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Displacement Chromatography of Proteins using Low Molecular Weight Anionic Displacers

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Abstract. A major impediment to the implementation of displacement chromatography has been the lack of suitable displacer compounds. Recently, it has been shown that low molecular weight dendritic polymers, protected amino acids and antibiotics can be successfully employed for displacement purification in cation-exchange systems. In this paper, a variety of low molecular weight anionic displacers are identified for the resolution of a bovine β-lactoglobulin mixture into two closely related forms (A and B). A Dynamic Affinity plot is employed to evaluate the affinity of these low molecular weight compounds under various displacement conditions. In contrast to large polyelectrolyte displacers, the efficacy of these low molecular weight displacers are shown to be dependent on displacer concentration. In fact, the Dynamic Affinity Plot qualitatively predicts the transition from a displacement to a desorption regime with these low molecular weight displacers. In addition to the fundamental interest generated by low molecular weight displacers, it is likely that these displacers will have significant operational advantages as compared to large polyelectrolyte displacers. Furthermore, the ability to carry out selective displacement chromatography with these low molecular weight displacers offers significant potential for developing robust large scale displacement processes.

Keywords:

Introduction

Ion-exchange chromatography is widely employed in the downstream processing of biopharmaceuticals. The modus operandi in preparative chromatography for commercial applications has primarily been continuous and step gradient modes of operation (Regnier, 1983; Cramer and Subramaniam, 1989). The major aversion towards the implementation of displacement chromatography for commercial applications has been the rather limited choice of displacer compounds.

Operationally displacement chromatography is performed in a manner similar to step-gradient chromatography, where the column is subjected to sequential step changes in the inlet conditions. The column is initially equilibrated with a relatively low ionic strength carrier buffer. The feed mixture is then introduced into the column followed by a constant infusion of the displacer solution. The displacer is selected such that it has a higher affinity for the stationary phase than any of the feed components. Under appropriate conditions, the displacer induces the feed components to develop into adjacent "square-wave" zones of highly concentrated pure material. After the breakthrough of the displacer from the column effluent, the column is regenerated and re-equilibrated with the carrier buffer.

The displacer, having a higher affinity than any of the feed components, competes effectively, under nonlinear conditions, for the adsorption sites on the stationary phase. An important distinction between displacement and step gradient chromatography is that the displacer front always remains behind the adjacent feed zones in the displacement train, while desorbents (e.g., salt, organic modifiers) move through the feed zones. The implications of this distinction are quite significant in that displacement chromatography can potentially concentrate and purify components from mixtures having low separation factors while step gradient often requires relatively large separation factors to give satisfactory resolution.

Ion-exchange displacement chromatography of proteins using large polyelectrolyte displacers has been studied by several investigators. Peterson and co-workers (Peterson, 1978; Torres et al., 1987; Torres and Peterson, 1992) have used carboxymethyl dextrans as displacers in anion-exchange displacement chromatography. Horvath and co-workers have employed chondroitin sulfate to displace β-galactosidase (Liao and Horvath, 1990) and β-lactoglobulins (Liao et al., 1987; Lee et al., 1988). Ghose and Mattiason (1991) have examined the purification of lactate dehydrogenase using a carboxymethyl-starch displacer. Jen and Pinto have employed polyvinyl sulfonic acid (Ghose and Mattiason, 1991) and Dextran sulfate (Jen and Pinto, 1990) to separate a mixture of moderately retained proteins, conalbumin and ovalbumin. Cramer and co-workers (Jayaraman et al., 1993; Gadam et al., 1993; Gerstner and Cramer, 1992a, 1992b, Gadam and Cramer, 1994) have identified a variety of efficient polyelectrolyte displacers for protein purification (DEAE-dextran, dextran sulfate, protamine, heparin, and pentosan polysulfate). Patrickios et al. (1995) have employed block methacrylic polyampholytes as protein displacers in anion-exchange systems.

Recently, several low molecular weight displacers (<2 kD) have been shown to be effective for displacing proteins in cation-exchange systems. Pentaerythritol based dendritic polyelectrolytes having quarternary amine functionalities and ranging in molecular weights from 480 to 5100 daltons were shown to be effective displacers of proteins in cation exchange systems (Jayaraman et al., 1995). Displacers containing a single arginine or lysine moiety have been shown to be effective for protein purification in cation exchange systems (Kundu et al., 1995b). Antibiotics such as Streptomycin A and Neomycin B have been shown to have sufficient affinity to displace weakly to strongly retained proteins (Kundu et al., 1995a). The ability of low molecular weight compounds to displace proteins is very significant in that it represents a major departure from the conventional wisdom that large polyelectrolyte polymers are required to displace proteins in ion exchange systems. In this paper, the displacement purification of β-lactoglobulins A and B employing a variety of low molecular weight anionic displacers is presented. Furthermore, the ability to transform a displacement separation into a selective displacement operation is presented using these low molecular weight displacers.

Experimental

Materials

Strong anion-exchange (SAX) (quarternary amine, 8 μm, 100 $\times$ 5 mm i.d.), IC-Pak anion column, and Nova-Pak reversed phase (C18) (300 $\times$ 3.9 mm i.d.) columns were donated by Millipore (Waters Chromatography Division, Millipore, Milford, MA, USA). Tris(hydroxymethyl)aminomethane hydrochloride, Tris(hydroxymethyl)aminomethane, β-lactoglobulin mixture (A and B), pure β-lactoglobulin A and β-lactoglobulin B, 1,2 benzene disulfonic acid (BDS), *p*-toluene sulfonic acid (PTS), 1,5 naphthalene disulfonic acid (NDS), pentane sulfonic acid, methane sulfonic acid and sodium sulfite, were purchased from Sigma Chemical Company (St. Louis, MO, USA). All the sulfonic acid based compounds are in the sodium salt form, except for BDS which is in the potassium salt form.

Apparatus

All displacement experiments were carried out using a model LC 2150 pump (LKB, Bromma, Sweden) connected to the chromatographic columns via a Model C10W 10-port valve (Valco, Houston, TX, USA). Fractions of the column effluent were collected using an LKB 2212 Helirac fraction collector (LKB). Protein analysis of the collected fractions was carried out using a Model Waters 590 HPLC pump (Waters, Milford, MA), a Model 7125 sampling valve (Rheodyne, Cotati, CA, USA), a spectroflow 757 UV-vis absorbance detector (Applied Biosystems, Foster City, CA, USA) and a Model C-R3A Chromatographic integrator (Shimadzu, Kyoto, Japan). Chloride analysis was done using a IC-Pak anion column, Waters 431 conductivity detector and a Waters 745B chromatographic integrator. The breakthrough fronts of methane sulfonic acid and pentane sulfonic acid were measured on-line using a Waters R401 Differential Refractometer.

Procedures

Determination of Adsorption Isotherms and Steric Mass Action Parameters. Adsorption isotherms of BDS, PTS, NDS and sodium sulfite were determined by frontal chromatography according to the method

of Jacobson et al. (1984). The steric factor was determined from the breakthrough volume of nonlinear frontal chromatographic experiments. The characteristic charge was determined from the induced salt gradients produced during these experiments. The equilibrium constant was determined by a best fit of the adsorption isotherm. A detailed description of these parameter estimation techniques is described elsewhere (Gadam et al., 1993).

Operation of Displacement Chromatography. A commercially available β-lactoglobulin mixture consisting of approximately equal amounts of the two forms A and B were displaced using the sulfonic acid based displacers in a SAX column. In all displacement experiments, the columns were initially equilibrated with the carrier (50 mM Tris buffer, pH 7.5) and then sequentially perfused with feed, displacer and regenerant solutions. The feed and the displacer solution were prepared in the same buffer as the carrier. The feed load, salt concentration, and displacer concentrations employed for each separation are given in the figure legends of the corresponding chromatograms. All experiments were carried out at room temperature (25°C) at a flow-rate of 0.2 ml/min unless specified otherwise. The pH of the carrier was maintained at 7.5. Fractions of 200 μl were collected directly from the column outlet for subsequent analysis of the protein, displacer and chloride ion concentrations in the effluent.

Protein Analysis by HPLC. Protein analyses of the fractions collected during the experiments were performed by ion-exchange HPLC under isocratic elution conditions. 140 mM NaCl in 50 mM Tris buffer at pH 8.0 was employed as the eluent. Displacement samples were diluted 10–100 fold with the eluent and 20 μl samples were injected at a flow rate of 0.5 ml/min. The column effluent was monitored at 235 nm.

Displacer Analysis. 1,2 Benzene disulfonic acid (BDS), and *p*-Toluene sulfonic acid (PTS), were analyzed using a Nova-Pak reversed phase column under isocratic conditions. The mobile phase used was 10%(v/v) of ACN in 50 mM sodium phosphate monobasic, pH 2.2. The column effluent was monitored at 260 nm for both BDS and PTS.

1,5 Naphthalene disulfonic acid was analyzed on a SAX column using a mobile phase containing 2.0 molar sodium chloride in 50 mM of Tris buffer at a pH of 7.0. The column effluent was monitored at 254 nm for 1,5 naphthalene disulfonic acid.

Sodium sulfite was analyzed on a SAX column using a mobile phase containing 140 mM sodium chloride in 50 mM of Tris buffer at a pH of 8.0. The column effluent was monitored at 235 nm.

The breakthrough volume of the displacers methane sulfonic acid and pentane sulfonic acid were determined from a separate frontal experiment using an on-line refractive index detector.

Analysis of Chloride Ion in the Mobile Phase. Effluent fractions from the displacement experiments were diluted 500 fold in plastic tubes in deionized water and their chloride content quantitated against known Cl^- ion standards (50–150 ppm) using an IC-Pak anion exchange column equilibrated in lithium borate gluconate eluent having a conductivity of 370 μs. The chloride peaks were detected using an on-line conductivity detector.

Results and Discussion

The results of the displacement experiments with low molecular weight anionic displacers can be qualitatively explained by the steric mass action (SMA) model developed by Brooks and Cramer (1992). This model explicitly accounts for steric effects in multicomponent protein equilibria and is able to predict complex behavior in ion-exchange displacement systems. The required model parameters for each component are the characteristic charge (ν), steric factor (σ), and equilibrium constant (K_{eq}). The characteristic charge represents the number of interactions between the adsorbent surface and the adsorbed solute. The steric factor represents the number of sterically hindered salt counterions on the adsorbent surface which are unavailable for exchange with the other solutes in solution. The equilibrium constant of the exchange reaction between the salt counter-ions and the solutes is represented by K_{eq}.

The steric mass action (SMA) parameters of the sulfonic acid displacers, 1,2 benzene disulfonic acid (BDS), 1,5 naphthalene disulfonic acid (NDS), *p*-toluene sulfonic acid (PTS), and sodium sulfite were determined from frontal experiments and are presented in Table 1. While BDS and NDS contain two sulfite groups, PTS and sodium sulfite have just one sulfite group. Furthermore, with the exception of sodium sulfite, the other three compounds have a benzene ring to which the sulfite group(s) is(are) covalently attached. As seen in Table 1, although the characteristic charge of these compounds are small (less than 2), their

Table 1. SMA parameters for proteins and displacers.

Protein/displacer	Characteristic charge (ν)	Equilibrium constant (K_{eq})	Steric factor (σ)
β-lactoglobulin A	7.5	0.0054	38.2
β-lactoglobulin B	6.3	0.0064	47.5
NDS	1.9	6.516	0.1
BDS	1.8	1.745	0.24
PTS	0.94	3.81	0.17
SS	1.66	0.372	N.D.

NDS: 1,5 Napthalene disulfonic acid, disodium salt.
BDS: 1,2 Benzene disulfonic acid, dipotassium salt.
PTS: *p*-Toluene sulfonic acid, sodium salt.
SS: Sodium sulfite.

equilibrium constants are several orders of magnitude greater than the proteins β-lactoglobulin B and β-lactoglobulin A. In addition, these low molecular weight displacers have negligible steric factors.

The SMA model has been used to develop a dynamic affinity plot for ion-exchange displacement systems (Brooks and Cramer, 1996). According to this analysis, the order in which the proteins elute in the displacement train is dictated by their dynamic affinites, where the dynamic affinities of the feed proteins and the displacer is given by the expression,

$$\lambda_i = \sqrt[\nu_i]{\frac{K_i}{\Delta}}$$

where λ_i is the dynamic affinity, ν_i is the characteristic charge of the species, K_i is the equilibrium constant of the exchange reaction with the salt counter-ions and Δ is the ratio of the stationary phase displacer concentration to the mobile phase displacer concentration.

The dynamic affinity plot (Fig. 1(a)) is a graphical representation of the dynamic affinites of the displacer and the two feed proteins. As seen in the figure, all the lines originate at the point Δ on the y-axis and passes through the point defined by the linear equilibrium properties of the solutes. The slope of these lines determines the dynamic affinity of the corresponding solutes. The greater the slope, the higher is the dynamic affinity (Note: the dynamic affinity increases in the direction as shown by the arrow in Fig. 1(a)). The dynamic affinity plot indicates that in order for a displacement separation to take place, the displacer must have a higher dynamic affinity than the feed proteins.

Displacement chromatography of a binary protein mixture of β-lactoglobulin A and B was carried out using NDS as a displacer and the corresponding dynamic

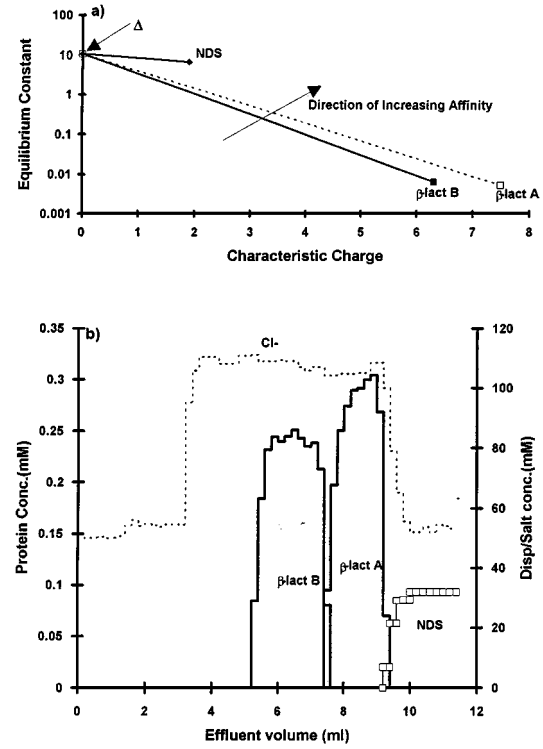

Figure 1. (a) Dynamic affinity plot under the displacement conditions. (b) Displacement separation of a β-lactoglobulin mixture using NDS as a displacer. Column: 100 × 5 mm i.d. strong anion exchanger (8 μm); carrier: 50 mM of tris buffer, pH: 7.5; feed: 1.8 ml of a mixture of the proteins at a total concentration of 0.53 mM in carrier. Displacer concentration, 32.0 mM; flow-rate: 0.2 ml/min; 200 μl fractions.

affinity plot is shown in Fig. 1(a). As seen in the figure, the order of increasing affinity in this system is β-lactoglobulin B, β-lactoglobulin A, and the displacer NDS. Thus, according to the theory, a displacement separation employing NDS as a displacer under these conditions, should result in a displacement of the two proteins. The displacement experiment was carried out and the results are shown in Fig. 1(b). The displacement chromatogram is a histogramatic representation of the protein, salt and displacer profiles determined from analysis of collected fractions. As seen in the figure, this low molecular displacer (280 daltons) with a characteristic charge of 1.9 was readily able to produce an efficient displacement separation of the model mixture. The separation was characterized by sharp displacement boundaries between the protein zones as well as the protein-displacer interface. Furthermore, these results corroborate the prediction of the dynamic affinity plot (Fig. 1(a)).

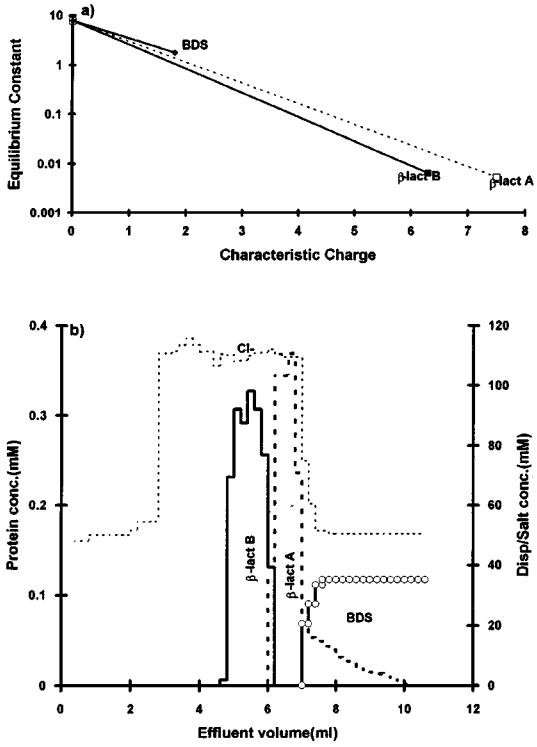

Figure 2. (a) Dynamic affinity plot under the displacement conditions. (b) Displacement separation of a β-lactoglobulin mixture using BDS as a displacer. Column: 100 × 5 mm i.d. strong anion exchanger (8 μm); carrier: 50 mM of tris buffer, pH: 7.5; feed: 1.8 ml of a mixture of the proteins at a total concentration of 0.425 mM in carrier. Displacer concentration: 35 mM; flow-rate: 0.2 ml/min; 200 μl fractions.

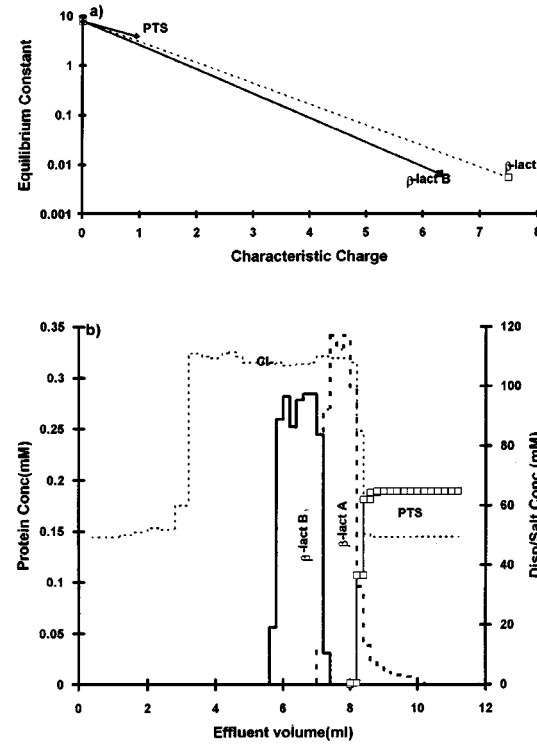

Figure 3. (a) Dynamic affinity plot under the displacement conditions. (b) Displacement separation of a β-lactoglobulin mixture using PTS as a displacer. Column: 100 × 5 mm i.d. strong anion exchanger (8 μm); carrier: 50 mM of tris buffer, pH: 7.5; feed: 1.8 ml of a mixture of the proteins at a total concentration of 0.42 mM in carrier. Displacer concentration: 64.88 mM; flow-rate: 0.2 ml/min; 200 μl fractions.

Displacement experiments were carried out with BDS and PTS and the corresponding dynamic affinity plots are presented in Figs. 2(a) and 3(a), respectively. While these plots indicate that these displacers should be able to displace the proteins under conditions corresponding to Figs. 2(a) and 3(a), it is clear that the dynamic affinities of these displacers are significantly lower than NDS. While the displacement experiments (Figs. 2(b) and 3(b)) resulted in displacement of the feed proteins, these separations were characterized by more diffuse protein-displacer boundaries in contrast to the displacement by NDS shown in Fig. 1(b). These results can be understood by examining the ratio of the dynamic affinities of these displacers to the proteins β-lactoglobulin A and B presented in Table 2. This ratio is a measure of the efficacy of a displacer to displace each of the proteins. If this ratio is less than one, then the displacer does not have sufficient dynamic affinity to displace the protein. As seen in Table 2, the ratio of affinities for the displacers relative to the more strongly retained β-lactoglobulin A was very close to 1 for the conditions corresponding to Figs. 2(a) and 3(a). Thus, as this ratio approaches 1, the displacers become less effective at displacing the protein. In contrast, the ratio for NDS is greater than 2, resulting in effective displacement of the proteins. These results are important in that they demonstrate the ability of the Dynamic Affinity plot to qualitatively predict the efficacy of a given displacement separation.

Sensitivity of the Displacement Process to Operating Conditions. The sensitivity of the dynamic affinity parameter λ to changes in the operating conditions is given by the following expression:

$$\frac{d\lambda_i}{\lambda_i} = -\frac{1}{\nu_i}\frac{d\Delta}{\Delta}$$

Table 2. Dynamic affinity under different operating conditions.

Disp. conc.	β-Lact B	β-Lact A	Disp.	Ratio of affinities: Disp/β-lact B	Ratio of affinities: Disp/β-lact A	Relevant figure
32 mM NDS	0.31	0.36	0.76	2.48	2.11	1a
35 mM BDS	0.32	0.38	0.43	1.34	1.14	2a
65 mM PTS	0.32	0.38	0.46	1.42	1.21	3a
20 mM BDS	0.30	0.35	0.33	1.10	0.93	5a
40 mM PTS	0.30	0.36	0.31	1.02	0.87	6a

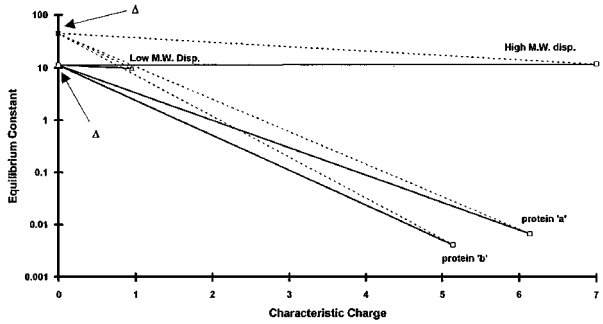

Figure 4. Comparision of the sensitivity of the dynamic affinity λ to the operating conditions (as characterized by slope of the operating line Δ) for low and high molecular weight compounds.

The sensitivity of λ to changes in the operating conditions (as characterized by a change in Δ) is inversely related to the characteristic charge (ν) of the displacer. Thus, the dynamic affinity of molecules which have low characteristic charge (e.g., low molecular weight displacers) would be expected to be more sensitive to changes in the operating conditions, than molecules possessing a relatively high characteristic charge (e.g., polyelectrolyte displacers). The relative sensitivity of high and low molecular weight displacers is illustrated in Fig. 4. As seen in the figure, while the high MW displacer will remain in the displacing region of the affinity plot, regardless of operating conditions, the low MW displacer is more sensitive to the Δ point. In fact, according to this analysis, appropriate changes in the operating conditions (i.e., salt and/or displacer concentrations) could potentially transform a low MW displacer into a desorbing agent.

A set of experiments were carried out with BDS and PTS as displacers to examine the sensitivity of the displacement process to the operating conditions. In both cases, the displacer concentration was changed while keeping the mobile phase salt concentration, the feed load and all other operating conditions the same. As described above (Fig. 2) 35 mM BDS was able to displace the model protein mixture, albeit with some tailing of the β-lactoglobulin A into the displacer zone. Again, this is due to the similarity of the dynamic affinities of BDS and β-lactoglobulin A under these conditions. In order to see the sensitivity of this displacement separation to changes in the displacer concentration, a displacement experiment was performed using a displacer concentration of 20 mM. As seen in the dynamic affinity plot (Fig. 5(a)) this change in the displacer concentration results in a reduction of the affinity of BDS relative to the proteins (Fig. 5(a)). In fact, as seen in Table 2, the dynamic affinity of BDS is now intermediate between the affinities of β-lactoglobulin A and B. Thus, according to the dynamic affinity analysis, BDS should be able to displace β-lactoglobulin B, but not β-lactoglobulin A. Indeed, the results as seen in the displacement experiment (Fig. 5(b)) corroborates this effect. While β-lactoglobulin B was displaced, β-lactoglobulin A was desorbed at a low concentration in the zone of the displacer. A similar trend was observed when the concentration of PTS was reduced to 40 mM from its initial value of 65 mM. This change in the displacer concentration lowered the dynamic affinity of PTS to a value intermediate between the two proteins as shown in Fig. 6(a). Again, according to this analysis a displacement experiment carried out under these conditions should only result in the displacement of β-lactoglobulin B. As shown in Fig. 6(b), the displacement separation confirms the predictions of the dynamic affinity plot. Interestingly, under these conditions the more retained β-lactoglobulin A remained on the column. These results indicate that it is indeed possible to carry out "selective displacement chromatography" where the more retained impurities are desorbed while the bioproduct of interest is displaced. This methodology may be particularly useful as it prevents the contamination of one protein with the other. In addition, a selective displacement requires less column

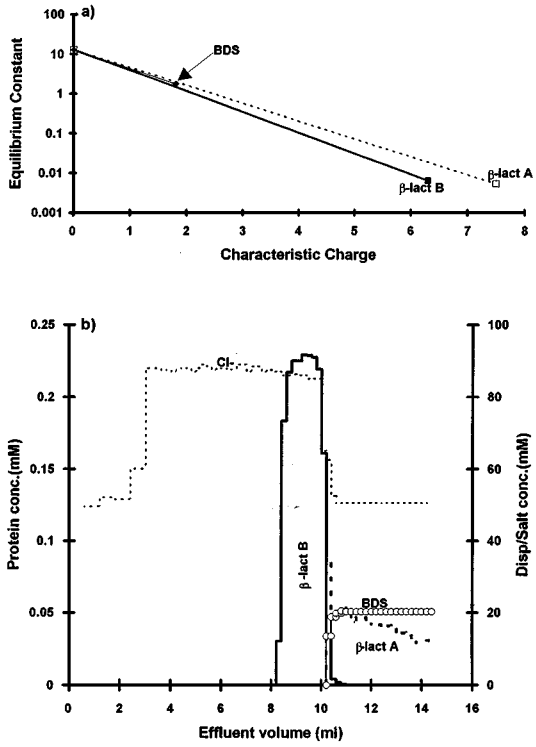

Figure 5. (a) Dynamic affinity plot under the displacement conditions. (b) Displacement separation of a β-lactoglobulin mixture using BDS as a displacer. Column: 100 $\times$ 5 mm i.d. strong anion exchanger (8 μm); carrier: 50 mM of tris buffer, pH: 7.5; feed: 1.8 ml of a mixture of the proteins at a total concentration of 0.42 mM in carrier. Displacer concentration: 20.05 mM; flow-rate: 0.2 ml/min; 200 μl fractions.

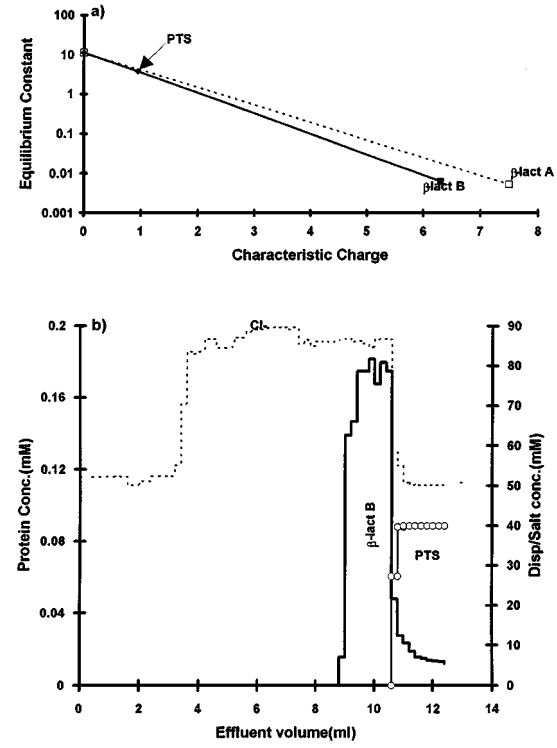

Figure 6. (a) Dynamic affinity plot under the displacement conditions. (b) Displacement separation of a β-lactoglobulin mixture using PTS as a displacer. Column: 100 $\times$ 5 mm i.d. strong anion exchanger (8 μm); carrier: 50 mM of tris buffer, pH: 7.5; feed: 1.8 ml of a mixture of the proteins at a total concentration of 0.42 mM in carrier. Displacer concentration: 39.93 mM; flow-rate: 0.2 ml/min; 200 μl fractions.

length for a desired separation as it involves the development of fewer components in a displacement train as compared to traditional displacement chromatography. It is to be noted that separating these displacers from the protein zones is a simple task, considering the vast difference in the molecular weight between the proteins and the displacers. These results confirm that the efficacy of low MW displacers is indeed sensitive to the displacer concentration employed in the experiment and that this sensitivity can be exploited to obtain selective displacement separations.

Effect of Displacer Chemistry on Affinity. It is instructive to compare the displacement separations obtained with these displacers in light of the number of sulfite groups present in each molecule. Comparing Figs. 2(b) and 6(b) we see that there is a significant difference in the efficacy of PTS and BDS to act as displacers. While 35 mM BDS, displaces both β-lactoglobulin B and A, a 40 mM concentration of PTS as shown in Fig. 6(b) results in displacement of β-lactoglobulin B, under the same mobile phase salt concentration. In fact, upon lowering the concentration of BDS to 20 mM, the displacement efficacy of BDS (Fig. 5(b)) is still higher than 40 mM of PTS, other operating conditions remaining the same. These results can be attributed to the additional sulfite group on BDS which is responsible for the elevated affinity of BDS relative to PTS. A comparison of the results obtained with BDS (Fig. 2) and NDS (Fig. 1) under similar conditions, indicates that NDS is a significantly more effective displacer than BDS. Since NDS and BDS each have two sulfite moities, these results indicate that the number of benzene rings present in the molecule may play a role in the affinity of these displacers for anion exchange stationary phase materials.

Since the charged moiety of BDS, PTS and NDS is sulfite, the natural question arises as to whether sulfite salts could act as displacers. A displacement

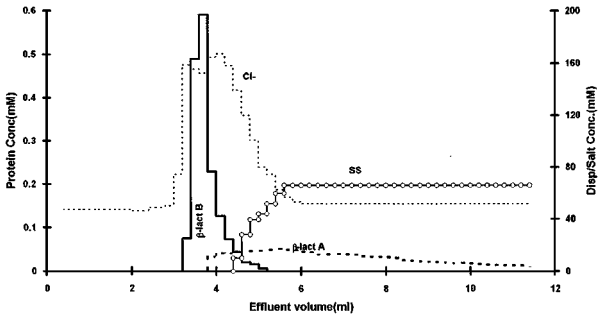

Figure 7. Displacement separation of a β-lactoglobulin mixture using sodium sulfite as a displacer. Column: 100 × 5 mm i.d. strong anion exchanger (8 μm); carrier: 50 mM of tris buffer, pH: 7.5; feed: 1.8 ml of a mixture of the proteins at a total concentration of 0.41 mM in carrier. Displacer concentration: 65.87 mM; flow-rate: 0.2 ml/min; 200 μl fractions.

experiment was carried out using a relatively high concentration (65 mM) of sodium sulfite. As seen in Fig. 7, this experiment resulted in the elution of β-lactoglobulin B and the gradual desorption of β-lactoglobulin A. The elution of β-lactoglobulin B occurred because of the high induced chloride gradient ahead of the displacer. This experiment indicates a marked difference in the efficacy of PTS and sodium sulfite to act as displacers, inspite of the charged moiety being the same for both compounds.

To address the question of hydrophobicity, experiments were carried out with the sodium salt of methane sulfonic acid and pentane sulfonic acid. While pentane sulfonic acid had a slightly higher affinity than methane sulfonic acid, neither of them were able to displace even the lesser retained protein β-lactoglobulin B. Thus, the fact that NDS, BDS and PTS have higher affinity than sodium sulfite and the alkyl sulfonic acids indicates that the benzene ring plays a positive role in the interaction with the surface. It is possible that the benzene ring, by virtue of its delocalized π-electrons is capable of consolidating the charged interactions with the oppositely charged stationary phase. This would explain why NDS has a higher affinity than BDS.

Conclusions

In this paper a variety of low molecular weight compounds have been shown to be effective displacers for protein purification in anion exchange systems. It has been shown that the displacer efficacy increases with an increase in the number of sulfite groups present in a molecule. Furthermore, the results indicate that the benzene ring plays a positive role in enhancing the electrostatic interactions in anion exchange by virtue of its delocalized π electrons. It has been demonstrated that changes in displacer concentration with these low molecular weight displacers can be exploited to obtain *selective* displacement chromatographic separations where the less retained protein β-lactoglobulin B was displaced, while the more retained protein β-lactoglobulin A was either desorbed or retained in the column. Experimental results were shown to corroborate the predictions of the dynamic affinity plot.

In addition to the fundamental interest generated by low MW displacers, it is likely that these displacers will have significant operational advantages as compared to large polyelectrolyte displacers. First and foremost, if there is any overlap between the displacer and the protein of interest, these low molecular weight compounds can be readily separated from the purified protein during postdisplacement downstream processing involving size-based purification methods (e.g., size exclusion chromatography, ultrafiltration). The relatively low cost of synthesizing low molecular weight displacers, can be expected to significantly improve the economics of displacement chromatography. Furthermore, as the affinity of these displacers are much less as compared to the polyelectrolyte displacers, the stationary phase can be regenerated by using mild regeneration schemes. Finally, the ability to carry out selective displacement chromatography with these low molecular weight displacers offers significant potential for developing robust large scale displacement processes. Clearly, in order to employ low molecular weight displacers in bioprocessing it will be important to have a sensitive, specific assay for the displacer and to validate the removal of the displacer from the bioproduct of interest.

Nomenclature

Greek Letters

Δ Slope of displacer operating line
ν Characteristic charge
σ Steric factor
λ Dynamic affinity (dimensionless)

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