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ORIGINAL CONTRIBUTION

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Interactions between dextran-modified poly(methyl methacrylate) latex particles and concanavalin A

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Abstract The electrostatic and affinity interactions between concanavalin A (Con A) and dextran-modified latex particles were investigated. The ratio of the initial number of dextran active sites to that of Con A molecules plays an important role in determining the structure of flocs formed during adsorption and, thereby, has an influence on the amount of adsorbed Con A and the subsequent elution yield. NaCl and D-glucose, used successively in the two-step desorption of Con A, were employed to study the relative importance of electrostatic and affinity interactions involved in the Con A adsorption process. A significant fraction of adsorbed Con A is attributed to the electrostatic interaction mechanism. The feasibility of using the dextran-modified latex products to purify Con A from Jack bean meal was demonstrated.

Key words Affinity/electrostatic interactions · Dextran-modified latex particles · Concanavalin A (Con A) · Adsorption/desorption

Introduction

A latex product comprises numerous submicron polymer particles dispersed in water. These latex particles have an extremely large surface area and can be used to purify protein from a crude biological mixture [1]. In our previous work, stable dextran-modified poly(methyl methacrylate) latex particles were prepared and characterized [2]. These dextran-modified latex products have an affinity for carbohydrate-binding proteins (lectins). Lectins are carbohydrate-binding proteins other than enzyme or antibody. Lectin (e.g., concanavalin A (Con A) used as a model compound in this study) shows an equal or better specific affinity for carbohydrates (e.g., dextran) as compared to antibodies for antigens [3]. Con A has dimeric ($\mathrm{pH} = 2.0$–5.8) or tetrameric ($\mathrm{pH} > 5.8$) structure [4]. Con A may thus specifically bind to the surface dextran molecules which are chemically coupled onto different latex particles. This results in a crosslinked network structure and, thereby, induces selective precipitation of the resultant large flocs. This postulation has been confirmed by the studies of coagulation kinetics [5] and isothermal equilibrium adsorption [6]. It was shown that the colloidal stability is primarily controlled by charge neutralization of the negatively charged dextran-modified latex particles toward adsorption of positively charged Con A molecules. The ratio of the initial number of dextran active sites to that of Con A molecules plays an important role in determining the crosslinked network structure formed during adsorption. The electrostatic interaction between Con A and latex particles in the absence of dextran is much weaker than the affinity interaction between Con A and dextran-modified latex particles. An empirical model was proposed to describe qualitatively the isothermal equilibrium adsorption data.

These studies show that adsorption of Con A on the dextran-modified latex particles is a very complicated process, which is dependent on such parameters as the latex particle surface charge density, the net charges on the surface of Con A, the dextran content, the molecular structure of Con A, the ratio of the initial number of dextran active sites to that of Con A molecules, the ionic strength, etc. The objective of this project was therefore to use designed adsorption/desorption experiments to gain a better understanding of the affinity and electrostatic interactions between the latex particles with various levels of dextran modification and Con A. In addition, the feasibility of using these dextran-modified latex particles to purify Con A directly from crude Jack bean extract was demonstrated. The results obtained from this work should be useful for successful development of the dextran-modified colloidal particles for affinity purification of lectins.

Experimental

Materials

The chemicals include the dextran-modified poly(methyl methacrylate) (PMMA) latex products D20 and D75 and, for comparison, the D0 PMMA latex particles stabilized only by the surface SO_4^- groups prepared in this laboratory [2]; sodium chloride (Riedel-de Haen); calcium chloride (J. T. Baker); manganous chloride (J. T. Baker); sodium phosphate, monobasic monohydrate (J. T. Baker); concanavalin A (Type III, Sigma); Jack bean meal (Sigma); D-glucose (J. T. Baker); deionized water (Barnsted, Nanopure Ultrapure Water System, specific conductance < 0.057 μS/cm).

Some chemical and physical properties of these latex products taken from Chern et al. [2, 5] are summarized in Table 1. The parameters d_w and d_n are the weight-average and number-average particle diameters, respectively. The ratio d_w/d_n represents the polydispersity index of the particle size distribution. The dextran content of the emulsion polymers (C*(Dextran)) represents the wt% dextran based on total polymer weight (PMMA + grafted dextran). The parameters CCC(NaCl) and CFC(Con A) are the critical coagulation concentration of the particles at pH 5 toward added NaCl and the critical flocculation concentration of the particles toward added Con A in a pH 5 phosphate buffer solution comprising 0.15 mol/l NaCl, 0.1 mmol/l $CaCl_2$, and 0.1 mmol/l $MnCl_2$. The electrolytes NaCl, $CaCl_2$, and $MnCl_2$ were added to ensure the complete dissolution of Con A in water.

Adsorption and desorption of Con A

Before the adsorption experiment was carried out, the latex product was separated into the supernatant phase and precipitate phase by centrifugation at 11,000 rpm for 15 min (Beckman, J2–21). The clear supernatant was withdrawn, followed by redispersion of the precipitate in fresh deionized water using a mini ultrasonic cleaner (Delta DG-1). This procedure was repeated at least three times to remove the free dextran molecules in water. The particle size data show that the dextran-modified particles are very stable toward at least five cycles of centrifugation-redispersion [2]. A latex sample was prepared by using a pH 5 phosphate solution comprising 0.15 mol/l NaCl, 0.1 mmol/l $CaCl_2$, and 0.1 mmol/l $MnCl_2$. The latex sample with a volume of 3 ml was then mixed with an equal volume of the pH 5 phosphate buffer solution comprising 17.62 μmol/l Con A, 0.15 mol/l NaCl, 0.1 mmol/l $CaCl_2$, and 0.1 mmol/l $MnCl_2$ to initiate the precipitation of Con A. The reaction mixture was stirred overnight at 25 °C. After centrifugation at 13,000 rpm for 10 min, the clear supernatant was filtered through a 0.2-μm membrane and the concentration of Con A in the supernatant ([Con A]) was determined by UV absorbance at 280 nm (Shimadzu UV-160A). The calibration curve established by a series of standards is: absorbance $= 1.1331 \times 10^{-3}$ [Con A] (mg/l) + 7.6513×10^{-3}.

Table 1 Some chemical and physical properties of the dextran-modified latex products

Latex ID	D0	D20	D75
d_w (nm)[a]	207	175	181
d_w/d_n[a]	1.007	1.016	1.016
C*(Dextran) (%)	–	1.24	2.15
CCC(NaCl) (mol/l)[b]	0.175	0.305	0.880
CFC(Con A) (μmol/l)[c]	18.6	16.4	17.1

[a] Particle size data based on transmission electron microscope

[b] Critical coagulation concentration of NaCl at pH 5

[c] Critical flocculation concentration of Con A in a pH 5 phosphate solution comprising 0.15 mol/l NaCl, 0.1 mmol/l $CaCl_2$, and 0.1 mmol/l $MnCl_2$

The procedure used to induce desorption of Con A from the particle surface is briefly described as follows. After the above adsorption experiment is complete, the precipitate was redispersed using a mini ultrasonic cleaner (Delta DG-1) for 2 h in a pH 5 phosphate solution comprising 0.1 mol/l D-glucose, 0.15 mol/l NaCl, 0.1 mmol/l $CaCl_2$, and 0.1 mmol/l $MnCl_2$. The redispersed latex sample with a volume of 6 ml was then subject to centrifugation at 13,000 rpm for 10 min. The clear supernatant was filtered through a 0.2-μm membrane and the concentration of Con A in the supernatant ([Con A]) was determined by UV absorbance at 280 nm (Shimadzu UV-160A).

Purification of Con A from Jack bean meal

First, 3 g Jack bean meal was dissolved in 40 ml pH 5 phosphate solution comprising 0.15 mol/l NaCl, 0.1 mmol/l $CaCl_2$, and 0.1 mmol/l $MnCl_2$ and the solution was allowed to stand at room temperature overnight. After centrifugation at 12,000 rpm for 1 h, the clear supernatant was filtered through a 0.45-μm membrane. A latex sample was prepared by using a pH 5 phosphate solution comprising 0.15 mol/l NaCl, 0.1 mmol/l $CaCl_2$, and 0.1 mmol/l $MnCl_2$. The latex sample with a volume of 5 ml was then mixed with an equal volume of the Jack bean meal solution to initiate the precipitation of Con A. The reaction mixture was stirred for 1 h at 25 °C. After centrifugation at 13,000 rpm for 15 min, the precipitate was collected and then mixed with 10 ml pH 5 phosphate solution comprising 0.1 mol/l D-glucose, 0.15 mol/l NaCl, 0.1 mmol/l $CaCl_2$, and 0.1 mmol/l $MnCl_2$. The flocculated colloidal system was thoroughly mixed using a mini ultrasonic cleaner (Delta DG-1) for 2 h. After centrifugation at 13,000 rpm for 15 min, the clear supernatant was filtered through a 0.2-μm membrane, and the UV absorbance at 280 nm determined.

Results and discussion

A schematic model of three intervals involved in adsorption of Con A on the dextran-modified PMMA particles is shown in Fig. 1 [6]. This empirical model is briefly described below. In Interval I, the amount of Con A initially added to the dextran-modified particles is not sufficient to saturate the latex particle surface. This interval represents a scenario that the competition among these latex particles for Con A in the aqueous solution is very keen. As a result, the probability of specifically binding the dimeric Con A molecules to the

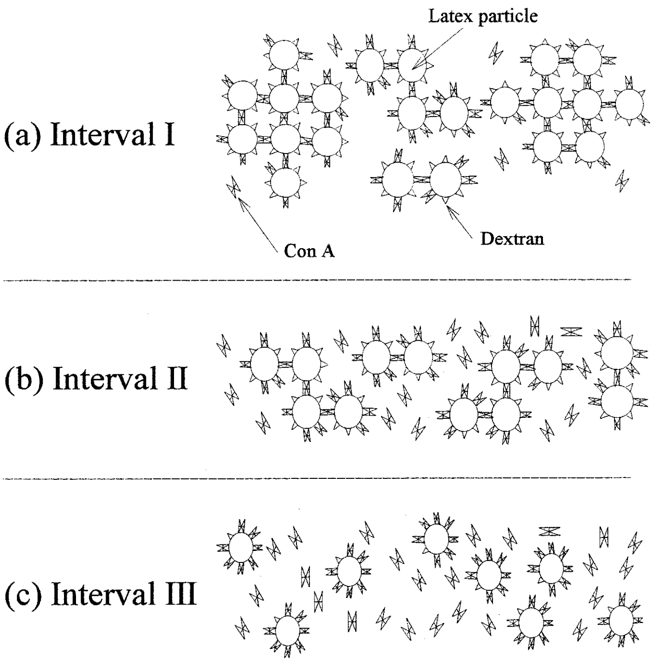

Fig. 1a–c A schematic model of three intervals involved in the adsorption of Con A on the dextran-modified particles: **a** Interval I; **b** Interval II; **c** Interval III. This empirical model is taken from Chern et al. [6]

surface dextran active sites coupled onto different particles increases significantly and an extensive crosslinked network structure forms during adsorption. In Interval II, the number of Con A molecules initially added to the adsorption system is comparable to the number of active binding sites on the particles. This interval is also characterized by formation of a crosslinked network structure, but the degree of crosslinking reaction is lower as compared to Interval I. This is simply because the competition among the particles for Con A in the aqueous solution becomes less severe. In Interval III, by contrast, the number of Con A molecules initially added to the adsorption system is much larger than the number of active binding sites on the particles. The initial Con A concentration in water is so high that the probability of specifically binding the dimeric Con A molecules to the surface dextran active sites grafted onto different particles is greatly reduced and, thus, formation of a crosslinked network structure is insignificant.

Figures 2 and 3 show the quantity of adsorbed Con A per gram PMMA particles (Q) and the percentage of Con A initially present in the colloidal system which is adsorbed on latex particles (P) as a function of the total solid content of D20 and D75, respectively. Note that the Con A concentration initially present in the system is kept constant in this series of experiments and, thus, variation in the total solid content of the colloidal system is equivalent to the study of the ratio of the initial

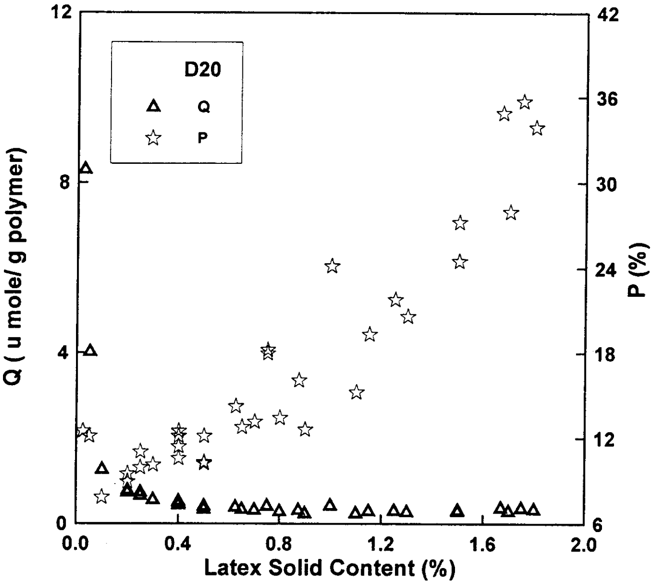

Fig. 2 Quantity of Con A per gram polymer particles (Q) and the percentage of Con A initially present in the system adsorbed on latex particles (P) as a function of the total solid content of D20: (△) Q; (☆) P

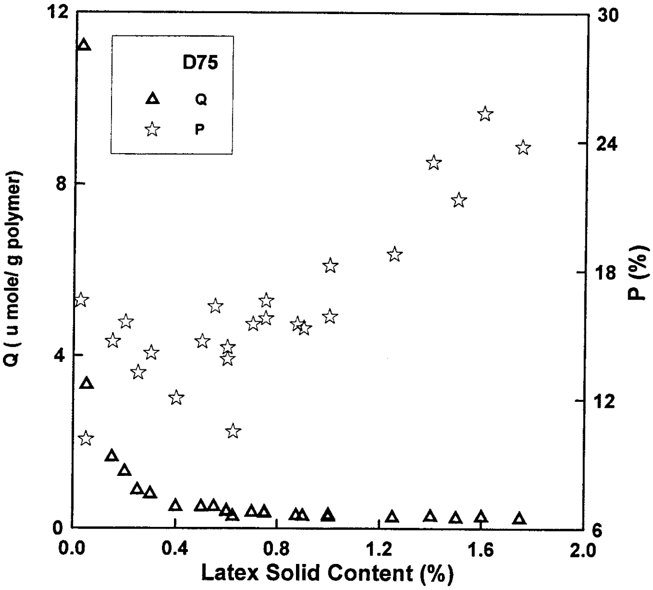

Fig. 3 Quantity of Con A per gram polymer particles (Q) and the percentage of Con A initially present in the system adsorbed on latex particles (P) as a function of the total solid content of D75: (△) Q; (☆) P

number of dextran active sites to that of Con A molecules. It is shown in Figs. 2 and 3 that Q decreases sharply and then levels off when total solid content increases. The rapidly decreased Q with total solid content is attributed to the transition from Interval III to Interval II and then from Interval II to Interval I. At very low total solid content, the number of Con A molecules initially added to the system is much larger than the number of active binding sites on the particles, thereby leading to a quite stable colloidal system (Interval III). This results in the condition that most of the active binding sites on the particles are available for adsorption of Con A (i.e., very large Q). Increasing the total solid content leads to the transition from Interval III to Interval II. In this case, a lightly crosslinked network structure forms as a result of the increased competition among the affinity particles for Con A in the aqueous solution. The number of effective binding sites on the particles available for adsorption of Con A may thus be greatly reduced due to the steric hindrance effect provided by the crosslinked network structure. This then causes a significant reduction in Q with total solid content. Upon further increasing the total solid content, the transition from Interval II to Interval I is achieved. Under the circumstances, the degree of crosslinking increases and, accordingly, Q continues to decrease with increasing total solid content. At still higher total solid content, the crosslinked network structure of flocs changes very little and, hence, the rate of change in Q with total solid content diminishes (Interval I). This result provides supporting evidence for the conclusion that the ratio of the initial number of dextran active sites to that of Con A molecules is the key parameter in controlling the adsorption behavior [6].

Although the adsorption data are rather scattered, Figs. 2 and 3 indicate that the percentage of Con A initially present in the colloidal system which is adsorbed on latex particles (P) increases gradually and then goes up more rapidly with increasing total solid content. At low total solid content, the small slope of the P vs total solid content curve is, again, attributed to the transition from a relatively stable colloid (Interval III) to the crosslinked network structure of flocs (Intervals II and I). This is because the number of binding sites on the particles available for adsorption of Con A increases with increasing total solid content. However, this effect is counterbalanced by the enhanced degree of crosslinking of flocs (i.e., the decreased number of effective binding sites on the particles) with total solid content. As a consequence, P only increases slowly when total solid content increases. At high total solid content, on the other hand, the crosslinked network structure does not change much and P increases at a faster rate with increasing total solid content. It is also interesting to note that the region of the slowly increased P with total solid content for the Con A/D20 system is narrower than that for the Con A/D75 system. This is simply because D20 has a lower dextran content and, therefore, the transition region from Interval III to Interval II and then from Interval II to Interval I is narrower as compared to the D75 counterpart.

According to Agrawal and Goldstein [7, 8], D-glucose was used to induce desorption of Con A out of the particle surface by competing with the surface dextran molecules for Con A. Figures 4 and 5 show the elution yield based on the adsorbed Con A (E) and recovery based on the fed Con A (R) as a function of the total solid content of D20 and D75, respectively. It is shown

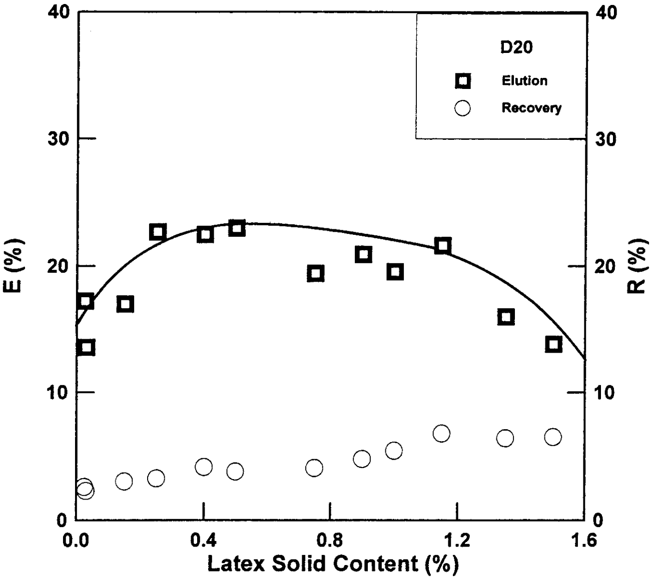

Fig. 4 Elution yield based on the adsorbed Con A (E) and recovery based on the source of Con A (R) as a function of total solid content of D20: (□) E; (○) R

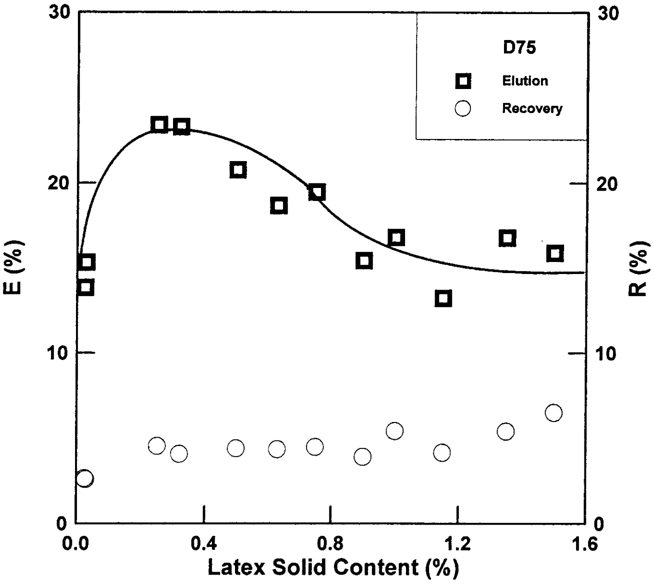

Fig. 5 Elution yield based on the adsorbed Con A (E) and recovery based on the source of Con A (R) as a function of total solid content of D75: (□) E; (○) R

that E first increases rapidly to a maximum and then decreases with increasing total solid content. At low total solid content, the adsorption system undergoes transition from a relatively stable colloid (Interval III) to the three-dimensional crosslinked network structure of flocs (Interval II) and, therefore, more Con A molecules are entrapped within the network structure during adsorption when total solid content increases. The steric hindrance effect provided by the network structure may also slow down the diffusion of D-glucose and make the entrapped Con A molecules not accessible to D-glucose. Another contributing factor is the reduced probability for the crosslinked Con A to desorb out of the particle surface because two D-glucose molecules are required to desorb one crosslinked Con A molecule. Thus, E is expected to decrease with increasing total solid content. The reason for the initial increase of E is not clear, but it is probably due to the very complicated affinity/electrostatic interactions between dextran-modified latex particles and Con A and/or the experimental error occurred when the amount of adsorbed Con A is rather limited at low total solid content upon further increasing total solid content (Interval II → Interval I), the crosslinked network structure continues to build up and R decreases with increasing total solid content. The difference between the E vs total solid content profiles of D20 and D75 reflects their different particle surface properties. The elution yield of Con A/dextran-modified latex particles is in the range of 12–24% and D-glucose is not as effective in inducing desorption of Con A out of the particle surface as might be expected, due to the greatly reduced diffusion of D-glucose molecules into the crosslinked network structure of flocs. Figures 4 and 5 indicate that R increases slowly with increasing total solid content and only less than 10% of Con A initially present in the adsorption system can be recovered. This is simply due to the following interactive effects: (i) the total particle surface area available for adsorption of Con A increases with total solid content, (ii) the crosslinked network structure of flocs becomes stronger when total solid content increases, and (iii) the competitive adsorption of Con A and D-glucose on the particle surface becomes diffusion-controlled when the crosslinked network structure of flocs forms. These experimental data imply that the colloidal stability of Con A/dextran-modified latex particles has a significant influence on the protein purification process. Further studies on the dimension and structure of flocs produced by adsorption of Con A on these dextran-modified latex particles are required to confirm the validity of using the empirical model (Fig. 1) to explain the experimental data presented in this work.

An optimum adsorption scheme for recovering the target protein from a crude biological mixture can be developed according to this empirical model. For example, if the recovery of the desired protein were the main concern, the affinity adsorption operation should be carried out in Interval I, where the amount of ligand on the latex particle surface is in much excess of the desired protein. In addition to the high protein adsorption yield, the relatively large floc size resulted from protein adsorption in Interval I will also facilitate the subsequent solid-liquid separation. On the other hand, if a higher ligand utilization efficiency were desired (in the case of very expensive ligand used), the operation should be performed in Interval III.

To gain a better insight into the mechanisms involved in the affinity/electrostatic interactions between dextran-modified latex particles and Con A, a series of elution experiments with various concentrations of NaCl ([NaCl] = 0.3–1.0 mol/l) were carried out. For comparison, the dextran-free PMMA particles (D0) were also included in this study. Increasing the ionic strength compresses the electric double layer of latex particles and then reduces the electrostatic force between Con A and latex particles [9]. This leads to desorption of Con A out of the particle surface. The resultant precipitate was then redispersed in pH 5 phosphate solution comprising 0.1 mol/l D-glucose, 0.15 mol/l NaCl, 0.1 mmol/l $CaCl_2$, and 0.1 mmol/l $MnCl_2$ by using a mini ultrasonic cleaner (Delta DG-1) for 2 h, and the elution yield attributed to affinity interaction was determined. The results are compiled in Table 2. The average total elution yield and average total recovery are defined, respectively, as the average elution yield and recovery achieved by using NaCl and then D-glucose as the elution agents for the series of experiments with [NaCl] = 0.3–1.0 mol/l. The average recovery attributed to affinity interaction then represents the average recovery achieved by using D-glucose as the elution agent for the series of experiments with [NaCl] = 0.3–1.0 mol/l. For example, for D20, the average total elution yield is [(23 + 27) + (23 + 27) +

Table 2 Effects of NaCl and D-glucose on the desorption of Con A out of the dextran-modified latex particles

Latex ID	D0	D20	D75
Adsorbed Con A (%)[a]	9.6	23.6	28.8
[NaCl] (mol/l)	E (%)[b]		
0.3	54 (25)	23 (27)	20 (26)
0.5	57 (25)	23 (27)	20 (27)
0.7	54 (23)	22 (26)	20 (25)
1.0	54 (24)	22 (27)	20 (25)
Average total elution yield (%)	79	49	46
Average total recovery (%)	7.6	11.6	13.3
Average recovery attributed to affinity interaction (%)	–	6.4	7.3

[a] Based on Con A initially added to the adsorption system

[b] Numerical value in the parenthesis represents the elution yield obtained from subsequent desorption of Con A with 0.1 mol/l D-glucose as the elution reagent

(22 + 26) + (22 + 27)]/4 = 49% and the average recovery attributed to affinity interaction is (27 + 27 + 26 + 27)/[(23 + 27) + (23 + 27) + (22 + 26) + (22 + 27)] × 11.6% = 6.4% (Table 2). The percentage of adsorbed Con A in the increasing order is D0 < D20 < D75, which is consistent with our previous work [6]. The elution yield of Con A seems insensitive to changes in [NaCl] (0.3–1.0 mol/l) for all the latexes (D0, D20, and D75) investigated. Thus, a significant fraction of adsorbed Con A via affinity interaction remains on the dextran-modified particle surface. The average total elution yield for D0 (79%, primarily electrostatic interaction) is much larger than that for D20 or D75 (~50%, electrostatic interaction + affinity interaction). This indicates that the bonding strength of the Con A-D0 pair is much weaker as compared to the Con A-D20 or Con A-D75 pair. The average total recovery of Con A in the increasing order is D0 (7.6%) < D20 (11.6%) < D75 (13.3%). As expected, the PMMA particles with the largest dextran content (D75) is the best candidate for the selective precipitation of Con A. In addition, the average total recovery attributed to affinity interaction is about 7% for the dextran-modified latex particles (D20 and D75). These data suggest that the fractions of adsorbed Con A caused by affinity interaction and charge neutralization for D20 and D75 are about 55% ((6.4 + 7.3)/(11.6 + 13.3) × 100%) and 45%, respectively.

Finally, for demonstration, the dextran-modified latex particles, D20 and D75, were used to purify Con A from Jack bean meal. The concentration of Con A eluted from the latex particle surface was determined by UV absorbance at 280 nm. Table 3 shows that, at constant total solid content, the UV absorbance at 280 nm for D75 is larger than that for D20. That is, the larger the dextran content, the larger is the amount of Con A recovered from Jack bean meal. As expected, the amount of recovered Con A increases significantly with increasing total solid content. Figure 6 shows the SDS-polyacrylamide gel electrophoresis patterns of the Con A obtained from the selective precipitation of Con A from Jack bean meal by the dextran-modified latex products (D20 and D75). Only using one purification step (affinity precipitation), the purity of Con A recovered from Jack bean meal is comparable to that obtained from Sigma Co. The feasibility of using these dextran-modified latex particles to purify Con A from Jack bean meal is thus verified. Since the process for purification of Con A from Jack bean meal by affinity precipitation with these dextran-modified latex products has not been optimized, a satisfactory Con A recovery yield cannot be expected. If the purification process were optimized, for example, using low pH (pH < 2) [8] instead of D-glucose to elute the adsorbed Con A molecules from the dextran-modified latex particle surface will improve the recovery yield significantly.

Table 3 Concentration of Con A eluted from the dextran-modified latex particle surface after the selective precipitation of Con A from Jack Bean meal extract

Latex ID	D20	D20	D75	D75
Total solid content (%)	0.25	1.0	0.25	1.0
Con A concentration (OD 280 nm)	0.044	0.138	0.048	0.161

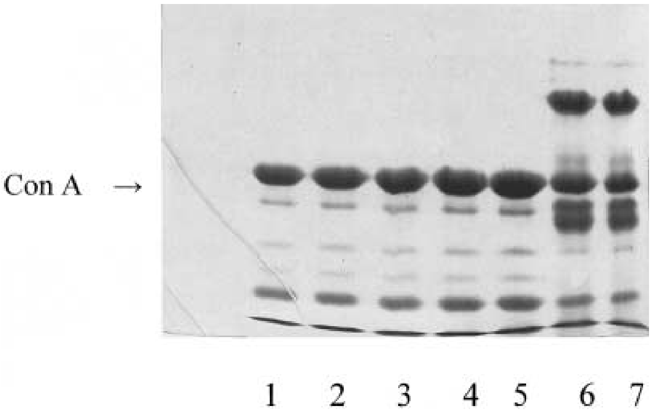

Fig. 6 SDS-polyacrylamide gel electrophoresis patterns of Con A purified from Jack bean meal by affinity precipitation. Lanes No.: (1) Con A obtained from Sigma Co.; (2) and (3) recovered from D20 precipitate; (4) and (5) recovered from D75 precipitate; (6) and (7) crude Jack bean meal extract

Conclusion

The dextran-modified latex particles show specific affinity for concanavalin A (Con A). The ratio of the initial number of dextran active sites to that of Con A molecules plays an important role in determining the structure of flocs formed during adsorption. This may thus have an influence on the amount of adsorbed Con A and the subsequent elution yield. A three-interval model proposed by Chern et al. [6] was used to explain the elution yield as a function of total solid content data. At very low total solid content, the colloidal system is quite stable (Interval III), thereby leading to the condition that most of the active binding sites on the particles are available for adsorption of Con A (i.e., very large amount of adsorbed Con A per gram PMMA particles (Q)). Increasing the total solid content, a lightly crosslinked network structure forms and this leads to the transition from Interval III to Interval II. The number of effective binding sites on the particles available for adsorption of Con A may thus be greatly reduced (i.e., a significant reduction in Q with total solid content). Upon further increasing the total solid content, the transition from Interval II to Interval I is achieved. The degree of crosslinking increases and, accordingly, Q continues to decrease with increasing total solid content. At still higher total solid content, the crosslinked network structure of flocs changes very little and, hence, the rate of change in Q with total solid content diminishes (Interval I). The elution yield based on the adsorbed Con A first increases rapidly to a maximum and then decreases with increasing total solid content. By contrast, the recovery based on the amount of fed Con A increases slowly with increasing total solid content and only less than 10% of Con A initially present in the adsorption system can be recovered.

NaCl and D-glucose, used successively in the two-step desorption of Con A out of the particle surface, were employed to study the mechanisms involved in the Con A adsorption process. The average total recovery of Con A (7.6–13.3%) increases with increasing dextran content of latex particles. In addition, the average total recovery attributed to affinity interaction is about 7% for the dextran-modified latex particles. This study indicates that the fraction of adsorbed Con A attributed to electrostatic interaction and affinity interaction is ca. 45% and 55%, respectively, for the dextran-modified latex particles. The feasibility of using the dextran-modified latex products to purify Con A from Jack bean meal was demonstrated.

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