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Isothermal equilibrium adsorption of concanavalin A on dextran-modified poly(methyl methacrylate) latex particles

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Abstract Isothermal equilibrium adsorption experiments were carried out to study the adsorption of concanavalin A (Con A) on dextran-modified poly(methyl methacrylate) (PMMA) latex particles. Three PMMA particles with various levels of dextran modification were selected for study: 0% (designated as D0), 1.24% (D20), and 2.45% (D75) based on total polymer weight. The Langmuir model is applicable to both D0 and D20 systems, although the data for the D20 system are somewhat scattered. On the other hand, the amount of Con A adsorbed per gram polymer particles (q^*) versus the Con A concentration in water (c^*) curve for the D75 system cannot be described by the Langmuir model. The deviation is attributed to the formation of a crosslinked network structure, caused by specific binding of the dimeric Con A molecules onto two neighboring particles with grafted dextran. The ratio of the initial number of Con A molecules to the initial number of active binding sites on the dextran-modified particle surface plays an important role in determining the structure of flocs formed. The maximum amount of Con A adsorbed on the particle surface (q_{max}) is of the order of 10^{-1} μmol per gram particles and q_{max} in decreasing order is D75 > D20 > D0. The dissociation constant of the Con A-D20 (or Con A-D75) pair is of the order of 10^{-1} μmol dm^{-3} which is 1 order of magnitude smaller than that of the Con A-D0 pair. Thus, the electrostatic interaction between Con A and D0 is much weaker than the affinity interaction between Con A and D20 (or D75). An empirical model is proposed to qualitatively explain the q^* versus c^* data.

Key words Isothermal equilibrium adsorption – Concanavalin A – Dextran-modified poly(methyl methacrylate) latex particles – Electrostatic/affinity interactions

Introduction

Various submicron latex particles have been used to isolate and recover proteins from crude biological mixtures due to their extremely large surface areas available for electrostatic interaction or specific ligand–protein interaction [1–13]. Generally, this purification process involves binding of the target protein onto the latex particle surface, sedimentation of the particles, removal of the supernatant, and dissociation and recovery of the protein product from the sediment. It may overcome some limitations associated with conventional affinity chromatography such as slow binding due to diffusion-controlled mass transfer, low available capacity due to steric hindrance in the association step, limited flow rates due to clogging, inefficient contact

due to channeling, and scale-up problems due to high pressure drop [14].

In a previous report [15], dextran-modified poly(methyl methacrylate) (PMMA) latex particles were synthesized and characterized. According to the color development with the phenol–sulfuric acid reaction [16], a significant fraction of dextran was shown to be chemically incorporated onto the latex particle surface, presumably via the grafting reaction. These dextran-modified particles may thus find potential application in affinity purification of carbohydrate-binding proteins (lectins). This is because lectins [e.g., concanavalin A (Con A) used as a model compound in this work] show an equal or better specific affinity for carbohydrates (e.g., dextran) in comparison with antibodies for antigens [17]. According to Edelman et al. [18], Con A has a dimeric structure (pH = 2.0–5.8) or a tetrameric structure (pH > 5.8). Consequently, these multimeric Con A molecules can specifically bind to the surface dextran active sites which are grafted onto different particles. This will result in a crosslinked network structure. Furthermore, the isoelectric point of Con A is 7.1 [19] and, hence, the net charge of Con A in a pH 5 phosphate buffer solution should be positive. Adsorption of the positively charged Con A onto the negatively charged dextran-modified particles is expected to occur due to electrostatic interactions. Both factors will contribute to destabilization of the dextran-modified particles toward added Con A and, thereby, induce the selective precipitation of the resultant large flocs. Recently, experiments of coagulation kinetics were carried out to study the colloidal stability of these dextran-modified particles toward adsorption of Con A [20]. It was shown that charge neutralization of the negatively charged particles by adsorption of the positively charged Con A is the predominant destabilization mechanism. The ratio of the number of dextran active sites to the number of Con A molecules plays an important role in the formation of the crosslinked network structure. In water the electrolytes cause a reduction in the electrostatic repulsion force among the particles, but this ionic-strength effect is not significant compared to charge neutralization. The objective of this work was to conduct isothermal equilibrium adsorption experiments [21] to gain a better understanding of the mechanisms involved in the adsorption of Con A on PMMA particles with different degrees of dextran modification.

Experimental

The dextran-modified PMMA latex products D20 and D75 employed in Ref. [15] were selected for study. For comparison, the latex product D0 exhibiting a comparable particle size and being stabilized only by the surface SO_4^- groups originating from the persulfate initiator, was also included in this work. The recipe for preparing D0 is exactly the same as that for D20 or D75 except that no dextran is incorporated into the D0 particles [15]. Other reagents used in this work include sodium chloride (Riedel-de Haen), calcium chloride (J. T. Baker), manganous chloride (J. T. Baker), sodium phosphate (J. T. Baker), monobasic monohydrate (J. T. Baker), Con A (type III, Sigma), and deionized water (Barnsted, Nanopure Ultrapure Water System, specific conductance < 0.057 μS/cm).

Some chemical and physical properties of these PMMA latex products taken from Refs. [15, 20] are summarized in Table 1. The parameters d_w and d_n are the weight-average and number-average particle diameters, respectively. The ration d_w/d_n represents the polydispersity index of the particle size distribution. The dextran content of the emulsion polymers [c*(dextran)] represents the weight percentage of dextran based on total polymer weight (PMMA + grafted dextran). The critical coagulation concentration (ccc) of the particles at pH 5 toward added NaCl and the critical flocculation concentration (cfc) of the particles toward added Con A in a pH 5 phosphate buffer solution comprising 0.15 M NaCl, 0.1 mM $CaCl_2$, and 0.1 mM $MnCl_2$ are represented by ccc (NaCl) and cfc (Con A), respectively. The electrolytes NaCl, $CaCl_2$, and $MnCl_2$ were added to ensure the complete dissolution of Con A in water.

Before the isothermal equilibrium adsorption experiments were carried out, the latex product was separated into the supernatant phase and the precipitate phase by centrifugation at 11000 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 3 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 [15]. A latex sample was prepared by using a pH 5 phosphate solution comprising 0.15 M NaCl, 0.1 mM $CaCl_2$, and 0.1 mM $MnCl_2$. The latex sample with a volume of 3 ml was then mixed with an equal volume of the pH 5 phosphate buffer solution comprising a prescribed amount of Con A (2.96–54.40 μM), 0.15 M NaCl, 0.1 mM $CaCl_2$, and 0.1 mM $MnCl_2$ to initiate the precipitation of Con A. The reaction mixture with a particle solid content of 1.5% was stirred overnight at 25 °C. After centrifugation at 13000 rpm for 10 min, the clear supernatant was filtered through a 0.2 μm membrane and the concentration of Con A in the supernatant 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}.

Results and discussion

Table 1 shows that latices D20 and D75 have similar particle sizes (d_w) and particle size distributions (d_w/d_n).

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

	d_w[a] (nm)	d_w/d_n[a]	c*(dextran) (%)	ccc(NaCl)[b] (M)	cfc(Con A)[c] (μM)
D0	207	1.007	0.00	0.175	18.6
D20	175	1.016	1.24	0.305	16.4
D75	181	1.016	2.15	0.880	17.1

[a] Particle size data based on transmission electron microscopy
[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 M NaCl, 0.1 mM $CaCl_2$, and 0.1 mM $MnCl_2$

Thus, the density of dextran grafted on the D75 particle surfaces should be greater than that on the D20 particle surfaces since the value of c^*(dextran) for D75 is about twice as large as that for D20 [see the c^*(dextran) data in Table 1]. The ccc(NaCl) data in Table 1 indicate that the colloidal stability of the latex products toward added NaCl decreases in the order D75 > D20 > D0. This is due to the fact that the D0 particles are stabilized only by the electrostatic repulsion force, whereas both the D20 and the D75 particles are protected more effectively by the synergetic electrostatic and steric stabilization mechanisms. The cfc(Con A) data are relatively insensitive to changes in c^*(dextran) (see Table 1). Nevertheless, Con A is much more effective in destabilizing these latex products than NaCl because the values of cfc(Con A) are about 4 orders of magnitude smaller than those of ccc(NaCl). Furthermore, a crosslinked network structure may form when the dimeric Con A molecules specifically bind to two neighboring particles [20]. Binding of Con A onto the tightly crosslinked particles may thus become diffusion-controlled and sterically hindered. This may result in a significant decrease in the binding capacity of the polymeric support. The isothermal equilibrium adsorption data presented later may provide useful information on the mechanism involved and the structure of flocs produced in the adsorption of Con A on the dextran-modified particles.

The Langmuir isotherm model [21] has been widely used to describe the adsorption of protein (e.g., Con A) on the particle surface when the system is at equilibrium:

$$q^* = q_{\max} c^* / (K_d + c^*) \ , \qquad (1)$$

where q^* is the amount of Con A adsorbed per gram polymer particles, $q_{\max}$ is the maximum amount of Con A that can be adsorbed on the particle surface, c^* is the Con A concentration in aqueous solution, K_d is the dissociation constant for the Con A–binding site pair. Rearrangement of Eq. (1) leads to the Scatchard (or Eadie-Hofstee) equation [22, 23]:

$$q^* = q_{\max} - K_d q^* / c^* \ . \qquad (2)$$

Thus, K_d and $q_{\max}$ can be obtained from the slope and intercept, respectively, of the q^* versus q^*/c^* curve.

The Langmuir isotherm curves and the Scatchard plots for Con A adsorbed on the D0, D20, and D75 latex particles are shown in Figs. 1–3. The values of K_d and $q_{\max}$ estimated from the Scatchard plots are listed in Table 2. Also included in this table is the maximum number of active binding sites per particle (n) which can be directly obtained from $q_{\max}$. It is shown in Fig. 1 that the Langmuir model adequately describes the adsorption of Con A on the D0 particles without grafted dextran, as shown by the coefficient of determination ($r^2 = 0.9802$) in Table 2. This result implies that flocculation among the D0 particles by charge neutralization does not block the access of Con A species to the particle surface. It is therefore postulated that the flocs produced during the adsorption process may exhibit a relatively loose structure. This speculation is further supported by the observation that the Con A/D0 system did not show apparent evidence of sedimentation during adsorption. A precipitate phase was not observed until the sample was allowed to stand at room temperature for some tens of minutes after the end of the isothermal equilibrium adsorption experiment. The sedimentation rate of the flocs was quite slow. The Langmuir model can still be used to predict the adsorption behavior for the Con A/D20 system, but the data are somewhat scattered (see Fig. 2). For the Con A/D75 system, the Langmuir model seems to fail to describe the q^* versus c^* data when c^* is above 1 μmol/l, as shown in Fig. 3. This observation is reflected in the coefficient of determination ($r^2 = 0.5735$) involved in computing $q_{\max}$ or K_d (see Table 2). The larger the deviation of the experimental data from the model prediction, the smaller the value of r^2. When the Langmuir model is applied to the dextran-modified particles, especially

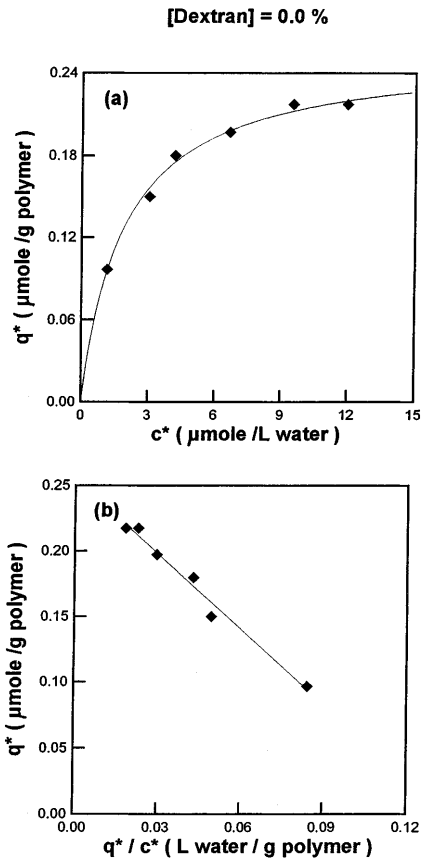

Fig. 1 **a** Langmuir isotherm curve and **b** Scatchard plot for concanavalin (*Con A*) adsorbed on the D0 latex particle surface

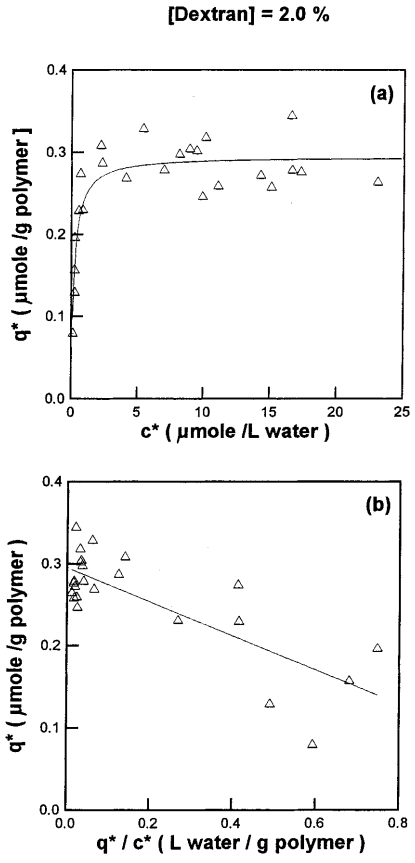

Fig. 2 **a** Langmuir isotherm curve and **b** Scatchard plot for Con A adsorbed on the D20 latex particle surface

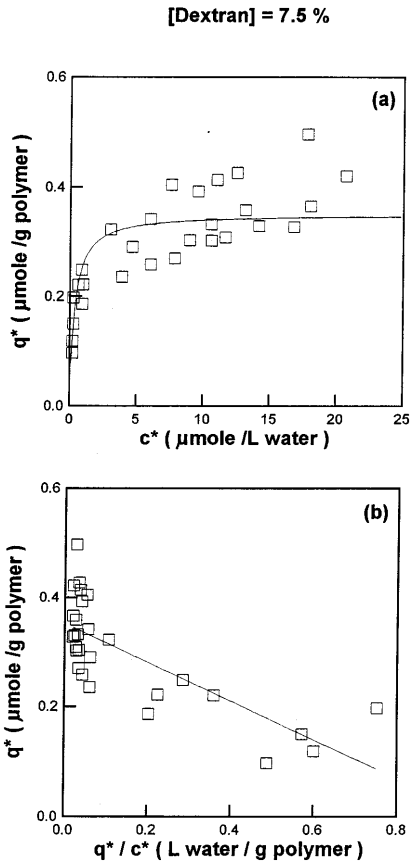

Fig. 3 **a** Langmuir isotherm curve and **b** Scatchard plot for Con A adsorbed on the D75 latex particle surface

D75, the poor performance is attributed to the formation of a crosslinked network structure during the adsorption of Con A on the dextran-modified particles. This speculation is further supported by the observation that the sedimentation rate of the particles with adsorbed Con A species upon standing at room temperature decreases in the order D75 > D20 ≫D0. This subject will be further discussed later.

Table 2 shows that the value of q_{max} for the Con A/D0 system is comparable to that for the Con A/D20 (or Con A/D75) system. Furthermore, the binding constant K ($= 1/K_d$) for the Con A/D0 system is 5.252×10^{-1} dm^3 μmol^{-1} which is about 1 order of magnitude smaller than that for the Con A/D20 or (Con A/D75) system ($K = 2.854$ and 4.864 dm^3 μmol^{-1} for the Con A-D20 pair and Con A-D75 pair, respectively). This result indicates that the electrostatic interaction between Con A and D0 is much weaker than the affinity interaction between Con A and D20 (or D75). The physically adsorbed Con A molecules can be eluted by washing the precipitated particles with a solution exhibiting high ionic strength. On the other hand, the specific Con A-dextran bond is expected to remain stable even in such a hostile environment. This makes the affinity purification of Con A from a crude biological mixture using the dextran-modified particles feasible. To the best of the authors' knowledge, the dissociation constant (K_d) of the Con A-dextran pair is not available in the literature; however, the value of K_d for the Con A-glucose pair is 1×10^3 μmol dm^{-3} [24]. This value of K_d is much larger than those obtained from the adsorption of Con A on the dextran-modified PMMA particles (see Table 2). This result indicates that the affinity interaction between Con A and the surface dextran is much stronger than for the Con A-glucose pair. This is because Con A is

Table 2 Langmuir parameters for adsorption of concanavalin A on the poly(methyl methacrylate) latex particles with various levels of dextran modification

	D0	D20	D75
q_{max} (μmol/g)	2.550×10^{-1}	2.954×10^{-1}	3.517×10^{-1}
n	358	499	657
K_d μmol dm^{-3}	1.904×10^{0}	2.056×10^{-1}	3.504×10^{-1}
$r^{2\,a}$	0.9802	0.6179	0.5735

[a] Coefficient of determination

divalent and dextran (a polymeric form of glucose) is polyvalent, whereas glucose is only monovalent. Adsorption of Con A on the dextran-modified PMMA particles is expected to result in a much larger binding constant K (i.e., much smaller K_d), as reported by Rao et al. [25] who obtained a value of K_d for a trivalent binding system 10 orders of magnitude smaller than for a monovalent binding system. It is also interesting to note that q_{max} (or n) increases with increasing c^*(dextran), as shown in Tables 1 and 2. This trend cannot be explained by variation in the total particle surface area per gram particles (a^*) available for adsorption. This is because d_w(D0) > d_w(D75) > d_w(D20) and, therefore, a^*(D0) < a^*(D75) < a^*(D20), but q_{max} (D0) < q_{max} (D20) < q_{max} (D75). It is then postulated that both the D20 and D75 particles are covered by a hydrophilic layer of grafted dextran and these dextran molecules exhibit a conformation comprising loops, trains, and tails on the particle surface [26]. These hairy particles may thus provide an abundant supply of specific binding sites for Con A.

The following presents an empirical model to qualitatively explain the isothermal equilibrium adsorption data described earlier. The percentage of Con A adsorbed on the latex particles (P_{ads}) versus c^* is shown in Fig. 4. A general feature is that P_{ads} first decreases rapidly and then levels off when c^* increases. Although the data are somewhat scattered, at constant c^*, P_{ads} increases with increasing c*(dextran). This trend is consistent with the q_{max} (or n) data listed in Table 2. The parameter P_{ads} was empirically set at 80% and then 30% to divide the series of isothermal equilibrium adsorption experiments into three intervals (see Fig. 4);

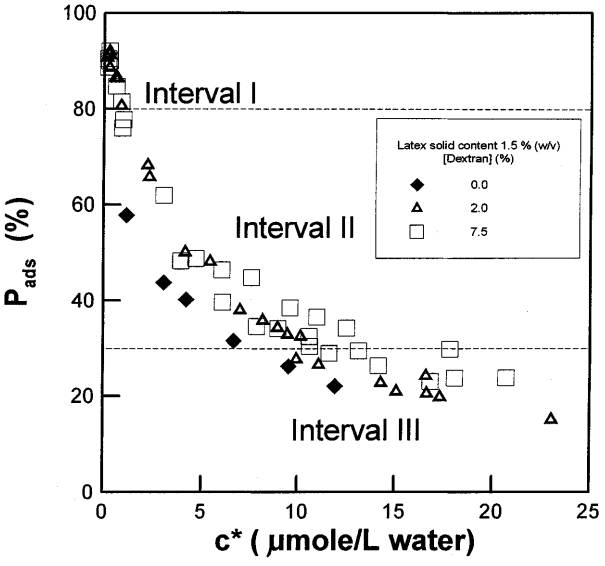

Fig. 4 Percentage of Con A adsorbed on the particle surface versus Con A concentration (c^*) profiles: D0 (◆), D20 (Δ), D75 (□)

that is, P_{ads} decreases rapidly with increasing c^* and, moreover, dP_{ads}/dc^* remains relatively constant in interval I (80% < P_{ads} < 100%). Subsequently, P_{ads} continues to decrease with increasing c^*, but in this case a continuous reduction in dP_{ads}/dc^* is observed (30% < P_{ads} < 80%, designated as interval II). In interval III (P_{ads} < 30%), P_{ads} starts to level off as c^* is further increased. The value of c^* corresponding to P_{ads} = 80% (or 30%) was then substituted into Eq. (2) along with the least-squares best-fitted apparent q_{max} and K_d to calculate q^* and the initial Con A concentration ($c = wq^* + c^*$), in which w is the total weight of polymer particles per liter water. The ratio of the initial number of Con A molecules to the initial number of active binding sites on the dextran-modified particle surface (r) can be calculated according to the relationship $r = 10^{-6} cN_A/[wn/(\pi d_w^3 \rho/6)]$, in which ρ is the density of polymer particles and N_A is Avogadro's number. The values of c^* obtained from Fig. 4 when P_{ads} = 80% and 30% and the corresponding values of r, which define the three intervals of the adsorption process, are compiled in Table 3. The schematic models of the three intervals involved in a series of isothermal equilibrium adsorption experiments are shown in Fig. 5.

In interval I, q^* increases rapidly with increasing c^* due to the fact that the amount of Con A initially added to the dextran-modified particles is not sufficient to saturate the particle surface (see the q^* versus c* data in the range c^* < 1 in Figs. 2a, 3a). As shown in Table 3 and Fig. 5a, this interval represents a scenario where the number of Con A molecules initially aded to the reaction system is relatively small in comparison with the number of active binding sites on the particles; that is, c^* < 1 and r < 1.071 for D20 or c^* < 1 and r < 1.187 for D75. Under these circumstances, competition among the particles for the Con A species in aqueous solution is very keen. As a result, the probability of specifically binding the dimeric Con A molecules to the surface dextran active sites coupled onto different particles increases significantly and an extensive crosslinked network structure will form during adsorption. In interval II, the rate of change in q^* with c* decreases significantly (see Figs. 2a, 3a). The adsorption system follows the behavior of interval II when 1 < c^* < 10 and 1.071 < r < 3.235 for D20 or 1 < c^* < 15 and 1.187 < r < 3.618 for D75; that is,

Table 3 Values of Con A concentration (c^*) obtained from Fig. 4 when P_{ads} = 80% and 30% and the corresponding values of r which define the three intervals of the adsorption process

	Interval I	Interval II	Interval III
D20	c^* < 1	1 < c^* < 10	c^* > 10
	r < 1.071	1.071 < r < 3.235	r > 3.235
D75	c^* < 1	1 < c^* < 15	c^* > 15
	r < 1.187	1.187 < r < 3.618	r > 3.618

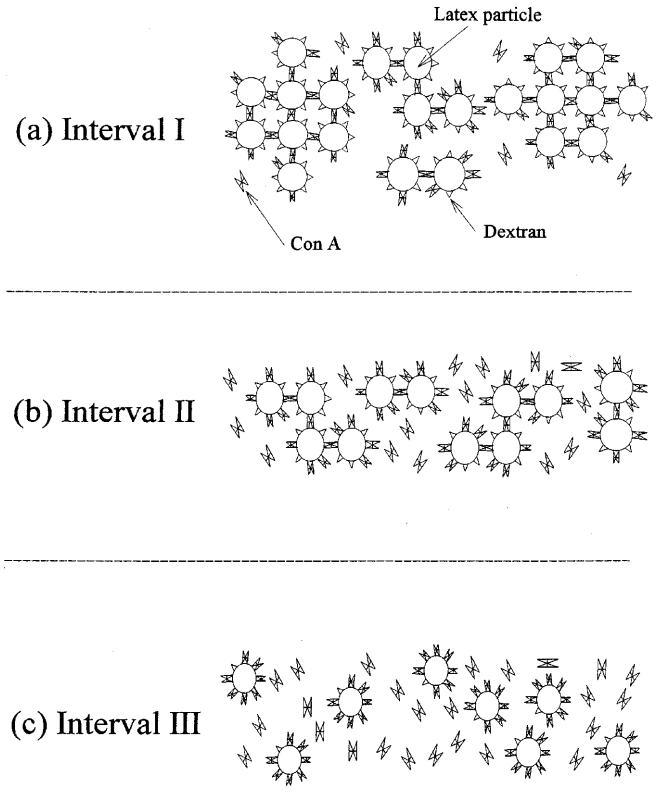

Fig. 5a–c A schematic model of the three intervals involved in the adsorption of Con A on the dextran-modified particles. **a** interval I, **b** interval II, and **c** interval III

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 the formation of a crosslinked network structure, but the degree of crosslinking is lower compared to interval I (see Table 3 and Fig. 5b). This is simply because competition among the particles for the Con A species in aqueous solution becomes less severe. When $c^* > 10$ and $r > 3.235$ for D20 or $c^* > 15$ and $r > 3.618$ for D75, the adsorption system can be placed in the category of interval III shown in Table 3 and Fig. 5c. In this case, 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. Thus, formation of a crosslinked network structure is insignificant.

It is also interesting to note that interval II for the Con A/D20 system is narrower than that for the Con A/D75 system because D20 has a lower dextran content. For example, the total number of active binding sites on the particles per liter water is 2.67×10^{18} and 3.17×10^{18} for D20 and D75, respectively. The range of the initial number of Con A molecules present in the reaction medium corresponding to interval II (i.e., $1.071 < r < 3.235$ for D20 or $1.187 < r < 3.618$ for D75) is then 2.86×10^{18}–8.64×10^{18} for D20 or 3.76×10^{18}–1.15×10^{19} for D75. This observation suggests that formation of a crosslinked network structure causes the significant deviation of the adsorption data from the Langmuir model for the D75 series of isothermal equilibrium adsorption experiments (see the q^* versus c^* data in the range $c^* < 15$ in Fig. 3a). On the other hand, the Langmuir model is capable of predicting the shape of the q^* versus c^* curve for the D20 series with better result (see Fig. 2a).

Based on the qualitative three-interval model proposed for describing the adsorption isotherm of these dextran-modified particles toward Con A, one can design an optimum adsorption scheme for recovering the target protein from a crude biological mixture. For example, if the desired protein adsorption yield were the main concern, the affinity adsorption operation should be carried out in the low-r region (interval I). In other words, a higher level of the dextran-modified polymeric support is required. On the other hand, the affinity adsorption operation performed in the high-r region (interval III) will result in an increase in the ligand utilization efficiency. Development of a mechanistic model which takes into account the diffusion-controlled and sterically hindered adsorption of Con A onto the dextran-modified particles is in progress in order to describe the isothermal equilibrium adsorption phenomenon in a quantitative fashion.

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