Concanavalin A Attached Poly(*p*-chloromethylstyrene) Beads for Glycoenzyme Separation

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ABSTRACT: Crosslinked poly(*p*-chloromethylstyrene) (PCMS) beads were produced by suspension polymerization. The beads had a nonporous but reasonably rough surface structure. Because of this property, a relatively high external surface area (i.e., 14.1 m^2/g) was obtained with the proposed carrier. A biospecific ligand commonly used in the affinity chromatography of various glycoenzymes, concanavalin A (Con A), was covalently attached onto the bead surface by a direct chemical reaction. PCMS/Con A beads were then used for the separation of a model glycoenzyme, invertase, from its crude solution. The appropriate invertase adsorption and desorption conditions of the affinity system were investigated. The desorption of invertase from the carrier was achieved by the use of methyl-α-D-mannopyranoside (MMP) as the counterligand. The effects of the MMP and salt concentrations and the temperature on the desorption behavior of invertase were investigated. The MMP concentration and temperature were found to be the dominant parameters controlling the desorption. Iterative experiments involving five reversible adsorption–desorption cycles were performed with the same particles to monitor the changes that occurred in the invertase adsorption–desorption capacities of Con A immobilized beads. The reversible adsorption–desorption stability was significantly improved by the crosslinking of Con A immobilized on the beads. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 2116–2124, 2004

Key words: chromatography; enzymes; functionalization of polymers; polystyrene; proteins

INTRODUCTION

One of the most important research areas in immobilization technology is the production of carriers, and much effort has been dedicated toward their development.[1,2] Suspension polymerization is a useful tool for the preparation of beaded support materials.[3–6] Covalent immobilization methods usually involve reactive functional groups on the surfaces of these beads. The functional groups can be incorporated into the bead structure by the use of functional monomers during the polymerization.[3,4] Polymeric supports with functional groups that have a direct reaction ability with the amine group have been widely used in the covalent attachment of enzymes. Functional monomers such as *p*-chloromethylstyrene (CMS), epoxy propyl methacrylate, *N*-acryloxysuccinimide, and acrolein can be considered in this class.[7–11] Poly(*p*-chloromethylstyrene) (PCMS) beads have attracted attention especially as carrier matrices for biotechnological applications because of their ability to directly bind amine groups. Several polymerization procedures have been proposed for the production of CMS-based beads with different bulk and surface properties.[12–14] We also developed some polymerization procedures for the synthesis of PCMS carriers.[15–18] In our previous studies, we investigated the DNA binding properties of these materials.[16,18]

The synthesis of PCMS beads carrying different functional groups can be accomplished by the covalent binding of ligands such as $NH_2(CH_2)_nX$, where *X* can be CH_2NH2, CH_2OH, or COOH.[13] Concanavalin A (Con A), a lectin from jack beans, is widely used for the purification and immobilization of glycoenzymes.[19,20] Con A is the most extensively studied lectin because of its ability to form complexes with molecules, including glycosyl and mannosyl residues. In an aqueous medium, Con A can be a monomer, dimer, or tetramer, depending on the pH and temperature.[19] Each subunit of Con A consists of 237 amino acid moieties and contains two metal sites for the binding of saccharides.[20] One of them is suitable for the binding of transition-metal ions, such as manganese, whereas the other one binds calcium. Complexes of Con A with different metal ions have also been identified.[21–24] The identification of the Con A/methyl-α-D-mannopyranoside (MMP) complex also provided a detailed description of the mannoside binding site.[25] Hydrophilic support materials precoupled with Con A have been extensively tested in the immobilization of glycoenzymes.[26–35] Invertase is one of the most commonly used glycoenzymes in immobilization and

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purification studies because of the commercial importance of sucrose hydrolysis.

In this study, a relatively hydrophobic carrier, PCMS, was used as a new support material for the separation of glycoenzymes by affinity chromatography. Spherical PCMS beads were obtained through the suspension polymerization of CMS. The ligand, Con A, was covalently immobilized onto the beads by a direct interaction taking place between the amino groups of Con A and the chloromethyl groups of the beads. Immobilized Con A was also crosslinked by glutaraldehyde (GA) for the enhancement of the ligand stability. Invertase was selected as a model glycoenzyme purified by the proposed affinity system. The adsorption and desorption behavior of the glycoenzyme and the reusability of the sorbent were investigated.

EXPERIMENTAL

Materials

The monomer CMS (98% purity), the crosslinker ethylene glycol dimethacrylate (EGDMA), and the stabilizer poly(vinyl alcohol) (PVA; 87–89% hydrolyzed, relative molecular mass = 85,000–146,000) were supplied by Aldrich Chemical Co. (Milwaukee, WI) and were used as received. The initiator 2,2′-azobisisobutyronitrile (AIBN) and monoethanol amine (MEA) were obtained from BDH Chemicals, Ltd. (Poole, England). Con A, MMP, invertase (EC 3.2.1.26, grade V), GA (grade II, 25% aqueous solution), and the Biuret reagent were purchased from Sigma Chemical Co. (St. Louis, MO). The substrate sucrose was supplied by the Ankara Sugar Plant (Ankara, Turkey). Distilled and deionized water was used in all the experiments.

Production of the PCMS beads

The crosslinked PCMS beads were prepared by a suspension polymerization method. The preparation procedure is described in detail elsewhere.[16,17] The method was developed through the combination of the principles of the polymerization method proposed by Nonaka et al.[14] and the principles of suspension polymerization investigated in our previous studies.[36–39] Typically, CMS (2.5 mL), EGDMA (0.75 mL), and AIBN (0.06 g) were dissolved in cyclohexane (3.6 mL). The resulting homogeneous phase was dispersed in an aqueous medium prepared by the dissolution of PVA (0.1 g) in water (40 mL). The polymerization was carried out in a magnetically stirred batch reactor (100 mL) at 78°C for 6 h. The stirring rate was kept constant at 300 rpm during the polymerization. At the end of the polymerization period, PCMS beads were extensively washed first with ethanol and then with water to remove the diluent and any possible unreacted monomer. The beads were screened in the presence of water, and an appropriately sized fraction (i.e., 147–297 μm) was isolated.

Con A attachment onto the PCMS beads

For the determination of the appropriate Con A immobilization conditions, the effects of the Con A concentration and pH on the Con A attachment onto the crosslinked PCMS beads were investigated. All the buffer solutions used in the Con A attachment experiments were included (1 m*M* $CaCl_2$, 1 m*M* $MnCl_2$, and 1 m*M* $MgCl_2$) because these ions were required for the stability and activity of Con A.[40–45] Typically, PCMS beads (1 g) were placed in a Con A solution (25 mL) at pH 7.0 (0.05*M* phosphate buffer). Con A immobilization was conducted at 4°C for 24 h through the shaking of the medium at 50 cpm. In the immobilization runs, the Con A concentration was varied between 0.5 and 4.0 mg/mL. After the immobilization, the PCMS beads were extensively washed with a buffer to remove physically adsorbed Con A. The amount of Con A immobilized on the beads was calculated by the determination of the initial and final protein concentrations by the Biuret method. For the determination of the amount of Con A covalently bound onto the beads, the protein concentration in the washing solution was also determined, and the amount of physically adsorbed Con A was subtracted from the total amount of Con A adsorbed onto the beads.

The subunits of Con A could leave each other in particularly acidic media. In the previous studies of the use of Con A as a ligand for glycoprotein isolation, it was observed that Con A lost its glycoprotein binding ability with repeated adsorption–desorption cycles. This behavior was explained by the removal of Con A subunits in the desorption process of the adsorbed glycoprotein. To overcome this problem, several authors have recommended the crosslinking of Con A.[44,45] Therefore, Con A immobilized onto the PCMS beads was also crosslinked with GA, a popular crosslinker for proteins. Because Con A is also a glycoprotein carrying many amino groups on its subunits, GA can be used for its crosslinking.[32] GA predominantly reacts with amino groups of proteins.[46] More details about GA protein reactions can be found in the literature.[46] The crosslinking protocol used in our study was as follows: 1 g of PCMS/Con A beads was added to a solution of 1% (w/w) GA (pH 7, 0.05*M* phosphate buffer including 1 m*M* $CaCl_2$, 1 m*M* $MnCl_2$, and 1 m*M* $MgCl_2$) and treated for 2 h at 25°C at a shaking rate of 50 cpm. After this process, the beads were washed extensively for the removal of unreacted GA. The blocking of possible residual aldehyde groups on the immobilized Con A was performed with MEA. For this purpose, a solution including 1% (w/w) MEA was prepared with a buffer solution at

pH 7.5 (phosphate buffer including 1 m*M* $CaCl_2$, 1 m*M* $MnCl_2$, and 1 m*M* $MgCl_2$). Crosslinked PCMS/Con A beads were treated with the MEA solution for 2 h at 25°C with a shaking rate of 50 cpm. Then, the beads were washed extensively with a buffer (pH 7). Con A immobilized PCMS beads were stored at 4°C in a buffer solution at pH 7. This procedure was also used to block any remaining chloromethyl groups on the bead surface. The beads carrying Con A in the crosslinked form were used in the invertase adsorption–desorption experiments.

Invertase adsorption–desorption experiments

These experiments were used to determine the invertase adsorption properties of Con A carrying PCMS beads. As previously described, 1 m*M* $CaCl_2$, 1 m*M* $MnCl_2$, and 1 m*M* $MgCl_2$ were included in all buffer solutions. Invertase adsorption onto PCMS/Con A particles was started through the addition of 1 g of beads into a 20-mL invertase solution, including 2 mg/mL crude invertase (at different pH values of 3.5–6.5). Adsorption experiments were performed in a batch reactor at 20°C at a shaking rate of 50 cpm. The adsorbed amount of invertase was found by the determination of the initial and final protein concentrations in the liquid part. In some cases, the initial and final invertase activities in the solution were determined for the expression of the enzymatic activity adsorbed or desorbed. To find the rate of the adsorption process, we followed the invertase adsorption against time.

A specific counterligand, MMP, was selected for the desorption of invertase from the beads. In the desorption experiments, the effects of the MMP and NaCl concentrations and the temperature on the desorption rate and desorption yield were investigated. A typical desorption experiment was performed as follows: 0.25 g of invertase-adsorbed beads were added to a desorption solution (5 mL, pH 4.7, 0.05*M* sodium acetate, 1 m*M* $CaCl_2$, 1 m*M* $MnCl_2$, and 1 m*M* $MgCl_2$) including 0.5*M* MMP and 0.68*M* NaCl. Unless stated otherwise, the desorption was conducted at 20°C in a batch reactor shaken at 50 cpm. The desorption rate and the desorption yield were determined by the measurement of the invertase activity of liquid samples withdrawn periodically. After a desorption period of 5 h, the particles were separated from the desorption solution, and a fresh medium with the same properties (5 mL) was added to the isolated particles. Then, the desorption experiment was continued up to 24 h. At the end of this period, the desorption yield was again determined.

Determination of the invertase activity

The enzymatic activity was determined in a stirred batch reactor by the measurement of the concentration of glucose with a glucose microanalyzer (Accutrend, Boehringer, Mannheim, Germany). The typical procedure used for the determination of the invertase activity was as follows: By means of a special pipette, a certain amount of invertase (0.5 mg based on the total protein content of invertase) was added to a batch reactor containing a 15% (w/w) sucrose solution (30 mL) at pH 4.7. The hydrolysis reaction was conducted at 55°C at a stirring rate of 200 rpm. Then, the concentration of glucose was determined against the time with the glucose microanalyzer. The invertase activity was expressed as the amount of hydrolyzed sucrose per unit of time (μmol of hydrolyzed sucrose /min).

RESULTS AND DISCUSSION

Characterization of the PCMS beads

A detailed characterization of the PCMS beads has been reported elsewhere.[16,17] In the suspension polymerization, the bead yield was determined to be 86% (w/w) on the basis of the mass of the monomer initially charged to the reactor. The PCMS beads obtained by the proposed suspension polymerization method were predominantly collected in the size range of 88–354 μm.[16,17] The average bead size was determined to be 186 μm by screen analysis.[16] The PCMS beads in the size range of 147–297 μm were isolated and used as carriers. An optical micrograph of the spherical beads is included in Figure 1. The opaque, dark view of the beads is probably an indication of a porous interior. Indeed, the presence of a porous internal structure in the beads has been shown elsewhere by a scanning electron microscopy (SEM) analysis of broken beads.[16] A SEM photograph showing the typical surface morphology of plain PCMS beads is also included in Figure 1. The particle surface was nonporous but reasonably rough. The specific surface area was determined to be 14.1 m^2/g by BET measurements.[16] The rough character of the bead surface probably provided this high specific surface area.

Con A immobilization

The chloromethyl groups of PCMS beads react with amino groups.[13] Therefore, it is possible to attach Con A onto PCMS beads without the application of any additional activation protocol via the reaction between the amino groups of Con A and the chloromethyl groups of the beads. The covalent attachment route is depicted in Figure 2. The charges of the functional groups on the protein molecules vary according to their dissociation constants with the pH of their environment.[47] This affects the total net charge of the protein molecule and the distribution of the charge on its exterior surface, in addition to the reactivity of the functional groups.[47] For this reason, Con A was im-

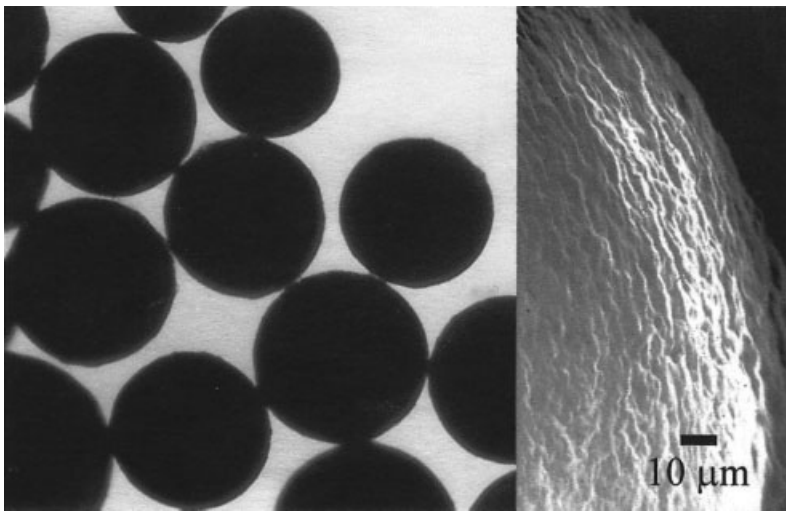

Figure 1 Optical (original magnification = 100×) and SEM (original magnification = 600×) micrographs of PCMS beads.

mobilized onto the PCMS beads at different pH values of 5.7–9 for the determination of the optimum pH. The variation of the immobilized Con A with the pH is illustrated in Figure 3. A rapid increase was observed in the immobilized Con A as the pH increased from 5.7 to 7.0. This behavior could be explained by the low reactivity of the chlorine groups in the acidic medium.[13] Maximum Con A attachment was observed at pH 7.0. A further increase in the pH (i.e., from 7 to 9) caused a decrease in the immobilized Con A. The pH providing the highest Con A immobilization under selected conditions was evaluated as the optimum value and was determined to be 7.0 from Figure 3. This pH was also suitable for the activity and stability of Con A.[44]

The maximum Con A binding capacity of the PCMS beads was determined by the assessment of the Con A adsorption isotherm. In these experiments, the initial Con A concentration of the immobilization medium was varied between 0.5 and 4.0 mg/mL (i.e., based on the total protein content of Con A), with the other immobilization conditions fixed at their appropriate values (i.e., pH = 7, immobilization time = 24 h, and temperature = 4°C). The variation of the immobilized Con A by the initial Con A concentration is given in Figure 4. The immobilized amount of Con A increased with the initial Con A concentration and then reached a plateau. The plateau value of the immobilized amount of Con A was determined to be approximately 18 mg of Con A/g beads in the immobilization media with initial Con A concentrations higher than 3 mg/mL. This concentration (i.e., 3 mg/mL) was selected as the optimum immobilization concentration of Con A because a further increase in the Con A concentration did not cause a significant increase in the immobilized amount of Con A.

Effect of the pH on the adsorption of invertase

Con A is commonly preferred as a ligand for the isolation and purification of glycoenzymes because of its ability to form complexes with the molecules carrying glycosyl and mannosyl groups such as glycoenzymes.[19,48–51] The pH of the adsorption medium may strongly affect the interaction between invertase and Con A. For this reason, the invertase adsorption experiments were carried out at different pHs of 3.5 to 6.5. In these experiments, the adsorbed amount of invertase was determined by the measurement of the initial and final protein concentrations in the adsorp-

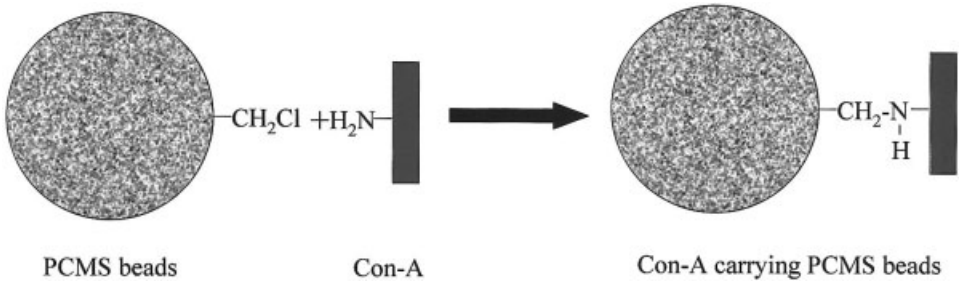

Figure 2 Con A immobilization schematics.

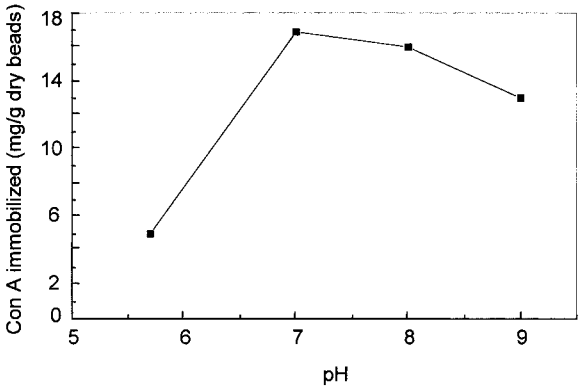

Figure 3 Effect of the pH on the Con A adsorption onto PCMS beads (temperature = 4°C, initial Con A concentration = 4 mg/mL, volume = 20 mL, and immobilization time = 24 h).

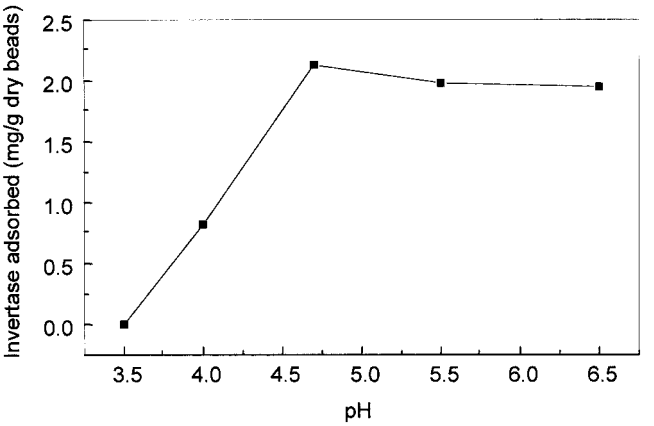

Figure 5 Effect of the pH on the invertase adsorption onto PCMS/Con A beads (temperature = 20°C, initial invertase concentration = 2 mg/mL, volume = 10 mL, and immobilization time = 24 h).

tion medium. The PCMS/Con A beads carrying 18 mg of Con A/g of dry beads in the crosslinked form (i.e., prepared at the initial Con A concentration of 4.0 mg/mL at pH 7) were used as a sorbent in the invertase adsorption and desorption experiments. The variation of the adsorbed amount of invertase with the pH is given in Figure 5. In the pH range of 3.5–4.7, a sharp increase in the adsorbed amount of invertase was observed with increasing pH. A further increase in the pH did not cause a significant change in the invertase adsorption. On the basis of this behavior, pH 4.7 was selected as the appropriate value for the other invertase adsorption experiments. This value was also reported to be an optimal value for the enzymatic activity and stability of invertase.[44,52] Optimal pH values, reasonably close to that found in our study, have also been used for similar Con A invertase affinity couples in the literature.[44,52]

The invertase adsorption at pH 4.7 was also followed dynamically by the determination of the residual activity in the adsorption medium. The variation of the adsorbed activity with the time is presented in Figure 6. Moreover, no significant decrease in the activity of invertase was detected during the time course of adsorption in a control experiment performed under adsorption conditions (i.e., 20°C and pH 4.7). As shown in Figure 6, the rate of the adsorption process was constant in the first 30 min. After this period, the rate gradually decreased. In other words, 75% of the equilibrium adsorption was achieved in the first 30 min. The adsorption experiment was continued for 24 h, but no significant increase was observed with respect to that obtained in the first 2 h.

Effect of the MMP concentration on the desorption of invertase

Because pH and temperature changes can damage some sensitive molecules, such as enzymes, one of the most popular methods for the desorption of glycoproteins bound to Con A is using a specific counterli-

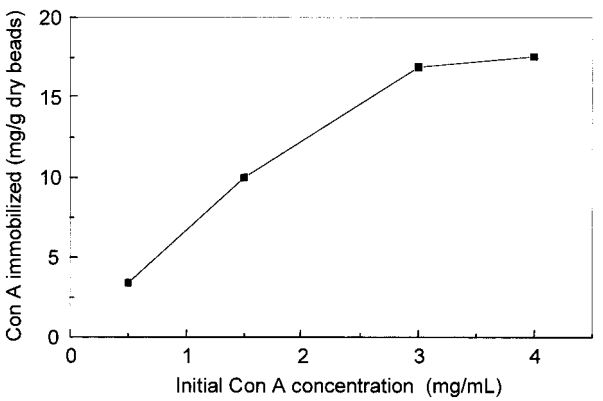

Figure 4 Effect of the initial Con A concentration on the Con A adsorption onto PCMS beads (temperature = 4°C, pH = 7, volume = 20 mL, and immobilization time = 24 h).

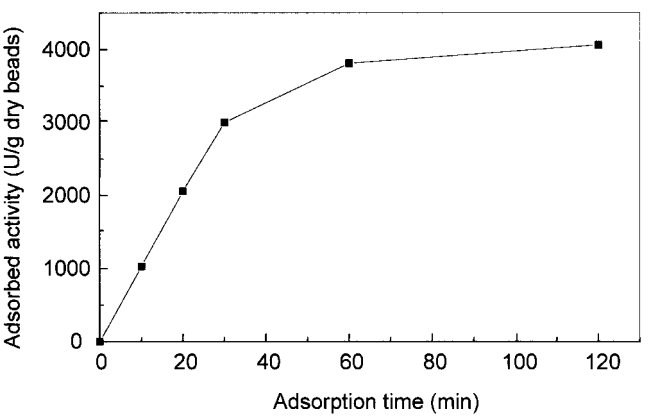

Figure 6 Dynamic adsorption behavior of invertase for PCMS/Con A beads (temperature = 20°C and pH = 4.7).

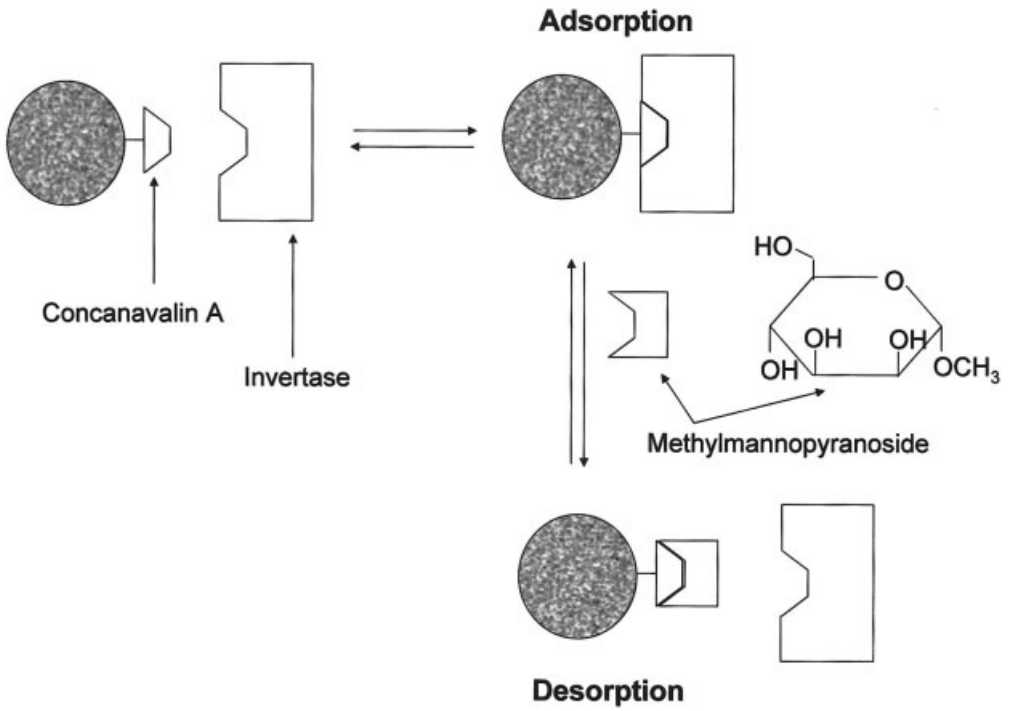

Figure 7 Adsorption and desorption of invertase by PCMS/Con A beads.

gand such as MMP.[41] The commercial availability and low price of MMP, along with its success in desorption, are the reasons for its popular usage.[41] A schematic representation of the invertase adsorption–desorption mechanism and the structure of MMP are given in Figure 7.

The effect of the MMP concentration on the desorption of invertase from the Con A attached PCMS beads was investigated. For this purpose, the desorption behavior of invertase was determined at different MMP concentrations varying in the range of 0.0–1.0*M*. In this set, the other conditions were kept constant (i.e., temperature = 20°C, pH = 4.7, NaCl concentration = 0.68*M*, amount of beads = 0.25 g, and amount of desorption medium = 5 mL). For the different MMP concentrations, the variation of the desorption yield with the time is given in Figure 8. Here, the desorption yield was defined as the ratio of activity released from the Con A attached PCMS beads in a certain period (U/g dry beads) to the enzymatic activity initially loaded to the same beads (U/g dry beads). Both the initially loaded activity and the activity desorbed from the beads in a certain period were determined by the measurement of the enzymatic activities of the liquid samples. In the absence of MMP, only 25% (w/w) of the adsorbed invertase could be desorbed in 5 h. However, the desorption yield obtained with the MMP concentration of 0.25*M* was approximately 60% in the desorption period of 5 h. This value could be increased up to 66% by the continuation of the desorption process for 24 h more. However, satisfactory desorption yields of up to 83% could be achieved with an MMP concentration of 0.5*M*. A further increase in the MMP concentration did not cause a significant increase in the desorption yield, and almost the same desorption behavior was observed for both 0.5 and 1.0*M* MMP concentrations. In other words, 85% of the invertase desorption was obtained in a desorption period of 5 h in the medium containing 1*M* MMP. This value could be increased up to 87% (w/w) by the continuation of the desorption process for another 24 h. On the basis of this behavior, an MMP concentration of 0.5*M* was selected as an appropriate value for the other experiments.

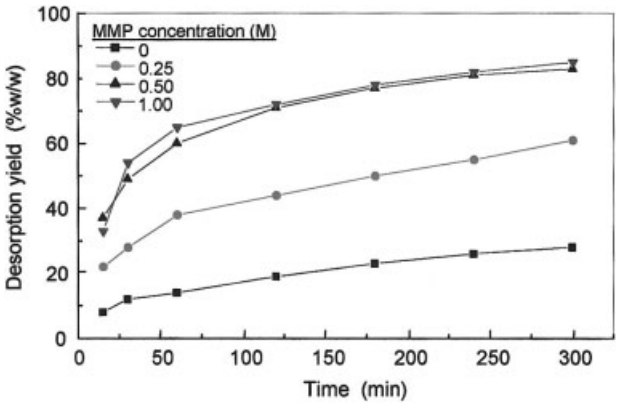

Figure 8 Effect of the MMP concentration on the desorption of invertase from PCMS/Con A beads (temperature = 20°C, NaCl concentration = 0.68–2*M* in the medium including no MMP, and pH = 4.7).

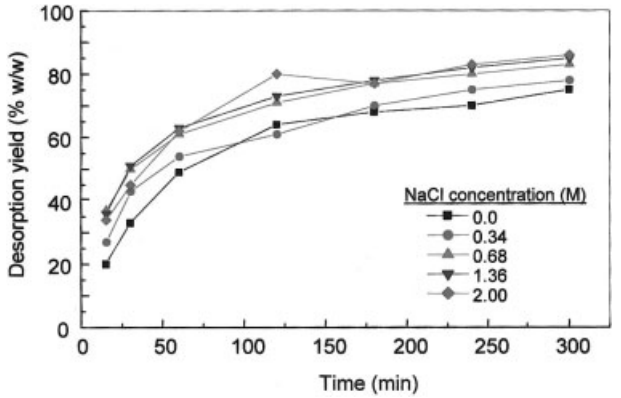

Figure 9 Effect of the NaCl concentration on the desorption of invertase from PCMS/Con A beads (temperature = 20°C, MMP concentration = 1*M*, and pH = 4.7).

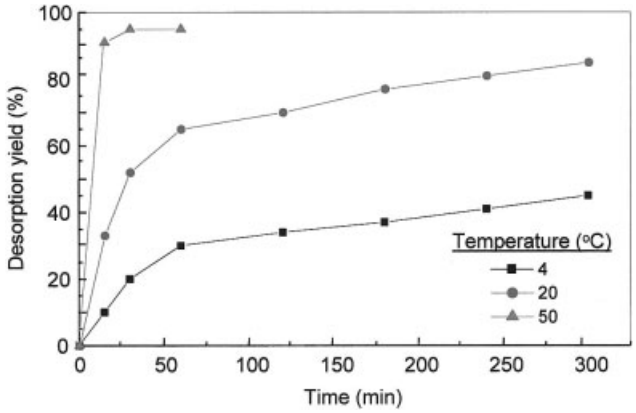

Figure 10 Effect of the temperature on the desorption of invertase from PCMS/Con A beads (MMP concentration = 1*M*, NaCl concentration = 0.68*M*, and pH = 4.7).

Effect of the salt concentration on the desorption of invertase

One common method for the desorption stage of affinity purification systems is changing the ionic strength of the desorption medium. For this reason, the desorption of invertase from the Con A attached PCMS beads was examined at different salt concentrations ranging from 0.0 to 2.0*M*. For this purpose, 0.25 g of invertase adsorbed beads was used in a desorption medium of 5 mL. The desorption experiments were performed at 20°C and at pH 4.7 for 5 h with an MMP concentration of 1.0*M*. The variation of the desorption yield with the time is shown in Figure 9 at different salt concentrations. The salt concentration was not so effective for improving the desorption yield. After a desorption period of 5 h, the desorption medium was refreshed, and the desorption process was continued for an additional 24 h. The obtained desorption yields at the end of this process are given in Table I. The results indicated that the 0.68*M* salt concentration was appropriate.

Effect of the temperature on the desorption of invertase

The temperature can have a drastic effect on both the thermodynamics and kinetics of protein surface interactions.[41] Some studies have shown that the dissociation rate constants of small solutes on Con A are sensitive to temperature,[53] and increasing the temperature in the range of 30–50°C results in the dissociation of the glycogen/Con A complex.[54] Con A selectively interacts with α-D-manno- and α-D-glucopyranoside moieties with unsubstituted hydroxyl groups or with glycoproteins and polysaccharides.[55] The reactions between lectins and simple sugars in aqueous media are usually rapid and exothermic.[56–59] To test the effect of the temperature on this affinity system, we performed desorption experiments by varying the temperature between 4 and 50°C. In these experiments, the dry weight of Con A attached PCMS beads and the volume of the desorption medium were 0.25 g and 5 mL, respectively. The MMP and NaCl concentrations in the desorption medium at pH 4.7 were 1.0 and 0.68*M*, respectively. The effects of the temperature on the variation of the desorption yield with the time are shown at different temperatures in Figure 10. The temperature was one of the most important variables controlling the desorption process. Nearly quantitative desorption with the highest rate was obtained at 50°C. This was probably due to the higher dissociation rate of the glycoprotein/Con A complex at higher temperatures. This has also been reported for several glycoproteins.[60] However, the thermal stability of the enzyme should be considered together with the yield of the desorption process. Because of the thermal deactivation, invertase lost 29% of its initial activity in the desorption medium at 50°C in 1 h. This value was considered in the desorption yield reported at this temperature. Another important point that should be taken into account is the thermal stability of Con A. It has been reported that Con A significantly loses its stability at temperatures higher than 50°C.[41] For all these reasons, although nearly quantitative desorption was observed at 50°C, this temperature should not be evaluated as an appropriate value for the desorption

TABLE I
Invertase Desorption Yields in the Desorption Media Prepared with Different Salt Concentrations

NaCl concentration (*M*)	Desorption yield (%)
0.00	78
0.34	82
0.68	85
1.36	87
2.00	88

Temperature = 20°C, MMP concentration = 1.00*M*, pH-4.7, period = 29 h.

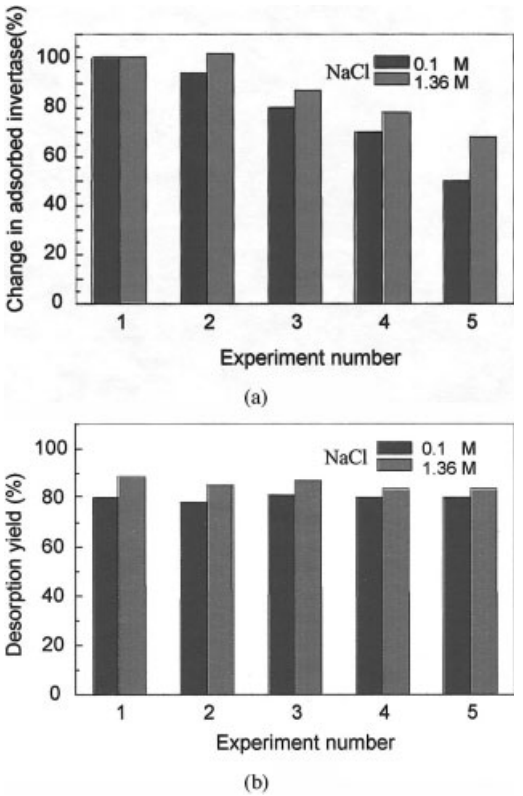

Figure 11 Changes in the invertase adsorption and desorption capacity of PCMS/Con A beads: (a) adsorption and (b) desorption.

of invertase from the Con A attached PCMS beads. However, a sufficiently high desorption yield (i.e., 83%) was obtained at 20°C within 5 h. No significant decrease in the enzymatic activity was also observed at that temperature during the desorption period. Then, 20°C seemed the most appropriate temperature at which the desorption process could be conducted with a satisfactory yield and without a significant decrease in the activity of the desorbed enzyme.

Iterated use of the PCMS/Con A beads

The stability of the PCMS/Con A beads was tested with the same beads in five successive adsorption–desorption experiments. In the adsorption part of these experiments, a 2 mg/mL crude invertase solution (20 mL) and 1 g of Con A attached PCMS particles were used. The Con A immobilized on the PCMS beads was crosslinked by GA. The adsorption was performed at 20°C at pH 7 for 3 h. The desorption was conducted with two different media prepared at different salt concentrations (i.e., 0.1 and 1.36*M*). The changes that occurred after five adsorption–desorption cycles in both the adsorption and desorption capacities of Con A attached PCMS particles are shown in Figure 11(a,b), respectively. As shown in Figure 11(a), the invertase adsorption exhibited a certain decrease in the successive use of the support. Here, the change in the adsorption capacity was defined as the ratio of the invertase adsorption capacity obtained in any experiment to that of the first one. However, the decrease in the adsorption capacity of the PCMS/Con A beads observed at a high salt concentration (1.36*M*) was lower than that observed at a low salt concentration (0.1*M*). Therefore, it can be concluded that the stability of Con A immobilized onto the PCMS beads was improved by the presence of salt in the desorption medium. After five adsorption–desorption experiments, the total decrease in the invertase adsorption capacity of the PCMS/Con A beads was found to be approximately 50 and 32% for the desorption media with 0.1 and 1.36*M* NaCl, respectively [Fig. 11(a)]. Con A was also immobilized onto a cellulose-based carrier, and this support was used for the affinity purification of invertase by Mislovicova et al.[44] They also tested the operational stability of the affinity system with iterated adsorption and desorption cycles. In their experiment, the successive use of the same particles resulted in a significant decrease in the binding capacity of the support. They found that 20% (w/w) of the initially loaded invertase could be adsorbed in the fourth iteration in a buffer containing 1.0*M* NaCl.[44] They concluded that there was noticeable deterioration in the binding properties of the carrier in the iterative use of the support. Josic et al.[61] also reported that the glycoprotein binding capacity of a carrier/Con A couple may decrease because of the unwanted loss of Con A (the dissociation of the Con A tetramer). The loss of Con A during elution, especially with MMP, accounted for serious complications.

The desorption was performed at 20°C with an MMP concentration of 1*M* at pH 4.7. As shown in Figure 11(b), no significant decrease was observed in the desorption yield with the iterative use of Con A attached PCMS beads. These behaviors and a comparison with the literature indicated that the crosslinking of Con A onto the bead surface resulted in a significant improvement in both the adsorption and desorption stability of the support.

CONCLUSIONS

In this study, an alternative support material was developed based on crosslinked PCMS beads produced by a suspension polymerization process. A commonly used lectin, Con A, was selected as the ligand and covalently attached onto the PCMS beads. Con A carrying PCMS beads were used in the purification of invertase as a model glycoenzyme. The developed support was also suitable for the isolation of other glycoenzymes with a pseudospecific interaction ability with the selected ligand (i.e., Con A). In the isolation of invertase, the counterligand, (i.e., MMP)

concentration and the temperature were determined to be the most important variables controlling the desorption process. However, the temperature should be assessed with respect to the thermal stabilities of both the selected enzyme and the ligand.

The structural integrity of four subunits of Con A was not stable in the acidic medium. By considering both the activity and stability, we found that the optimum pH of invertase (i.e., 4.7) was not suitable for the structural integrity of Con A. In some of the previous studies, it was reported that Con A lost its binding ability against the target glycoprotein in repeated adsorption–desorption cycles.[44,61] This behavior was explained by the removal of noncrosslinked Con A subunits during the desorption of adsorbed glycoprotein. In our study, Con A was crosslinked onto the PCMS beads after the covalent attachment stage. The results indicated that the crosslinking of Con A led to the successive use of the support without a significant decrease in the isolation yield of invertase at 20°C. Hence, the stability of Con A on the beads was probably improved and the removal of Con A subunits from the support material was prevented.

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