to added activity.

Table 2 Effect of degree of deacetylation of chitosan on the immobilization of glucoamylases on chitosan beads BCW 3510

Degree of deacetylation of chitosan	*R. niveus* glucoamylase			*Asp.* sp. K-27 glucoamylase		
	Unbound activity (units)	Immobilized activity (units)	Activity yield (%)	Unbound activity (units)	Immobilized activity (units)	Activity yield (%)
20%	2.4	26.9	33.6	1.9	23.1	28.9
20%+GA	1.5	31.8	39.8	1.8	23.9	29.9
50%	2.0	30.7	38.3	1.4	28.1	35.1
50%+GA	1.5	35.8	44.8	1.4	29.4	36.8
80%	1.4	37.4	46.8	1.3	28.1	35.1
80%+GA	1.1	44.1	55.1	1.2	32.9	41.1

Eighty units of glucoamylases were added. After immobilized beads were collected by filtration, immobilized and unbound activities were determined by the assay of glucoamylase activity of beads and filtrate, respectively.

increased the activity yields in BCW 3510, but did not increase those in BCW 2510 and 3010.

Chitosan is generally a compound with different degree of deacetylation. Therefore, the effect of the degree of deacetylation of chitosan beads on the immobilization of glucoamylases was studied using BCW 3510. As presented in Table 2, an increase in the degree of deacetylation of chitosan brought about an increase in the activity yields in both GA-treated and -untreated beads. GA treatment resulted in a marked increase in activity yields for *R. niveus* glucoamylase than in those for *Aspergillus* sp. glucoamylase.

Table 3 Effect of the amount of *R. niveus* glucoamylase on the immobilization onto chitosan bead BCW 3510

Added activity (units)	Treatment with glutaraldehyde	Unbound activity (units)	Immobilized activity (units)	Activity yield (%)
20	−	0.3	12.5	62.5
20	+	0.2	15.5	77.5
40	−	0.9	21.1	52.8
40	+	0.6	29.2	73.0
80	−	1.8	32.3	40.4
80	+	1.5	43.6	54.5
120	−	3.8	39.7	33.1
120	+	2.5	46.9	39.1
160	−	6.3	40.0	25.0
160	+	4.5	48.0	30.0

The enzyme (20~160 units) was incubated with 2g of chitosan bead BCW 3510 which were either treated with GA or untreated.

Table 4 Effect of the amount of chitosan bead BCW 3510 on the immobilization of *R. niveus* glucoamylase

Amount of beads (%)	Treatment with glutaraldehyde	Unbound activity (units)	Immobilized activity (units)	Activity yield (%)	Activity yield per g beads
1	−	1.70	21.0	25.6	25.6
1	+	1.50	25.0	30.5	30.5
2	−	1.30	34.0	41.5	20.8
2	+	1.10	42.0	51.2	25.6
3	−	0.35	37.8	46.2	15.4
3	+	0.12	53.7	65.5	21.8
4	−	0.10	36.8	44.9	11.2
4	+	0.06	48.0	58.5	14.6
5	−	0.06	35.0	42.7	8.5
5	+	0.05	49.5	60.4	12.1

The enzyme (82 units) was incubated with 1~5g of chitosan beads BCW 3510 which were either treated with GA or untreated.

Optimal condition for the immobilization of glucoamylase on chitosan beads

The optimal condition for the immobilization of *R. niveus* glucoamylase on chitosan beads BCW 3510 was studied. The effect of the amount of enzyme on the immobilization onto GA-treated and -untreated chitosan beads is presented in Table 3. As the amount of the enzyme added increased, that of the enzyme immobilized also increased. However, the activity yield gradually decreased with increasing the enzyme added and it was the highest when 20 units of the enzyme was added to 2 g of citosan beads.

The effect of pH during GA treatment of chitosan beads and immobilization was examined. The activity yields in GA-treated and -untreated beads were about 50 and 40%, respectively, during GA treatment and immobilization at pH 4.5, 6.0, and 7.0. At pH 8.0, the activity yield in GA-untreated beads was reduced to 34%, although that in GA-treated

beads retained 50%. However, the activity yields in both GA-treated and -untreated beads at pH 9.0 were very low.

Table 4 shows the effect of the amount of GA-treated and -untreated chitosan beads on the immobilization of enzyme. The activity yield increased with increasing the amount of beads from 1 g to 3 g. However, further increase in the amount of beads did not bring about the increase in the activity yield. When 1 g of chitosan beads was used for 82 units of the enzyme, the activity yield per g of beads was the highest.

Stability of immobilized glucoamylases

The stability of *R. niveus* glucoamylase immobilized on chitosan beads BCW 3510 with or without GA treatment was studied. For this purpose, the immobilized enzyme was suspended in 0.05 M acetate buffer (pH 4.5) containing 1.0 M NaCl and kept at 37°C for 1/2 to 6 hr, and residual activity was determined. The result is presented in Table 5. The immobilized glucoamylase was found to be very stable in 1.0 M NaCl. After incubation for 6 hr in 1.0 M NaCl, the immobilized enzyme retained about 84% of the initial activity in GA-untreated beads and about 90% in GA-treated ones.

The effect of 1.0 M NaCl on the stability of *Aspergillus* sp. K-27 glucoamylase immobilized on the chitosan beads BCW 2510, 3010, and 3510, with or without GA treatment was studied. As shown in Table 6, glucoamylase immobilized on BCW

Table 5 Stability of *R. niveus* glucoamylase immobilized on chitosan bead BCW 3510 in 1.0 M NaCl

NaCl treatment	Time (hr)	Enzyme activity (units)	Residual activity (%)
Original		38.0	100
Control	1/2	38.0	100
	1	37.0	97.4
	2	36.8	96.8
	3	37.0	97.4
	6	37.0	97.4
NaCl	1/2	37.0	97.4
	1	34.2	90.0
	2	32.8	86.3
	3	31.8	83.7
	6	32.0	84.2
Glutaraldehyde Original		43.6	100
Control	1/2	43.6	100
	1	43.2	99.1
	2	42.8	98.2
	3	43.0	98.6
	6	41.7	95.6
NaCl	1/2	43.6	100
	1	42.6	97.7
	2	42.6	97.7
	3	41.4	95.0
	6	39.2	89.9

The immobilized enzymes (100 mg) were suspended in 2 ml of 0.05M acetate buffer (pH 4.5) containing 1.0 M NaCl at 37°C. In the control the immobilized enzymes were suspended in 0.05M acetate buffer (pH 4.5).

3510 was found to be stable in 1.0 M NaCl in both GA-treated and -untreated beads. The enzyme immobilized on GA-untreated beads of BCW 3010 and 2510 lost 23% and 61% of the original activity in the buffer solution, and 65% and 83% in 1.0 M NaCl solution, respectively. However, the enzyme immobilized on GA-treated BCW 3010 and 2510 was found to be stable even in 1.0 M NaCl.

R. niveus glucoamylase immobilized on GA-treated and -untreated beads BCW 3510 was stored at 4°C in 0.05 M acetate buffer (pH 4.5) and then the residual activity was assayed every month. As shown in Table 7, the immobilized glucoamylase gradually lost its activity, but retained more than 65% of the initial activity even after storage for 9 months.

The stability of *R. niveus* glucoamylase immobilized on GA-treated and -untreated beads BCW 3510 on repeated use was studied. As shown in Fig. 1, the immobilized enzyme retained about 75% of the ini-

Table 6 Stability of *Aspergillus* sp. K-27 glucoamylase immobilized on different types of chitosan beads in 1.0 M NaCl

Chitnsan bead		Enzyme activity (units)	Residual activity (%)
3510	Original	26.0	100
	Control	26.0	100
	NaCl	22.0	84.6
3510	Original	32.0	100
+Glutaraldehyde	Control	32.0	100
	NaCl	30.0	93.8
3010	Original	26.0	100
	Control	20.0	76.9
	NaCl	9.0	34.6
3010	Original	24.0	100
+Glutaraldehyde	Control	24.0	100
	NaCl	22.0	91.7
2510	Original	28.8	100
	Control	11.2	38.9
	NaCl	5.0	17.4
2510	Original	26.0	100
+Glutaraldehyde	Control	26.0	100
	NaCl	25.0	96.2

The immobilized enzymes were suspended in 2 ml of 0.05M acetate buffer (pH 4.5) containing 1.0M NaCl at 37°C for 1 hr. In the control the immobilized enzyme was suspended in 0.05M acetate buffer (pH 4.5).

Table 7 Storage stability of *R. niveus* glucoamylase immobilized on chitosan bead BCW 3510

Storage period (months)	With glutaraldehyde		Without glutaraldehyde	
	Activity (units)	Residual activity (%)	Activity (units)	Residual activity (%)
0	34.2	100	45.0	100
1	33.4	97.7	38.0	84.4
2	29.0	84.8	34.3	76.2
3	26.6	77.8	32.7	72.7
4	26.0	76.0	32.4	72.0
5	25.4	74.3	32.0	71.1
6	25.1	73.1	31.5	70.0
7	24.0	70.2	31.7	70.4
8	23.2	67.8	30.0	66.6
9	23.0	67.3	30.2	67.1

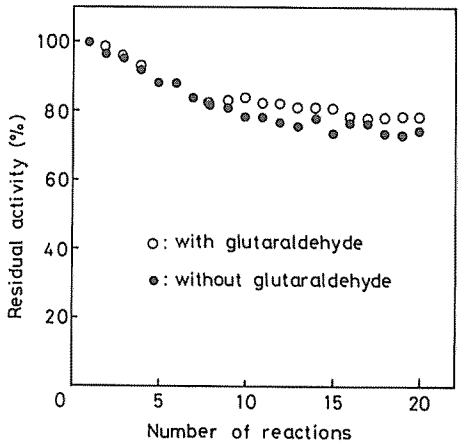

Fig. 1 Repeated use of *R. niveus* glucoamylase immobilized on BCW 3510.
The immobilized enzyme (20 mg) was suspended in 1 ml of 0.05M acetate buffer (pH 4.5) and 1 ml of 1% soluble starch. The mixture was incubated for 15 min at 37°C in reciprocal shaker and the amount of reducing sugars produced was determined. The beads were separated from the reaction mixture by filtration, washed with deionized water and used again.

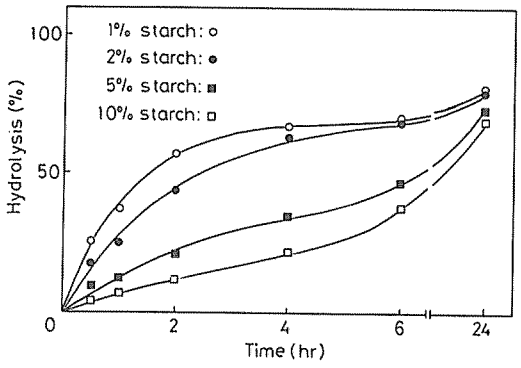

Fig. 2 Hydrolysis of soluble starch by *R. niveus* glucoamylase immobilized on BCW 3510 in batch reactor.
The preparation of immobilized enzyme and the procedure for hydrolysis of soluble starch was described in Materials and Methods.

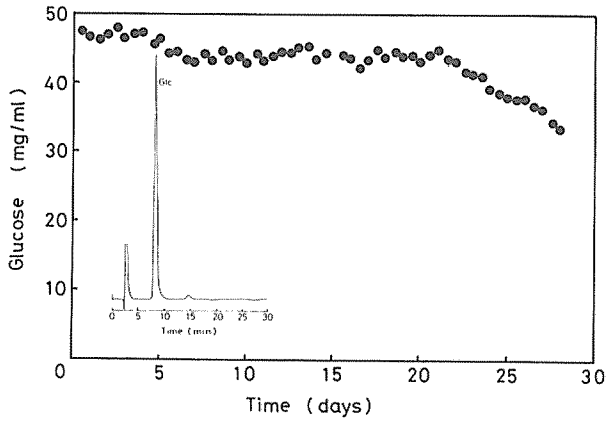

Fig. 3 Conversion of starch to glucose in column reactor packed with *R. niveus* glucoamylase immobilized on BCW 3510.
The preparation of immobilized enzym and liquefied starch solution was described in Materials and Methods. The three batches of 1 litre of liquefied starch solution continuously passed through the column at the flow rate of 4.3 ml per hr.

tial activity after repeated use of 20 times regardless of GA treatment.

Conversion of starch to glucose by immobilized glucoamylase

The hydrolysis of soluble starch by *R. niveus* glucoamylase immobilized on BCW 3510 in batch reactor is shown in Fig. 2. When 1% and 2% starch solution were used, more than 60% of the starch was hydrolyzed by the enzyme within 4 hr and about 80% after incubation for 24 hr. When 5% and 10% starch solution were used, the rate of the hydrolysis was slow in the initial stage, but it gradually increased as the reaction proceeded and attained to 74% and 69%, respectively, after incubation for 24 hr.

Three litres of 5% liquefied starch solution was passed through the column which contained *R. niveus* glucoamylase immobilized on BCW 3510 for 28 days. Only glucose was detected in the eluate by HPLC, so the amount of glucose in the eluate was determined twice for a day by the method of Somogyi-Nelson. As shown in Fig. 3, the amount of glucose in the eluate was above 43 mg/ml for 20 days of continuous operation, altough glucose production decreased after 20 days.

Discussion

Glucoamylase from *R. niveus* is a basic protein with the isoelectric point at pH 8.4, while glucoamylase from *Aspergillus* sp. K-27 is an acidic enzyme judging from its chromatographic behaviour[11]. Glucoamylase of *Aspergillus* sp. K-27 immobilized on chitosan beads BCW 2510 and 3010 was sensitive to change in ionic strength, as had been found in α-galactosidase from *P. cinnabarinus*[10]. Therefore, it is assumed that this enzyme is bound to these chitosan beads through weak forces involving ionic bonding. On the contrary, glucoamylase of *R. niveus* is not adsorbed on these chitosan beads, because of its positive charge at acidic pH. However, both glucoamylases immobilized on chitosan beads BCW 3510 were stable in high ionic strength. This may be attributed to the formation of hydrophobic bonding between the enzymes and the aromatic spacers crosslinked with the beads. When GA is used as a cross-linking reagent for immobilization, it is probable that the enzymes are covalently bound to the chitosan beads through GA to give stable enzymes.

Glucoamylases immobilized on chitosan beads BCW 3510 were stable even without GA treatment and could be stored for several months without significant loss of the immobilized activity. The relative surface area, apparent density and compressive strength of the chitosan beads are not varied by sterilization in autoclave. Therefore, glucoamylase of *R. niveus* immobilized on autoclaved BCW 3510 is useful as a reactor for conversion of starch to glucose.

Acknowledgments

We wish to sincere thanks to Fuji Spinning Co. Ltd. and Daikin Kogyo Co. Ltd. for supplying chitosan beads, chitopearl BCW 2510, 3010, and 3510, and crude glucoamylase

from *Aspergillus* sp. K-27, respectively.

References

1) W.L. Stanley, G.G. Watters, S.H. Kelly and A.C. Olson; *Biotechnol. Bioeng.*, **20**, 135-140 (1978).
2) S. Hayashida and P.Q. Flor; *Agric. Biol. Chem.*, **46**, 1639-1645 (1982).
3) P.Q. Flor and S. Hayashida; *Biotechnol. Bioeng.*, **25**, 1973-1980 (1983).
4) R. Yamaguchi, Y. Arai, T. Kaneko and T. Itoh; *Biotechnol. Bioeng.*, **24**, 1081-1091 (1982).
5) E. Bon, D.G. Freire, M.F. Mendes and V.F. Soares; *Biotechnol. Bioeng. Symp.* No. 14 (1984).
6) E. Bon, D.G. Freire, M.F. Mendes, C.P. Moreira and V.F. Soares; *Biotechnol. Bioeng. Symp.* No. 14 (1984).
7) M.T. Xavier, V.F. Soares, D.G. Freire, C.P. Moreira, M.F. Mendes, and E. Bon; *Biomass*, **13**, 25-32 (1987).
8) D.G. Freire, A. Sant and L. Geraldo Jr.; *Biomass*, **23**, 71-78 (1990).
9) M. Mitsutomi, Y. Uchida and A. Ohtakara; *J. Ferment. Technol.*, **63**, 325-329 (1985).
10) A. Ohtakara and M. Mitsutomi; *J. Ferment. Technol.*, **65**, 493-498 (1987).
11) J. Abe, K. Nakajima, H. Nagano and S. Hizukuri; *Carbohydr. Res.*, 175, 85-91 (1988).

グルコアミラーゼのキトサンビーズへの固定化とデンプンのグルコースへの変換への固定化酵素の利用

Gouri MUKERJEE DHAR・光富　勝・大宝　明
(食糧化学研究室)

平成4年10月29日受理

摘　　要

グルタルアルデヒド (GA) 処理および非処理のもとで，グルコアミラーゼのキトサンビーズへの固定化を行った．その結果，*Rhizopus niveus* のグルコアミラーゼは，芳香族化合物で架橋されたキトサンビーズ BCW 3510にのみ固定化されたが，*Aspergillus* sp. K-27のグルコアミラーゼは BCW 3510のほかに，脂肪族化合物で架橋されたキトサンビーズ BCW 2510と BCW 3010にも固定化された．BCW 3510への両酵素の固定化量は，キトサンビーズの脱アセチル化度が増大するに従って増加した．GA 処理なしの BCW 2510および3010に固定化された *Aspergillus* sp. K-27グルコアミラーゼは，1.0M 食塩溶液中で酵素が漏出して容易にそれらの活性を失ったが，BCW 3510に固定化された両酵素は，GA 処理なしでも1.0M 食塩溶液中に漏出しなかった．BCW 2510および3010に固定化された酵素の漏出は GA 処理によって防止された．キトサンビーズ BCW 3510に固定化された *R. niveus* のグルコアミラーゼは，9か月後も約67%の活性を残存しており，カラムリアクター中で20日間連続使用後もデンプン分解活性を保持していた．