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Review

Polymer displacement/shielding in protein chromatography☆

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Abstract

An overview of different applications of polymer interactions with ion-exchange and dye-affinity chromatographic matrices is presented here. The strength of interaction between the ligand and the polymer plays a crucial role in deciding the mode of chromatographic application. Charged, non-ionic and thermosensitive polymers such as poly(ethylene imine), poly(*N*-vinyl pyrrolidone) and poly(vinyl caprolactam) respectively, show different degrees of interaction with the dye molecules in dye ligand chromatography. Polymers, with their ability of multipoint and hence strong attachment to the chromatographic matrices, were used as efficient displacers in displacement chromatography. The polymer displacement resulted in better recoveries and sharper elution profiles than traditional salt elutions. The globule–coil transition of the thermosensitive reversible soluble–insoluble polymer, poly(vinyl caprolactam), can be exploited in dye-affinity columns for the temperature induced displacement of the bound protein. In another situation, prior to the column chromatography of crude protein extract, polymers formed complexes with the dye matrix and ''shielded'' the column. The polymer shielding decreased the nonspecific interactions without affecting the specific interactions of the target protein to the dye matrix.

Keywords: Reviews; Displacement chromatography; Polymers; Proteins; Lactate dehydrogenase

Contents

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1. Introduction

Preparative and process scale separations of biomolecules have been of considerable interest in recent years [1]. In this context, liquid chromatography has remained a main tool to accomplish wider application. All the efforts in the past have been concentrated in increasing the efficiencies of various chromatographic techniques to achieve better results. With the advent of high-performance liquid chromatographic techniques, much emphasis has been placed on improving the efficiency of chromatographic matrices. Modern biochromatography demands highly sophisticated packing materials in terms of biocompatibility, protein selectivity and recovery. Polymers can be designed in a wide variety and therefore can deliver solutions to specific chromatographic problems. Thus, tailor-made polymer coatings or polymer modifications of the matrices have shown tremendous success and can be a better alternative to the classical chemically bonded stationary phases [2]. There are several classical applications where polymer modifications of the stationary chromatographic supports have provided rapid, efficient and better recoveries of the proteins [3–6].

In our laboratory, we have looked for the application of polymers in chromatography with a rather different approach. Our main emphasis for polymer application in protein chromatography has focused on the use of binding strength of the polymer with the chromatographic matrix. This can be addressed as follows:

I) Polymers, with their ability of multipoint attachment and hence strong binding to the chromatographic matrices, can be utilized as efficient displacers of the bound protein.
II) Whenever the complexing efficiency of the polymer with the chromatographic ligands is not strong enough to displace the bound protein, the polymer binding to the matrix prior to the protein loading could be used for ''shielding'' of the matrix.
III) Polymer binding to the matrix can be tuned from less strong binding to more strong binding or vice versa by a shift in environmental conditions. This approach can be utilized with thermoresponsive polymers in temperature induced elution of the bound protein.

2. Polymer shielded dye-affinity chromatography

The application of affinity chromatography at the early stages of protein purification processes promises significant advantages by reducing the overall working volumes and thereby processing costs [7]. In such processes, the requirement is to use the stable ligands which must be resistant to chemical and biological degradation, since the conditions used in the initial stages are much harsher than those used in the later stages. Group-specific ligands such as triazine dyes are thus preferred in such applications and are widely used for the purification of dehydrogenases and kinases [8–10]. However, the major drawback has been their low selectivity. Dye-affinity interactions provide group specific isolation of the proteins and in most of the cases nonspecific interactions are very significant since the molecular structure of the dye offers multiple possibilities for interaction at many sites [11]. While most of the interactions occur at the natural ligand binding site (specific interactions), some similar interactions (electrostatic, hydrophobic, hydrogen bonding and charge transfer) can also take place at sites away from the natural ligand binding site [12]. Such interactions are termed nonspecific and elimination of these nonspecific interactions has been one of the major objectives in the development of new affinity supports. In this direction, the polymer shielding of the dye-affinity matrix has been successful in reducing such nonspecific interactions.

The idea of polymer shielding in dye-affinity chromatography is to apply a polymer to an affinity matrix, whereby the polymer molecules would bind to the matrix. The polymer is applied to the column before the protein loading. There are at least two different modes of applying the shielding polymers to the matrix. If a solution of polymer is passed over the column, binding to the ligand will take place via non-covalent interactions. The polymer is kept on the support via multipoint of attachment, even under eluting conditions for the target protein. Alternatively, the shielding polymer can be covalently attached to the polymer backbone. When a protein is intro-

duced on such a polymer shielded column, a competition between the protein and the bound polymer for binding to the ligand takes place [13]. If the interactions of the dye with the incoming protein are stronger than those with the polymer, then the protein will bind to the column, resulting in a local displacement of the bound polymer. The presence of the polymer however will prevent the weak binding of the protein to the dye matrix which can be mainly nonspecific. A schematic diagram of the concept of polymer shielding is shown in Fig. 1. On an unshielded column the target protein can be retained by both specific and nonspecific interactions with the immobilized dye ligands. Also sometimes some protein impurities can bind through nonspecific interactions with the dye ligand. On a polymer-shielded column, the nonspecific interactions of the target protein and the impurities with the ligand are blocked by the presence of the shielding polymer, and therefore only the target protein interacts mainly by the stronger specific interactions. Polymer shielding modulates the protein–ligand interactions. The protein binding capacity of the column is decreased but the recoveries are higher than those from an unshielded column since the overall retention force is decreased. Also, since the weak nonspecific interactions are masked by the polymer, the protein elutes in a narrow band. Thus, sharper elution peaks have been obtained using dye-affinity columns which have been pretreated with polymer [14,15].

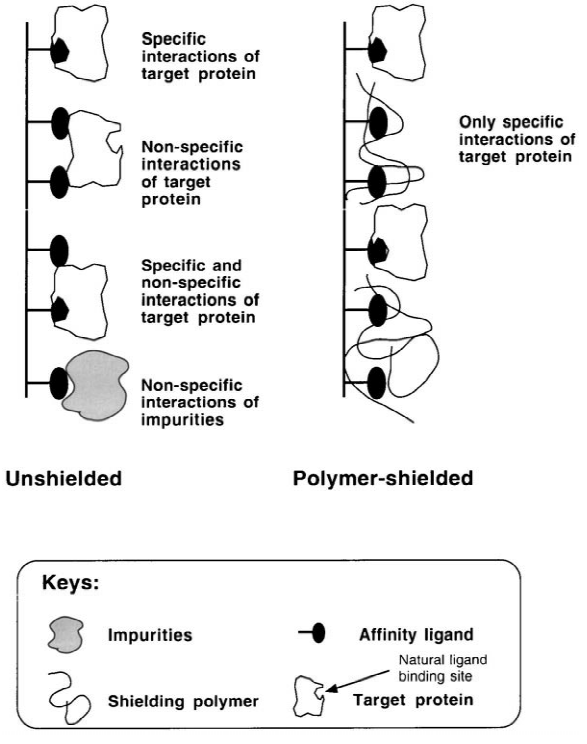

Fig. 1. Schematic representation of the concept of polymer-shielded dye-affinity chromatography.

2.1. Selection strategy of shielding polymer

The chemical nature of the polymer plays an important role when considering for the shielding purpose, since it would determine the nature and extent of its interactions with the protein and the dye molecules. A suitable shielding polymer must fulfill certain requirements:

I) It should bind to the ligand-matrix via multipoint attachment i.e., several ligand molecules should bind to the same polymer molecule. This would ensure that the polymer is not displaced by either the protein or the eluting agents used to recover the protein.
II) The bound polymer should restrict the number of interactions between the target protein and the ligand. This can reduce the nonspecific interactions with the ligands without impairing the specific interactions.
III) The shielding polymer should not interact with the proteins.
IV) It should be cheap, chemically stable, nontoxic and non-immunogenic.

Water soluble, nonionic, moderately hydrophobic polymers such as poly(vinyl pyrrolidone) (PVP) and poly(vinyl alcohol) (PVA) appear to be well suited for shielding purposes since they fulfill most of these criteria.

Another important aspect has been the selection of dyes having suitable interaction with the target protein and also with the shielding polymer. An extensive study has been carried out in this laboratory [13,16] in reaching the rational choice of dye–protein and dye–polymer combination in polymer shielded dye-affinity chromatography. Difference

spectroscopy provides a good estimate of the strength of these complexes and allows for the screening of a number of dyes in a simple way [16,17]. Dyes whose complexes with the target protein have dissociation constants in the range of 0.1–1 μM were useful in dye-affinity chromatography and hence in polymer shielding chromatography. A higher binding strength (K_d<0.1 μM) can also be of interest for shielding purposes but stronger binding consequently poses difficulties in protein elution. A lower binding strength (K_d>1.0 μM) would result in very weak protein binding and can pose the problem of protein leaching from the column. On the other hand, the binding constant for the polymer–dye complex should be in the range of 10–20 μM. This makes it quite suitable for the ''shielding'' of the dye-matrix. However, a moderate binding between the polymer and the dye (K_d≈1.0–10.0 μM) can be useful in some cases. Stronger polymer–dye interactions (K_d<5 μM) prevent the binding of the target protein to the column, while the weaker interaction (K_d>25 μM) may result in some cases in displacement of the polymer by the target protein. However, it is also possible that the strength of the polymer–dye complex can be modulated by varying the molecular weight of the polymer. A stronger complex is obtained with a high molecular weight polymer and a weak complex with a lower molecular weight polymer.

The binding interactions which are rendered, though unsuitable for shielding purposes, can however be made useful in other chromatographic systems. Stronger binding of the polymer can be useful in displacement chromatography whereas weak polymer binding can be modulated to suit the specific applications e.g., temperature regulated binding. These applications are also discussed separately in this review. Considering such binding interactions between the polymer, dye and target protein a general strategy was proposed for designing various polymer pulsed dye-affinity chromatographic systems (Fig. 2).

2.2. Applications of polymer shielding

As an important example, a purification procedure for lactate dehydrogenase (LDH) from porcine muscle extract was developed using the polymer shield-

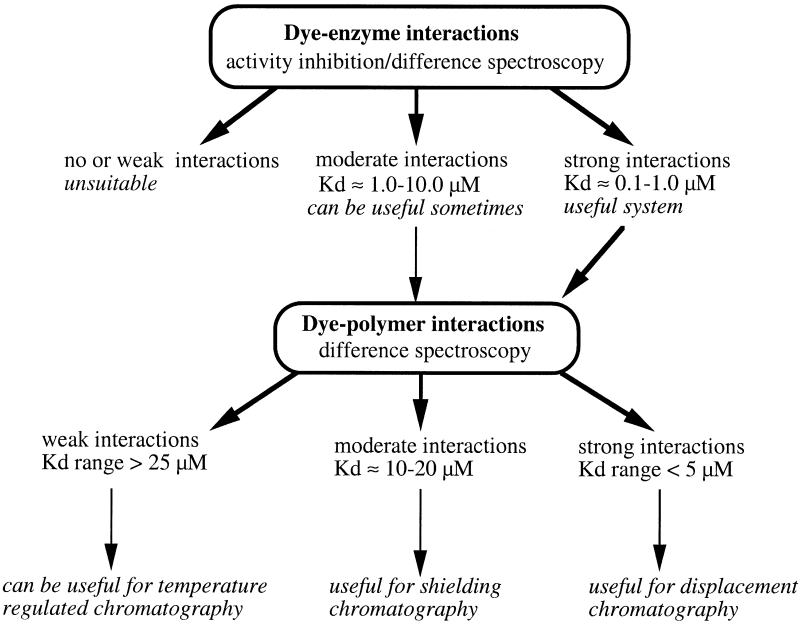

Fig. 2. The proposed strategy for the choice of polymer–dye combination in dye-affinity chromatographic systems. Modified with permission from Mattiasson et al. [13].

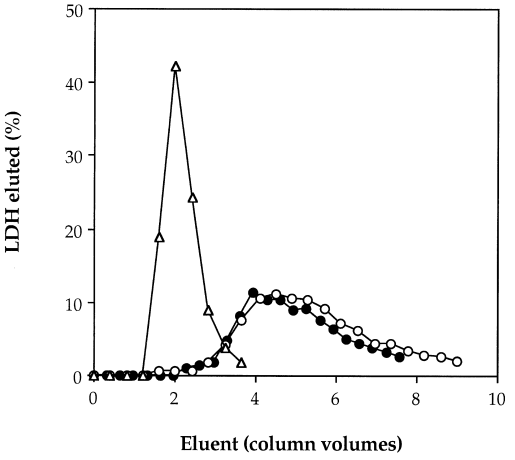

Fig. 3. Elution profile of LDH from a Procion Blue HERD–Sepharose column. (○) unshielded, (●) PVA shielded, (△) PVP-10 shielded. The total amount of enzyme eluted from the column was taken as the 100% value, and the amount in each fraction was calculated as a percentage of this value. This was done since the amounts of enzyme bound on the unshielded and polymer shielded columns were different. The column had an I.D. of 1.0 cm and a bed volume of 4.9 ml for the unshielded and PVP-10 shielded column and 5.2 ml for the PVA shielded column. Reproduced with permission from Mattiasson et al. [13].

ing strategy outlined above [16]. The enzyme was purified on Procion Blue HERD affinity column, shielded by PVP-10. Binding constant of the dye for the enzyme is about 0.27 μ*M* and the dye forms the complex with the shielding polymer with K_d of about 10 μ*M*. Thus, the binding interactions are in the favorable range for polymer-shielding dye chromatography. Almost quantitative recovery (94%) of the bound enzyme was achieved from the polymer shielded column, which gave a 30-fold purification of the enzyme. The enzyme was eluted as a narrow zone in an elution volume which was half the volume of that from an unshielded column (Fig. 3). When PVA (K_d 20 μ*M*) was applied as a shielding polymer, it did not have such an effect on the purification process. The PVA–dye complex has a K_d value in the correct range. However, the $n<5$ value (the number of dye molecules bound by one polymer molecule) calculated for the complex [16], may be lower indicating fewer points of interaction, and therefore a weaker overall interaction. This can explain the poor shielding effect of PVA in this system. Other useful examples where polymer shielding was applied successfully to the dye affinity chromatography of various enzymes are summarized in Table 1. Polymer shielding improved the efficiency of the purification process (in terms of smaller elution volumes and higher recoveries) in all cases. It was also applied successfully in an expanded bed chromatographic system for the isolation of lactate dehydrogenase from crude porcine muscle

Table 1
Reported examples of polymer-shielded dye affinity chromatographic systems [13]

Matrix	Polymer	Enzyme	Ref.
Procion Blue HERD–Sepharose	PVP	Lactate dehydrogenase (porcine muscle)	[16]
Cibacron Blue 3GA–Sepharose	PVP	Phosphofructokinase (baker's yeast)	[17]
Cibacron Blue 3GA–Streamline (Expanded bed chromatography)	PVP	Lactate dehydrogenase (porcine muscle)	[18]
Cibacron Blue 3GA–Sepharose	PVP	Lactate dehydrogenase (porcine muscle)	[19,20]
Cibacron Blue 3GA–Sepharose	PVCL	Lactate dehydrogenase (porcine muscle)	[19]
Procion Scarlet H2G–Sepharose	PVP	Secondary alcohol dehydrogenase (*Thermoanerobium brockii*)	[20]
		Lactate dehydrogenase (porcine muscle)	[20]
Procion Red HE3B–Sepharose	PVA	Pyruvate kinase (porcine muscle)	[21]

extract [18]. This clearly presents its potential application when applying dye-affinity chromatography in the initial stages of purifications.

Although polymer shielding has so far been applied successfully to dye-affinity chromatography only, recently we have seen in our laboratory that this concept can also be applied to immobilized metal affinity chromatography (unpublished results).

3. Polymer displacement chromatography

Displacement chromatography in protein purification attracts increasing attention due to its obvious advantages: sufficient resolving power at high column loading and sharper elution profiles (absence of peak tailing). Proteins opposite to low molecular weight compounds interact with the chromatographic matrix via multipoint interaction at several sites on the surface of the protein molecule. An efficient strategy to elute such proteins is to use a substance called a displacer with stronger binding to the matrix than that of the target protein [22]. Binding of such an agent results in the displacement of the bound protein and is therefore called displacement chromatography. Polymers with their ability of multipoint and hence strong attachment to the chromatographic matrices proved to be efficient protein displacers in different types of chromatography. Some important examples where both natural and synthetic polymers have been applied in displacement chromatography are presented in Table 2. Most of the works show by tradition greater application of displacement mode in ion-exchange chromatography. These have recently been summarized by Chen and Scouten [23]. Contrary to polymer shielding chromatography (where the polymer is applied to the column before the protein is loaded), in polymer displacement chromatography the polymer is applied to the column either with the protein loading or after the target protein is bound to it.

Earlier in this laboratory, different polymers e.g., chondroitin sulphate C, alginate, Eudragit and carboxymethyl starch were applied as displacers for the purification of lactate dehydrogenase on an ion-exchange column [34,35]. Again the chemical nature of the polymers decide how efficiently they can be used in displacement chromatography. The polymers differ in molecular weight, viscosity and solubility properties. Higher recoveries and well defined peaks

Table 2
Application of polymers in displacement chromatography for protein separation

Polymer	Chromatography	Proteins	Ref
Nalcolyte 7105, Poly(ethylene imine)	Cation exchange	Cytochrome *c*, lysozyme, ribonuclease, α-chymotrypsinogen	[22]
Alginate	Anion exchange	Ovalbumin, β-lactoglobulin	[23]
Methacrylic block copolymers	Hydrophobic interaction chromatography	Trypsin/chymotrypsin]	[24]
Poly (ethylene glycol)	Hydrophobic interaction chromatography	Monoclonal antibodies/ Lactate dehydrogenase	[25,33]
Polyacrylic acid	Hydroxyapetite chromatography	Immunoglobulin A	[26]
Dendritic polymers	Cation exchange	α-chymotrypsinogen A/ cytochrome *c*	[27]
Protamine	Cation exchange	Recombinant protein therapeutics	[28]
DEAE-dextran	Anion exchange	α-chymotrypsinogen, Cytochrome *c*	[29]
CM-dextran	Anion exchange	guinea pig serum	[30]
Poly(vinyl pyrrolidone)/ Poly(vinyl caprolactam)	Affinity chromatography (Blue Sepharose)	Lactate dehydrogenase	[19,31]
Poly (ethylene imine)	Affinity chromatography (Red Sepharose)	Lactate dehydrogenase	[31,32]

were obtained when enzyme was displaced with chondroitin sulphate and Eudragit -*S* and -*L*. It was shown that because of higher charge density on Eudragit-*L* as compared to Eudragit-*S*, its protein displacement efficiency was more. The -*L* and -*S* forms of Eudragit differ in the ratio of the free carboxyl groups to esters, which is approximately 1:1 in the former and 1:2 in the latter. The lowered recoveries obtained using alginate as displacer is attributed more to its poorly defined character. The heterogeneity of a displacer can be defined in terms of its affinity distribution which, for a charged polymer, would be a function of its molecular weight distribution and charge density. Such a polymer heterogeneity would, in general, lead to a much higher degree of band-spreading and its dispersion within the protein peaks. The choice of carboxymethyl starch (CMS) which worked well in these studies was based on the use of similar substituted polymers, carboxymethyl dextrans and carboxymethyl cellulose by other workers [30,36].

3.1. Displacement in dye-affinity chromatography

Application of polymer displacement in affinity chromatography is rather complex because of the strong protein binding to the affinity ligand. It is desirable to find a polymer which could mimic such interaction and ensure the even stronger binding required for displacement. There are few examples where different displacers have been used in affinity chromatography purifications. Highly purified cathapsin G was obtained from an affinity column by elution with poly-L-lysine [37]. Even pure proteins RNase [38] and myoglobin [39] were used as displacers in immobilized metal affinity chromatography.

Polymer displacement in dye-affinity chromatography was initiated in our laboratory (Table 2) [19,31,32,40]. Poly(*N*-vinyl pyrrolidone) (PVP), a weakly hydrophobic polymer interacts with Cibacron Blue 3GA in solution with binding constants of 2–6 μ*M* depending on molecular weight of the polymer. The more hydrophobic polymer, poly (*N*-vinyl caprolactam) (PVCL), interacted more strongly with the dye. Thus both PVP and PVCL at high concentrations could interact strongly with the dye–ligand matrix to displace bound proteins. When used for the purification of LDH from porcine muscle from a Blue Sepharose column, 1% solutions of PVP of molecular weight 40 000 and PVCL of molecular weight 80 000 displaces the enzyme efficiently (Fig. 4). PVCL proved to be slightly more efficient than PVP, which was expected from the stronger PVCL–dye interactions than those between PVP and dye. Both polymers displaced LDH more efficiently than when it was eluted with 1.5 *M* KCl, a traditionally used strong nonspecific eluent of enzymes bound to dye-ligand matrices.

However, using polymers as displacers, the two major problems that can arise need to be tackled appropriately. First, the recovery of the displaced protein from the polymer and furthermore the regeneration of the column after the polymer is bound to it. In situations where polymer removal is deemed necessary, one can introduce a gel filtration step to separate the polymer from the protein. However, application of PVCL provided a very simple and good solution to this problem, since PVCL is a thermosensitive phase separating polymer. Aqueous solutions of PVCL are characterized by a low critical solution temperature (LCST) [41]. It can be made insoluble by increasing the temperature to around 35°C and can be separated from the protein by centrifugation. However, because of the non-charged

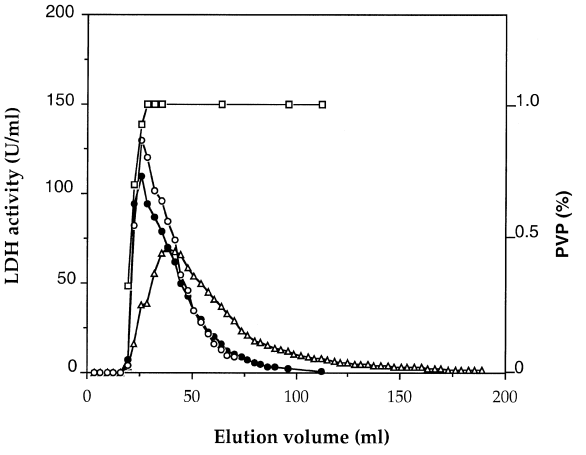

Fig. 4. LDH elution profiles from Blue Sepharose with 1% PVP (●), 1% PVCL (O) and 1.5 *M* KCl (△). The PVP concentration in the eluent is presented as (□). Reproduced with permission from Galaev and Mattiasson [19].

nature of these polymers (PVP and PVCL), the regeneration of the column is rather difficult. The interactions with the dye-matrix are not affected by the change in pH or ionic strength. Thus this general mode of column regeneration does not seem to work in such conditions. In such a case, the application of ionic polymers is much suitable.

The interactions of the charged polymers with the matrix can be modulated by changing pH or ionic strength. As such the matrix can be regenerated after polymer application. One example can be the use of polymer, poly(ethylene imine) (PEI). PEI interacts quite strongly with the dye-ligands at neutral conditions. It complexes with Cibacron Blue about 10 times more strongly than the dye complexing with non-ionic polymer PVP [31]. The binding constant was estimated to be much less than 0.1 μ*M*. Such strong binding makes it an efficient displacer in protein chromatography, rather than being used as a shielding polymer. For example, LDH was displaced completely with 1% PEI and eluted as a sharp peak without the tailing usually observed during non-specific elution with high salt concentration and especially during specific elution with nucleotide solution [31,40]. The enzyme was eluted from Blue Sepharose column in 2.5-fold less volume than with 1.5 *M* KCl. The enzyme however could not be eluted with spermine, which is regarded as a low molecular weight PEI analogue with a degree of polymerization of four. Thus the polymeric nature of PEI and the possibility of multipoint interaction with the dye matrix were crucial for an efficient LDH elution.

Now recently we have also extended this approach of PEI polymer displacement for the elution of a rather different class of protein, the lactose repressor from an ion-exchange column [42]. This presents a good system to analyse the application of polymer displacement mode on a protein which binds strongly to the matrix. The lac repressor protein from an overproducer strain of *E. coli* BMH8117 binds strongly to a cellulose phosphate matrix. The matrix functions as an ion exchanger with high affinities for multivalent cations and affinity for small biopolymers. The repressor protein bound on the matrix could be eluted quantitatively with 1% PEI-600 at pH 8.5 (Fig. 5). The protein was also effectively eluted here with high molecular weight polymer (PEI-50 000) or spermine. Lac repressor was displaced with 1% PEI and eluted as a sharp peak in 3-fold less volume than with the conventional salt elution (Fig. 5). PEI bound strongly to the column after polymer elution and did not allow protein to bind again. It was necessary to remove the bound polymer from the matrix. Polycation displacers are washed out from the cation exchangers with high pH–high salt buffer [29,43]. PEI ionization is suppressed at high pH and high salt concentration decreases efficiency of ionic interaction. The polymer was removed efficiently from the cellulose phosphate column by washing with 0.1 *M* glycine solution, pH 12.0 containing 1 *M* NaCl. After regenerating the column and equilibrating with the excess of equilibrating buffer, the protein could bind to the ion exchanger with the same efficiency as in the initial use. In all three cases where different polymers e.g., PEI-600, PEI-5000 or spermine were used for protein displacement, the regeneration procedure was equally efficient for removing the bound polymer from the matrix.

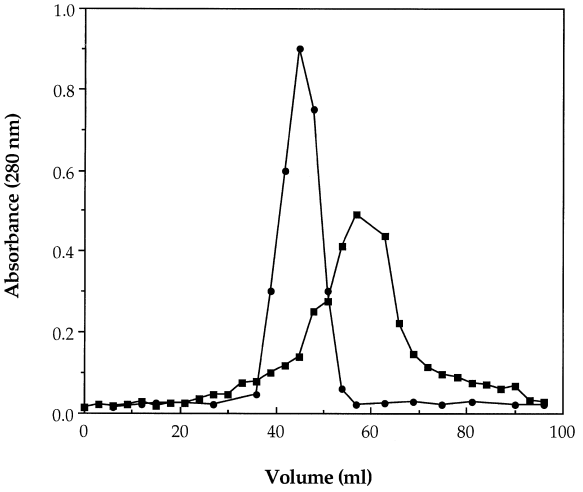

Fig. 5. Elution of lac repressor protein from phosphocellulose column with (■) 1.5 *M* KCl in 0.075 *M* KPG, pH 7.2 and polymer displacement with (●) 1% poly(ethyleneimine)-600 in 0.075 *M* KPG, pH 8.5. Experimental conditions: column, 10×1.5 cm; flow-rate 0.5 ml min^{-1}; fraction volume 3 ml. Four milliliter of cell free protein extract (20–33% ammonium sulphate precipitated) from *E. coli* BMH8117 harboring plasmid pWB1000 was applied to 10 ml phosphocellulose column. KPG is potassium phosphate buffer containing 0.1 *M* EDTA, 5% (w/v) glucose, 0.3 m*M* DTT and 1 m*M* NaN_3.

3.2. Temperature-induced displacement of proteins

Reversible phase transitions in polymer solutions and critical phenomena in polymer gels have already gained great attention in drug delivery, biocatalysis and bioseparation. These transitions, which are initiated by small changes of temperature, pH, ionic strength, light, electric or magnetic fields, can be utilized for specific applications. Temperature change seems to be one of the most attractive triggers of such transitions, because of its simple operational conditions. Polymers which respond to these triggers, called thermoresponsive polymers, are showing great potential for the preparation of biocatalysts [44] and for the design of intelligent materials [45].

In fact, another fascinating approach using these polymers can be the regulation of polymer interactions with the chromatographic matrix from stronger to less strong and vice versa by temperature induced shift. Thus, this behaviour of the temperature responsive polymers has tremendous potential in the development of chromatographic systems, as a small temperature shift can modify the binding and elution of target substances. One such attempt was made by Gewehr et al. [46] in gel permeation chromatography. The gel matrix made of porous glass was modified with temperature responsive polymer, poly-(*N*-isopropylacrylamide). The effective pore size of the matrix changed with the change in the temperature. This strongly affected the elution pattern, when dextrans of various molecular masses were applied to the column. This effect was largely attributed to the transition of polymer molecules from coils to globules on the surface of the pores of the glass beads.

The globule–coil transition of the polymer caused by the temperature shift was recently used by our group [40] for the displacement of bound proteins without changing the buffer composition, and hence as the only eluting factor. LDH from porcine muscle was conveniently eluted from the poly(vinyl caprolactam) (PVCL) treated Blue-Sepharose affinity column by a simple temperature shift. One can note that the same polymer was used for the displacement of LDH from Blue-Sepharose column because of its strong interaction with the dye molecules (Fig. 4). Here in this particular case, the polymer interactions

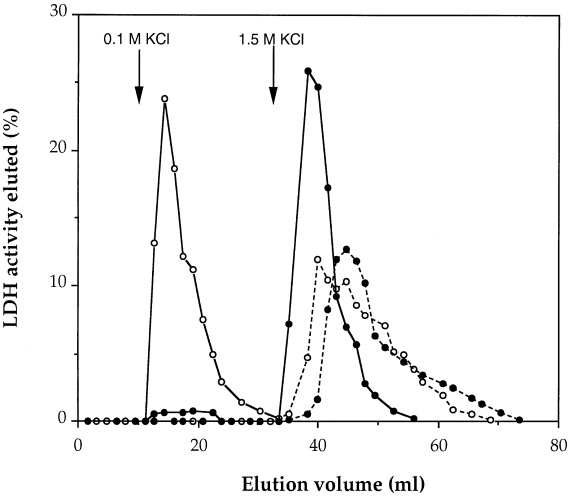

Fig. 6. Elution profile of LDH at (O) room temperature and (●) 40°C from untreated (shown as broken lines) and PVCL-treated Blue Sepharose (shown as solid lines) with 0.1 *M* KCl, followed by elution with 1.5 *M* KCl. Arrows indicate when elution started. Experimental conditions: column, 1.3×0.9 cm; flow-rate, 0.17 ml min^{-1}. The total amount of LDH eluted from the column was taken as 100% in all instances for the sake of comparison.

are so regulated by temperature shift that the same polymer–dye interactions can be used in shielding and displacement mode together. Fig. 6 presents elution profiles (with 0.1 *M* KCl followed by 1.5 *M* KCl) of pure LDH from untreated and PVCL-shielded columns at room temperature (23°C) and at 40°C. The elution profiles at room temperature and at 40°C from the untreated column were similar, thus explaining no temperature effect on the behaviour of the untreated column. However, the elution behaviour using PVCL treated column was different. At 40°C (where the LDH extract was loaded on the column), enzyme was not eluted at all with 0.1 *M* KCl. But as the column was cooled to room temperature (23°C), the enzyme was easily eluted with the same eluent. The lowering of temperature causes the PVCL polymer to solubilize and change to coil conformation along the matrix. This gives stronger binding of the polymer to the dye-matrix and simultaneously weakens the enzyme binding. This eventually leads to the easy elution of enzyme with 0.1 *M* KCl. Also with 1.5 *M* KCl at 40°C the enzyme is eluted with a sharper elution profile than as seen in

untreated column. This may be an effect attributed to the polymer shielding of the column.

4. Conclusions

The pulsing of a protein chromatography column with a polymer after or before the protein is bound to it can lead to specific advantages in chromatography. Polymer shielding of a dye-affinity column prevents the non-specific binding of the proteins, and polymer displacement allows the protein to be recovered in concentrated form. Also, attractive advantages can be achieved while using thermosensitive reversible polymers as coating materials of the columns. The obvious applications of polymers in these chromatographic systems depends upon the binding strength of the polymer to the matrix. It is strongly felt that these chromatographic processes can show greater advantages in large scale applications. The use of temperature sensitive polymers as coating materials in various forms of liquid chromatography can be a challenging area to investigate.

5. Abbreviations

PVP	Poly(vinyl pyrrolidone)
PVA	Poly(vinyl alcohol)
PEI	Poly(ethylene imine)
PVCL	Poly(vinyl caprolactam)
LDH	Lactate dehydrogenase
Lac	Lactose
K_d	Dissociation constant

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