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Ion-exchange displacement chromatography of proteins Dendritic polymers as novel displacers

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Abstract

While the ability to carry out simultaneous concentration and purification in a single displacement step has significant advantages for downstream processing of biopharmaceuticals, a major obstacle to the implementation of displacement chromatography has been the lack of appropriate displacer compounds. All protein displacement separations reported to date have employed relatively high-molecular-mass (>2000) polyelectrolyte displacers. In this paper, results are presented on the discovery that low-molecular-mass dendritic polymers can be successfully employed as efficient displacers for protein purification in ion-exchange systems. Pentaerythritol-based dendritic polyelectrolytes ranging in molecular mass from 480 to 5100 were investigated as potential displacers for the purification of proteins in cation-exchange systems. The adsorption properties of these dendrimers were investigated using the steric mass action (SMA) model of non-linear ion-exchange chromatography. An analysis of the resulting SMA parameters using a dynamic affinity plot indicated that these dendrimers should have sufficient affinity to act as protein displacers. Displacement separations of protein mixtures in cation-exchange systems were carried out using zero-, first- and second-generation dendrimers. These experiments demonstrate that this new class of dendritic polyelectrolytes can indeed act as efficient protein displacers. The ability of a low-molecular-mass compound such as the "zero"-generation dendrimer (M_r 480) 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 addition to the fundamental interest generated by low-molecular-mass displacers, it is likely that these displacers will have significant operational advantages as compared to large polyelectrolyte displacers.

1. Introduction

The design of efficient and cost-effective downstream processes for the large-scale purification of biomolecules from complex biological mixtures continues to be one of the major challenges facing the biotechnology industry [1]. In recent years, displacement chromatography has attracted considerable attention as a promising preparative technique for protein separations [2–5]. Displacement chromatography can be carried out using existing chromatographic systems with minor modifications to enable the sequential perfusion of the column with the carrier, feed, displacer and regenerant solutions [6]. The key operational feature which distinguishes displacement from step-elution chromatography is the use of a high-affinity displacer compound. In displacement chromatography, the column is first equilibrated with a carrier

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under conditions wherein the components to be separated have a relatively high affinity for the stationary phase. A large volume of the feed mixture is then loaded onto 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. (Note: a more detailed discussion of affinity in displacement systems is given below). Under appropriate conditions, the product components exit from the column as adjacent "square wave" zones of highly concentrated pure material, in the order of increasing affinity of adsorption. Finally, after the breakthrough of the displacer from the column outlet, the high-affinity displacer must be desorbed from the stationary phase by employing an appropriate regeneration protocol, followed by re-equilibration with the carrier.

The displacement mode of chromatographic operation offers tremendous potential for simultaneous concentration and purification of biomolecules. The peak tailing observed in overloaded elution chromatography is greatly reduced in the displacement mode due to self-sharpening boundaries formed in the process. Since the process takes advantage of the non-linearity of the adsorption isotherms, a chromatographic column operated in the displacement mode can process higher feed loads, enabling the purification of large amounts of material with relatively small chromatographic columns [6–8].

Displacement chromatography is fundamentally different from desorption chromatography (e.g., affinity chromatography, step-gradient chromatography). The displacer, having an affinity higher than any of the feed components, competes effectively for the adsorption sites on the stationary phase. An important distinction between displacement and desorption 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 in the case of desorption chromatography, relatively large separation factors are generally required to give satisfactory resolution.

Anion-exchange displacement chromatography has been studied by several investigators. Carboxymethyl-dextrans have been used in carrier displacement chromatography by Peterson and co-workers [8–10] to separate protein mixtures with anion-exchange columns. They have also reported the purification of monoclonal antibodies by "complex-displacement chromatography" on a cation-exchange column: CM-cellulose [11]. Torres and Peterson [12] have recently reported on the purification of guinea pig serum proteins and mouse liver cytosol proteins by anion-exchange displacement chromatography using a heterogeneous mixture of carboxymethyl dextrans as spacer displacers. In these carrier displacement systems the feed components co-elute with the heterogeneous displacer compounds.

Horváth and co-workers have employed chondroitin sulfate to displace β-galactosidase [13] and β-lactoglobulins [14]. They have also employed displacement chromatography in concert with frontal chromatography and an intermittent column flush to separate relatively large quantities of the proteins β-lactoglobulin A and B [15]. Ghose and Mattiasson [16] have examined the purification of lactate dehydrogenase using a carboxymethyl-starch displacer. Jen and Pinto have employed the smallest polyelectrolyte displacers to date, using a 2000 molecular mass sodium salt of polyvinyl sulfonate [17] and dextran sulfate [18] as displacers in anion-exchange systems.

Cramer and co-workers [19–23] have identified a variety of efficient polyelectrolyte displacers for protein purification including DEAE-dextran, dextran sulfate, protamine, heparin and pentosan polysulfate. Patrickios et al. [23] have employed block methacrylic polyampholytes as protein displacers in anion-exchange systems. To date, all reports in the literature on protein purification by ion-exchange displacement chro-

matography have employed relatively large polyelectrolytes ($M_r > 2000$) as the displacers.

The displacement mode of chromatography, while receiving attention from the academic community has not been widely employed in the biopharmaceutical industry to date. This inertia in industry with respect to displacement chromatography has been attributed to: a dearth of efficient displacers for protein purification, the lack of an appropriate theoretical framework for the rational design of displacement separations, and a paucity of case studies which demonstrate the utility of displacement chromatography for difficult bioseparation problems. A particular problem with the implementation of this technology for biotherapeutics has been the reluctance to employ high-molecular-mass displacers due to regulatory constraints and cost requirements.

As described above, all studies on ion-exchange protein displacement chromatography reported in the literature to date, have employed relatively high-molecular-mass polyelectrolytes as displacers. In this paper, a novel class of displacers, low-molecular-mass pentaerythritol-based dendrimers, are investigated for their relative efficacy in displacement purification of proteins in cation-exchange systems. These molecules have a distinctly different conformation as compared to "linear-chain molecules". Dendritic polymers (also known as starburst polymers) are three-dimensional, highly ordered oligomeric and polymeric compounds formed by reiterative reaction sequences starting from smaller molecules —"initiator cores" such as ammonia or pentaerythritol [24]. The syntheses proceed via discrete stages referred to as generations. The syntheses and structural characterization of the zero- (PE–TMA4), first- (PE–TMA12) and second- (PE–TMA36) generation pentaerythritol-based dendrimers are described elsewhere [25]. The architectural features of the cationic dendrimers employed in this study are presented in Fig. 1. In this manuscript the nonlinear adsorption behavior of these dendrimers are examined and their efficacy as displacers for ion-exchange displacement chromatography of proteins is investigated.

2. Theory

2.1. Dynamic affinity in ion-exchange displacement chromatography

The steric mass action (SMA) ion-exchange model developed by Brooks and Cramer [26] explicitly accounts for *steric effects* in multicomponent protein equilibria and is able to predict complex behavior in ion-exchange displacement systems. In order to present the dynamic affinity plot [27] (described below) for displacement chromatography it is first useful to briefly review the SMA model. Consider an ion-exchange resin with a total capacity, Λ, equilibrated with a carrier buffer solution containing salt counterions. Upon adsorption, the protein interacts with ν_i stationary phase sites (characteristic charge), displacing an equal number (ν_i) of monovalent salt counterions. In addition, the adsorption of the protein also results in the steric hindrance of σ_i salt counterions (steric factor). These *sterically* hindered salt counterions are *unavailable* for exchange with the protein in free solution. For a system of n proteins and a single mobile phase modifying salt, n equilibrium expressions can be written to represent the stoichiometric exchange of each individual protein with the salt.

$$C_i + \nu_i \bar{Q}_1 Q_i + \nu_i C_1 \quad i = 2, 3, \ldots, n+1 \tag{1}$$

where C and Q are the mobile and stationary phase concentrations, and the subscripts i and 1 refer to the protein and salt, respectively. The overbar, $\bar{}$, denotes bound salt counterions *available* for exchange with the protein. The equilibrium constants, K_{1i}, for the ion-exchange process is defined as:

$$K_{1i} \equiv \left(\frac{Q_i}{C_i}\right) \cdot \left(\frac{C_1}{\bar{Q}_1}\right)^{\nu_i} \quad i = 2, 3, \ldots, n+1 \tag{2}$$

Electroneutrality on the stationary phase requires:

$$\Lambda \equiv \bar{Q}_1 + \sum_{i=2}^{n+1} (\nu_i + \sigma_i) Q_i \tag{3}$$

For a single protein, substituting Eq. 3 into

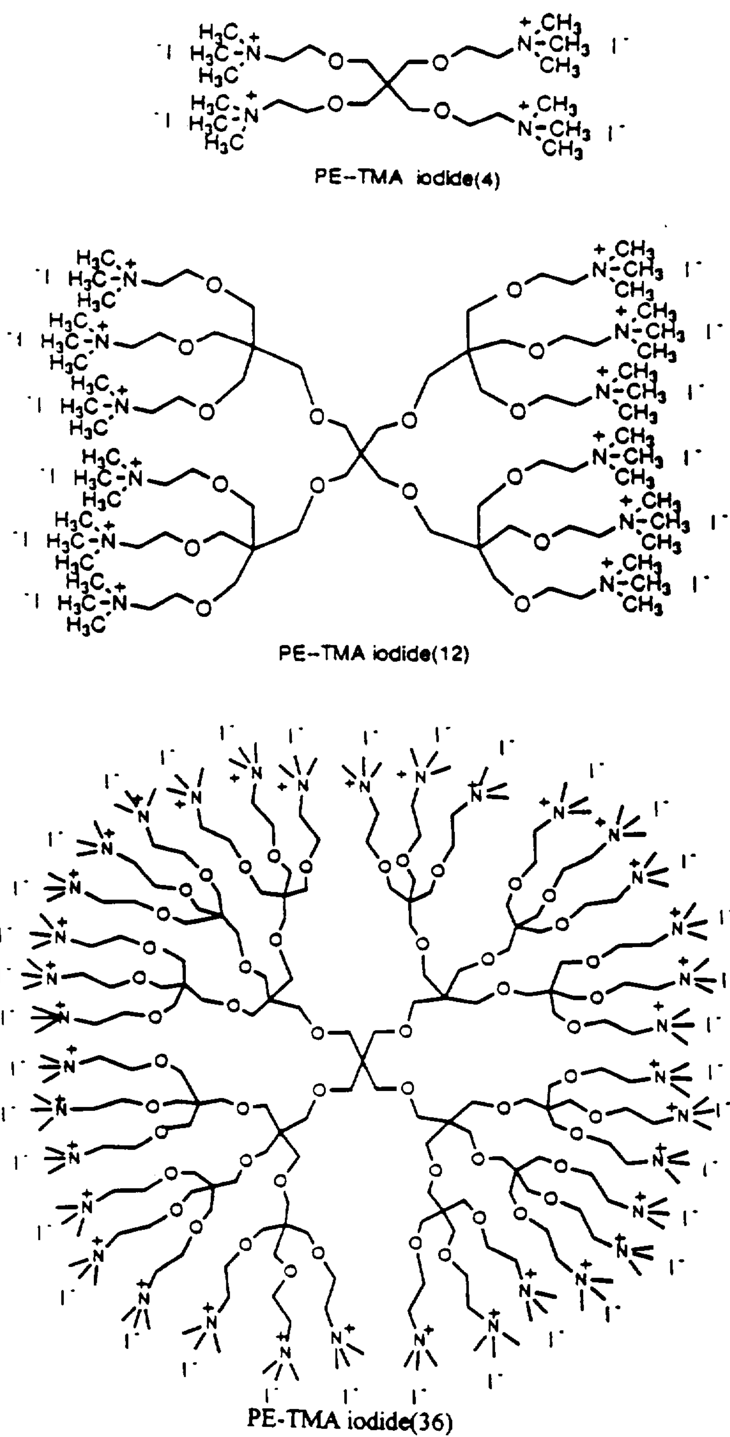

Fig. 1. Architectural features of dendrimer displacers.

Eq. 2 and rearranging yields the following implicit isotherm.

$$Q_i = K_i C_i \cdot \left(\frac{\Lambda - (\nu_i + \sigma_i)Q_i}{C_1}\right)^{\nu_i} \quad (4)$$

The equilibrium stationary phase concentration of the protein, Q_i, is implicitly defined in terms of its mobile phase concentration and the concentration of the salt. Thus, once the SMA equilibrium parameters of the protein are determined [28], the isotherm of the protein can be constructed at any mobile phase salt condition.

The velocity of the displacer as well as the feed components in the isotachic displacement train is determined from a solute movement analysis to be:

$$u_d = \frac{u_0}{1 + \beta \cdot \frac{Q_d}{C_d}} \quad (5)$$

The slope of the displacer operating line, Δ, is calculated using the displacer's implicit single component isotherm.

$$\Delta = \frac{Q_d}{C_d} = K_{1d} \cdot \left(\frac{\Lambda - (\nu_d + \sigma_d)Q_d}{(C_1)_d}\right)^{\nu_d} \quad (6)$$

Consider the displacement separation of a feed solution containing two proteins: a and i. The separation results in the formation of an isotachic displacement train with the two feed components traveling at a velocity u_d characterized by Δ. We have shown through a stability analysis [26] that the order of elution of feed components in isotachic displacement train can be determined from the following expression:

$$\sqrt[\nu_a]{\frac{K_{1a}}{\Delta}} < \sqrt[\nu_i]{\frac{K_{1i}}{\Delta}} \quad (7)$$

The elution order of the feed components in the isotachic displacement train will be a followed by i when Eq. 7 is satisfied. In general, we wish to determine the set of all species i with characteristic charge ν_i and equilibrium constant K_{1i}, that will displace feed component a in an isotachic displacement train traveling at a velocity defined by Δ. The left side of Eq. 7 can be written as the parameter λ_a (Eq. 8), which characterizes the dynamic affinity of species a traveling with a velocity characterized by Δ.

$$\lambda = \sqrt[\nu_i]{\frac{K_{1i}}{\Delta}} \quad (8)$$

After some rearrangement and taking the logarithm of both sides, the following relation can be written:

$$\log K_{1i} > \log \Delta + \log (\lambda_a)\nu_i \quad (9)$$

Thus, on a plot of log K_{1i} versus ν_i (Brooks dynamic affinity plot), Eq. 9 defines two regions demarcated by a line with slope log λ_a and intercept log Δ. As seen in Fig. 2 [27], the line originates at the point Δ on the ordinate axis and passes through the point defined by the linear equilibrium properties, K_{1a} and ν_a, of species a. The region above the *affinity line* includes all solutes which will displace solute a when traveling at a velocity characterized by Δ. Conversely, solutes in the region below the *affinity line* will be displaced by solute a under these conditions.

The Brooks dynamic affinity plot provides a simple method for determining the elution order of feed components in isotachic displacement separations. In addition to providing insight into the dynamic affinity of displacement systems, the Brooks dynamic affinity plot also indicates that low-molecular-mass molecules with sufficiently high equilibrium constants and relatively small characteristic charges may have utility as displacers for protein purification systems. In this paper we will explore the utility of low-molecular-mass

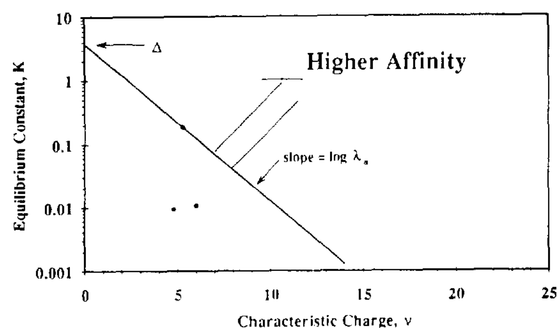

Fig. 2. Brooks dynamic affinity plot [27].

dendrimers as displacers for ion-exchange displacement chromatography of proteins.

3. Experimental

3.1. Materials

Strong cation-exchange "SCX" (sulfopropyl, 8 μm, 50 × 5 mm I.D.) columns were donated by Millipore (Waters Chromatography Division, Millipore, Milford, MA, USA). Sodium chloride, sodium monobasic and dibasic phosphate and all proteins were purchased from Sigma (St. Louis, MO, USA). All the pentaerythritol-based dendrimers were synthesized according to the procedures described elsewhere [25]. M_r 500 cut-off cellulose ester disc membranes were obtained from Spectrum (Houston, TX, USA). Reagent-grade cesium chloride (99.999%) was obtained from Aldrich (Milwaukee, WI, USA). Polyvinylsulfuric acid potassium salt (PVSK) and *o*-toluidine blue indicator were obtained from Nalco (Naperville, IL, USA).

3.2. Apparatus

Diafiltration of the first- and second-generation dendrimers was carried out using an Amicon 8050 stirred cell (Amicon, Danvers, MA, USA). Lyophilization of the diafiltered compounds was carried out using a Model Lyph Lock 4.5 Freeze Dry System (Labconco, Kansas City, MO, USA). All frontal and elution chromatographic experiments were carried out with a modular chromatographic system consisting of a Model LC 2150 pump (LKB Produkter, Bromma, Sweden) and a Spectroflow 757 UV–Vis detector (Applied Biosystems, Foster City, CA, USA) connected to a Waters Maxima 820 chromatographic workstation. Fractions of the column effluent were collected using an LKB 2212 Helirac fraction collector (LKB Produkter). Sodium ion analysis was performed using a Perkin-Elmer Model 3030 (Norwalk, CT, USA) atomic absorption spectrophotometer. A 10-port valve Model C10W injector (Valco, Houston, TX, USA) with multiple loops was used to conduct all the frontal experiments.

3.3. Procedures

Purification of polyelectrolytes

All the dendritic polymers employed in this study were subjected to elemental analysis and linear chromatographic characterization to examine the purity and the linear retention behavior of each compound. The zero-generation dendrimer, PE–TMA-(4) iodide, had a very high degree of purity after synthesis as deemed from the elemental analysis of the compound [25] and therefore was not subjected to further purification. The first- and second-generation dendritic polyelectrolytes, on the other hand contained some impurities and were subjected to diafiltration with a M_r 500 cut-off cellulose ester membrane (Spectrum) in an Amicon ultrafiltration cell to remove the low-molecular-mass impurities. The purified compounds were then lyophilized.

Estimation of SMA equilibrium parameters

The SMA parameters [i.e. characteristic charge (ν), steric factor (σ) and equilibrium constant (K)] for proteins and the zero-, first- and second-generation dendritic displacers were determined using the protocols developed by Gadam et al. [28]. Briefly, the characteristic charge and the equilibrium constant for the proteins were determined using linear elution techniques, while the steric factor of the proteins was determined using a single non-linear frontal chromatographic experiment. The SMA parameters of the dendrimers were determined using the method of induced salt gradient and sequential frontal chromatographic techniques.

Operation of the displacement chromatograph

In all displacement experiments, the columns were initially equilibrated with the carrier buffer and then sequentially perfused with feed, displacer and regenerant solutions (see figure legends for details). The feed and displacer solutions were prepared in the same carrier buffer. Fractions were collected directly from the

column effluent to avoid extra-column dispersion of the purified components.

Regeneration

The cation-exchange column was regenerated after each displacement experiment by passing 10–15 column volumes of 1 M NaCl in 100 mM phosphate buffer, pH 11.0, to assure complete regeneration of the column.

Protein analysis by HPLC

Protein analyses of the fractions collected during the displacement experiments were performed by ion-exchange HPLC under isocratic elution conditions. 150 mM NaCl in 50 mM sodium phosphate buffer at pH 6.0 was employed as mobile phase. Displacement fractions were diluted 10–100-fold with the mobile phase and 20-μl samples were injected into a 100×5 mm I.D. SCX column at a flow-rate of 0.5 ml/min. The column effluent was monitored at 280 nm. The protein compositions of the fractions were then used to construct a histogram of the displacement profiles.

Dendrimer analysis

All dendrimers were analyzed using a colloidal titration assay provided by Nalco. A known amount of sample was dissolved in 100 ml of deionized water and the solution was titrated against 0.001 M PVSK in presence of the *o*-toluidine blue indicator. Linear calibrations for amount of sample with titrant volume were obtained for these titrations.

Analyses for sodium counterions

Sodium was analyzed using atomic absorption spectrometry. Effluent fractions were diluted 2000–3000-fold in tubes containing 5 g/l cesium chloride solution (to minimize background noise) and their amounts calculated against known Na^+ ion standards (10–50 μM). The sodium compositions of the fractions were then used to construct a histogram of the salt profiles during the displacement experiments.

4. Results and discussion

4.1. SMA parameters of dendritic displacers

Zero-, first- and second-generation dendrimers were synthesized as described elsewhere [25]. The molecular masses and net charges of the zero-, first- and second-generation dendrimers were 480, 1620 and 5128; and 4, 12 and 36, respectively. The characteristic charge (ν), steric factor (σ), σ/ν ratio and equilibrium constant (K) of the dendrimers were determined by frontal chromatography and are presented in Table 1. The SMA parameters were found to be insensitive to both salt and solute concentration for these dendrimers. As seen in the table, the characteristic charges were 1.7, 4.2 and 11 for the zero-, first- and second-generation dendrimers. Thus, only 30–40% of the charged groups on the dendrimers are bound to the surface. This is in contrast to polyelectrolytes such as dextran-based displacers, where 60–80% of the charged moieties are bound to the surface of the SCX

Table 1
SMA parameters for proteins and dendrimers

Protein/ displacer	Characteristic charge (ν)	Steric factor (σ)	σ/ν ratio	Saturation capacity (mM)	Equilibrium constant (K_i)
α-Chymotrypsinogen A	4.8	49.2	10.3	10.4	$9.2 \cdot 10^{-3}$
Cytochrome *c*	6.0	53.6	8.9	9.4	$1.1 \cdot 10^{-2}$
PE–TMA 4	1.7	1.5	0.88	185	11
PE–TMA 12	4.2	6.5	1.55	55.3	22
PE–TMA 36	11	16.7	1.52	20.9	2.5

Chromatographic conditions: SCX column (bed capacity, $\Lambda = 580$ mM); sodium phosphate buffer, pH 6.0.

resin [28]. The lower percentage of surface-bound groups for the dendrimers is a reflection of the inflexible nature of these molecules relative to "linear-chain" polyelectrolytes such as dextrans.

Surprisingly, even the relatively small zero- and first-generation dendrimers exhibited a measurable steric factor. It is important to note that the steric factor is a measure of steric hindrance effects which can occur both "underneath" as well as "around" the adsorbed molecule. It is possible that the steric factor for these dendrimers is determined to a large extent by charge repulsive forces between the adsorbed highly charged dendrimers. As seen in the table, the σ/ν values are considerably different for the proteins and dendrimers. While the σ/ν ratios are on the order of 7–10 for proteins, they are almost an order of magnitude lower for the dendrimers. The first-and second-generation dendrimers exhibited similar σ/ν values (approximately 1.5), while the lower-molecular-mass zero-generation dendrimer exhibited a σ/ν value of 0.88. These data suggest that the lower-molecular-mass zero-generation dendrimer exhibits a relatively higher surface coverage under non-linear conditions. It turns out that the σ/ν ratio is an important parameter for displacer design since it determines the level of induced salt gradient produced upon the adsorption of the displacer [29]. A higher value of σ/ν for a displacer molecule corresponds to lower levels of induced salt-gradient in ion-exchange displacement systems for a given breakthrough volume. The results presented here indicate that the zero-generation dendritic displacer will produce a correspondingly higher induced salt gradient as compared to the higher-generation dendrimers.

Table 1 also presents the equilibrium constants for the proteins and dendrimers. While the characteristic charges of the proteins and the dendrimers are comparable, the equilibrium constants for the dendrimers are two to three orders of magnitude higher than the proteins. These results are very important with respect to the ability of these dendrimers to act as protein displacers. In addition, it is interesting to note that the equilibrium constant of the second-generation material is an order of magnitude lower than the zero- and first-generation dendrimers. One possible explanation for this difference is that the larger second-generation dendrimer is unable to get as close to the chromatographic surface as the smaller dendrimers. The relationship between the physico-chemical properties of the displacers and the SMA parameters is the subject of active research in our laboratory.

According to the conventional wisdom, based on results observed with derivatized polysaccharide displacers, a high-molecular-mass compound with a relatively high characteristic charge is required for protein displacement chromatography. However, as described in the Theory section, the dynamic affinity plot of Brooks and Cramer [27] indicates that the ability of a given compound to act as a displacer is determined by its dynamic affinity parameter (Eq. 8) which is a function of the equilibrium constant, characteristic charge, and slope of the displacer operating line.

A dynamic affinity plot was constructed for the zero-, first- and second-generation dendrimers and the feed proteins α-chymotrypsinogen A, cytochrome *c*. According to this analysis (Fig. 3), it can be predicted that both the zero-, first- and second-generation dendrimers should act as effective displacers for these proteins. Although the zero-generation dendrimers has a lower characteristic charge than either of the two proteins, its higher "affinity" can be attributed to the equilibrium constant which is 3 orders of magnitude higher than those of the proteins. The ability of these dendritic molecules to displace proteins would provide a good test case for the dynamic affinity plot as well as a significant departure from the conventional wisdom that high-molecular-mass polyelectrolytes are required for protein displacements.

4.2. Displacement chromatography of proteins by dendritic displacers

Displacement chromatography of a binary protein mixture of α-chymotrypsinogen A and cytochrome *c* was carried out with each generation of the dendritic displacers. In order to

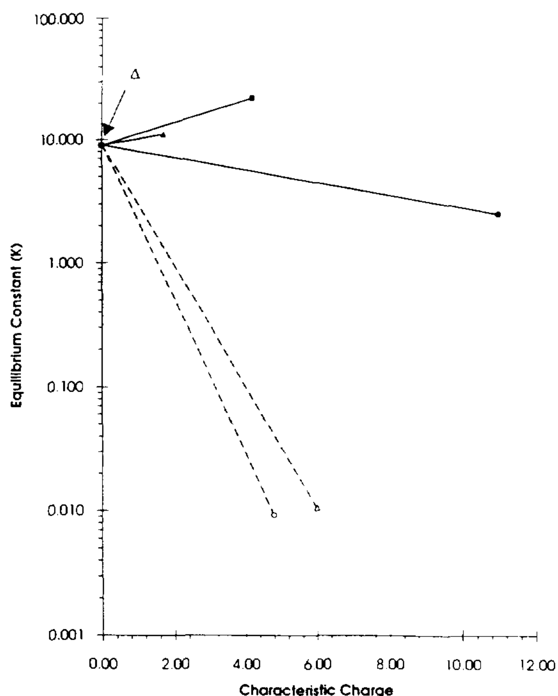

Fig. 3. Dynamic affinity plot for dendrimers and feed proteins. ○ = α-Chymotrypsinogen A; △ = cytochrome c; ▲ = PE–TMA 4; ■ = PE–TMA 12; ● = PE–TMA 36.

compare the relative efficacies of the various generations of displacers, the inlet concentration of each displacer on a mass basis was kept the same. In addition, the initial salt concentration in the carrier solution was reduced for the zero-generation experiment in order to maintain comparable salt microenvironments in the displacement zones. (Note: this is due to the elevated induced salt gradient produced by the zero-generation dendrimer). The displacement experiment with the second-generation dendrimer displacer is shown in Fig. 4. As expected, the second-generation dendrimer (M_r 5280) with a characteristic charge of 11 was readily able to displace the proteins. The displacement experiment with the first-generation dendrimer was then carried out. As seen in Fig. 5, this relatively low-molecular-mass dendrimer was able to produce an efficient displacement of the model proteins. This separation was characterized by very sharp displacement boundaries and the separation of trace impurities associated with the α-chymotrypsinogen A [19]. The zero-generation dendrimer was then investigated for its ability to act as a displacer for protein separation. The results of this experiment are presented in Fig. 6. As seen in the figure, this low-molecular-mass dendrimer (M_r 480) with a characteristic charge of 1.7 was indeed able to displace the proteins. This result is quite significant in that it is in contrast to the conventional wisdom that large polyelectrolytes are required for protein displacement. Furthermore, it confirms the predictions of the dynamic affinity plot (Fig. 3) that low-molecular-mass displacers with small characteristic charges and sufficiently high equilibrium constants can indeed function as protein displacers.

These displacements clearly demonstrate that increasing the molecular mass and number of charged groups on these dendrimers has little effect on their efficacy as displacers. Furthermore, the ratio of steric factor to characteristic charge for the first- and second-generation dendrimers (about 1.5) is comparable to that of the higher-molecular-mass dextran polyelectrolytes [19,28]. Therefore, for the same breakthrough volumes, these dendritic displacers induce the same level of induced salt gradient as the dextran displacers. In other words, the first- and second-generation dendrimers have the same efficacy as high-molecular-mass "linear-chain" polyelectrolytes for protein displacement separations in ion-exchange systems.

The level of induced salt gradient obtained with the zero-generation displacer was significantly higher than those obtained with the first- and second-generation dendrimers. Again, this is due to the relatively low σ/ν ratio (0.88) of the zero-generation dendrimer. Nevertheless, it is more advantageous to employ a low-molecular-mass displacer due to several distinct operational advantages which are discussed below.

5. Conclusions

This research has resulted in the discovery that low-molecular-mass dendritic polymers can in-

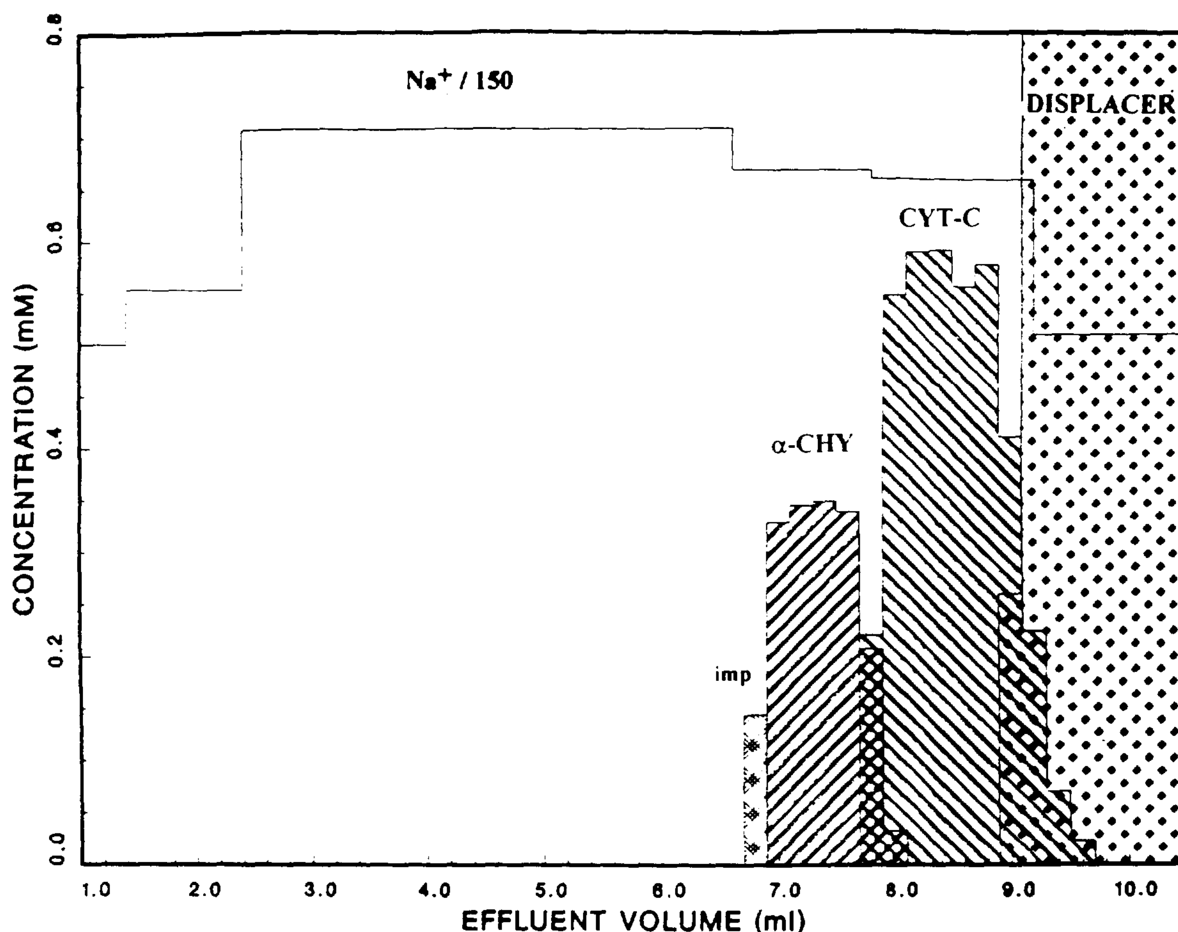

Fig. 4. Displacement separation of a two component protein mixture using second generation (PE–TMA 36) dendritic polyelectrolyte as displacer. Column: 100 × 5 mm I.D. strong cation exchanger (8 μm); Carrier: 75 mM sodium phosphate buffer, pH 6.0; Feed: 1.0 ml of 0.35 mM α-chymotrypsinogen A (α-CHY) and 0.7 mM cytochrome c (CYT-C) in carrier. Displacer concentration, 1.96 mM; flow-rate 0.1 ml/min; 200-ml fractions. imp = Impurity.

deed be employed as efficient displacers for protein purification. The ability of a low-molecular-mass compound such as the zero-generation dendrimer (M_r 480) 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 addition to the fundamental interest generated by low-molecular-mass 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-mass materials can be readily separated from the purified protein during post-displacement downstream processing involving size-based purification methods. (e.g. size-exclusion chromatography, ultrafiltration) Furthermore, the relatively low cost of synthesizing low-molecular-mass displacers, can be expected to significantly improve the economics of displacement chromatography. It is also likely that column regeneration with these materials will require less extreme conditions and reduced regenerant volumes. Additional potential advantages include: the ability to precisely control molecular design parameters such as size, shape,

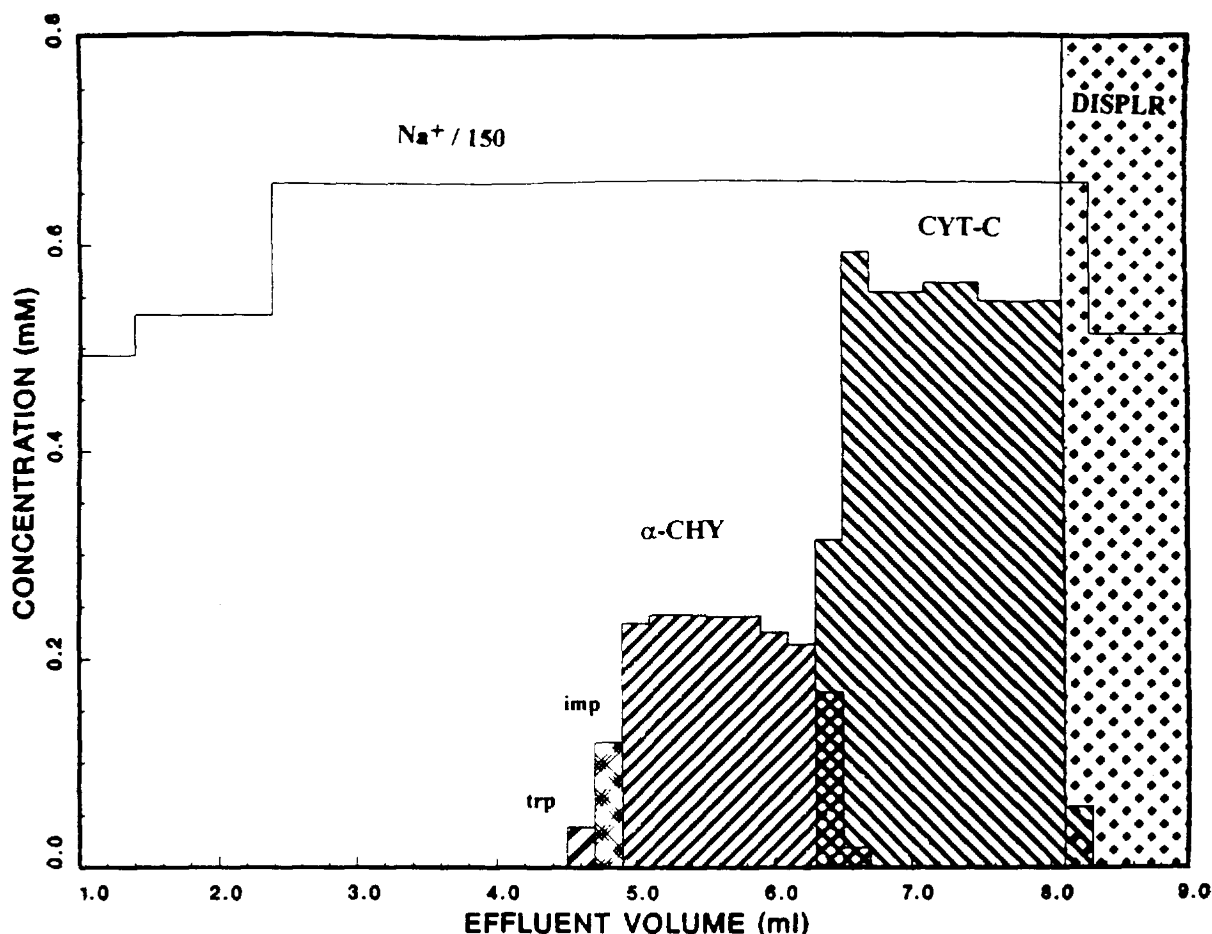

Fig. 5. Displacement separation of a two-component protein mixture using first generation (PE–TMA 12) dendritic polyelectrolyte as displacer. All conditions as in Fig. 4 except feed: 1.0 ml of 0.4 mM α-chymotrypsinogen A and 0.8 mM cytochrome c in carrier; and displacer (DISPLR) concentration, 6.17 mM. trp = Trypsin.

topology, flexibility and degree of functionalization for the dendritic molecules; the rapid mass transport and relatively low viscosities of these small high affinity displacers will facilitate the use of these displacers in high throughput, fast flow chromatographic systems. Clearly, it is very important to consider what other low-molecular-mass compounds could also be employed as displacers for protein purification. We are carrying out a detailed investigation into the design of efficient low-molecular-mass displacers for protein purification. Research on how systematic changes in the displacer chemistry and structure affect important displacer chromatographic properties such as dynamic affinity, induced gradients, and effective operating regimes will be the subject of a future report.

6. Symbols and abbreviations

6.1. Symbols

C	mobile phase concentration (mM)
K	non-dimensionalized equilibrium constant (dimensionless)

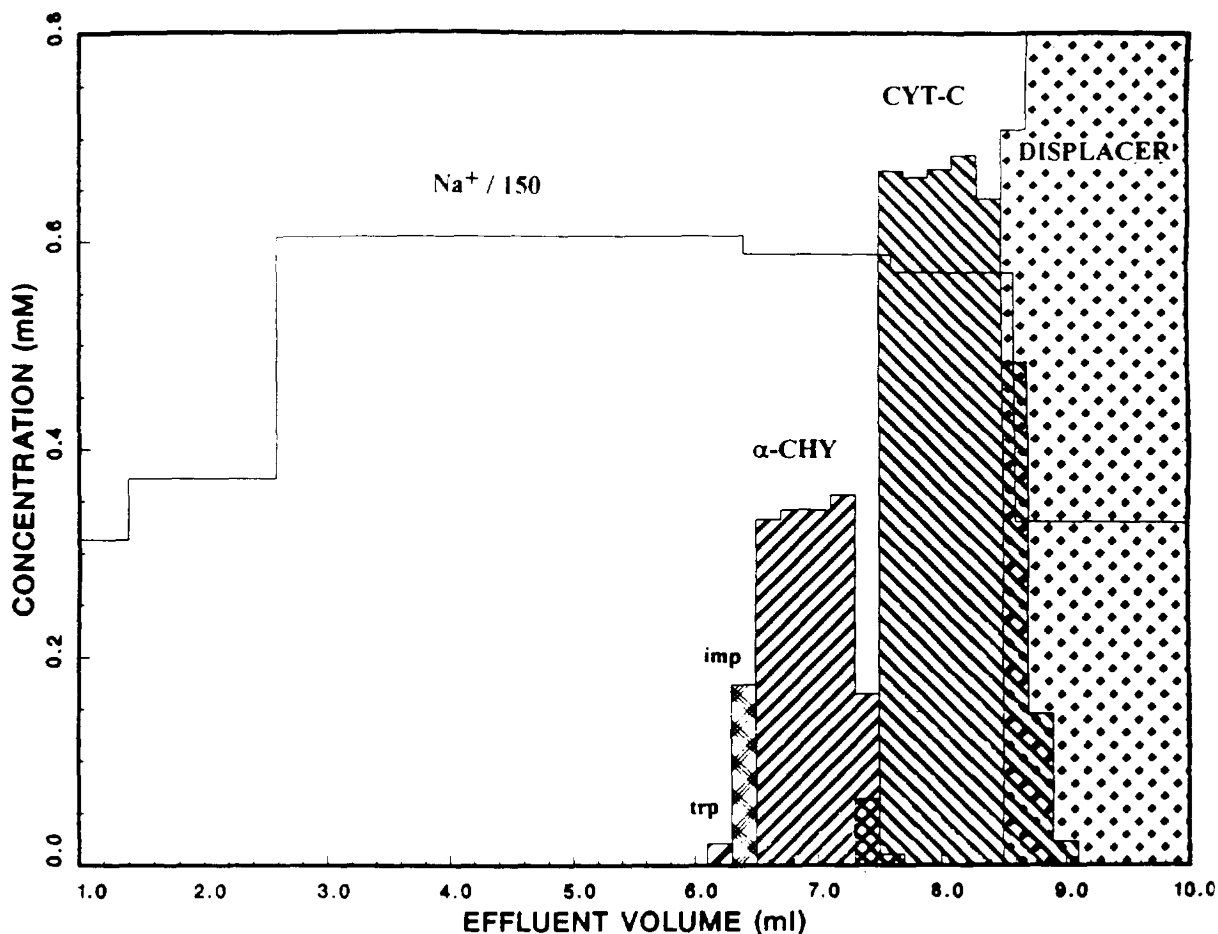

Fig. 6. Displacement separation of a two-component protein mixture using zero generation (PE–TMA 4) dendritic polyelectrolyte as displacer. All conditions as in Fig. 4 except carrier: 50 m*M* sodium phosphate buffer, pH 6.0; feed: 1.2 ml of 0.35 m*M* α-chymotrypsinogen A and 0.7 m*M* cytochrome *c* in carrier; displacer concentration, 21 m*M*.

Q	stationary phase concentration (m*M*)
u	velocity (cm/s)

Greek letters

β	column phase ratio, $(1-\varepsilon)/\varepsilon$
Δ	slope of displacer operating line
ϵ	total porosity
Λ	stationary phase capacity (monovalent salt counterion)
ν	characteristic charge
σ	steric factor

Subscripts and superscripts

$-$	sterically non-hindered
0	unretained solute
d, D	displacer
i	solute
1	salt

6.2. Abbreviations

SMA	steric mass action model
CM	carboxymethyl
DEAE	diethylaminoethyl
PE–TMA	pentaerythritol–trimethylamine

SCX	strong cation exchanger
PVSK	polyvinylsulfuric acid potassium salt

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