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COMPARISON OF WEAK AND STRONG HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY

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SUMMARY

A comparison of the chromatographic characteristics of weak anion exchangers with those of strong anion exchangers was made for a series of proteins. On both SynChropak AX300 and Q300, the resolution of bovine serum albumin from its dimer was better at pH 6 than at pH 8. Conversely, catalase components separated better at pH 8 than at pH 6. A pH effect which may be due to hydrophobicity was observed for ovalbumin and lactate dehydrogenase on the weak anion exchangers. A protein with a molecular weight of 140 000 shows equivalent separations on both 300-Å and 1000-Å column materials, whereas smaller proteins are fractionated better on the 300-Å columns.

INTRODUCTION

In traditional anion-exchange chromatography on carbohydrate gels, most analyses have utilized a weak functionality of diethylaminoethanol because the strong quaternized anion exchangers have had deleterious effects on labile proteins. High-performance liquid chromatography (HPLC) methods have used both weak and strong anion-exchange mechanisms for protein purification, with no adverse effects on the proteins being noted from either functionality. Although work has been reported which illustrates the effects of salt[1–4] and pH[5,6] in protein purification, no one has examined the relative chromatographic merits of weak and strong anion exchangers in terms of selectivity, resolution, capacity, and enzymatic recovery.

This paper describes the chromatographic behavior of several proteins on weak and strong anion exchangers at pH values from 6 to 8. Pore diameters of 300 Å and 1000 Å were both investigated to obtain optimum resolution for proteins ranging in molecular weight from 45 000 to 230 000 daltons.

EXPERIMENTAL

Chemicals

Tris(hydroxymethyl)aminomethane (Tris) was purchased from Aldrich (Milwaukee, WI, U.S.A.). Ovalbumin, bovine serum albumin (BSA), lactate dehydrogenase (LDH), catalase, and lactate were all purchased from Sigma (St. Louis, MO, U.S.A.).

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Apparatus

SynChropak AX300 and Q300 columns, 250 × 4.1 mm I.D. (particle size 6.5 μm), SynChropak AX1000 and Q1000, 250 × 4.6 mm I.D. (particle size 10 μm) and a SynChropak PCR column, 400 × 5.0 mm I.D. were obtained from SynChrom, (Linden, IN, U.S.A.). A Varian Model 5000 gradient high-performance liquid chromatograph with a Valco Model CV-6-UHPa-N-60 injection valve (Varian, Walnut Creek, CA, U.S.A.) and a Chem Research Model 2020 multiple-wavelength detector (Instrumentation Specialties, Lincoln, NE, U.S.A.) or an Anspec AN-203 UV detector (Anspec, Ann Arbor, MI, U.S.A.) were used for the analyses. A Linear Model 1200 recorder (Linear, Irvine, CA, U.S.A.) was used.

Methods

The buffers were prepared by adding the appropriate amount of sodium acetate to 0.02 *M* Tris in deionized water. Hydrochloric acid was used to adjust the pH. Standards were dissolved in the initial buffer.

Post-column reaction

The SynChropak PCR reactor was attached to the exit of the analytical column with a tee, into which a solution of the substrate was pumped at 0.8 ml/min.

The substrate for LDH is lactate, which is converted to pyruvate by LDH. Reduced nicotinamide–adenine dinucleotide (NADH) is simultaneously produced from the oxidized form (NAD) and is detected at 340 nm. The substrate solution consisted of 1.0 m*M* NAD and 0.75 *M* lactate in 0.2 *M* Tris, pH 7.

RESULTS AND DISCUSSION

The general procedure in this study was to vary the pH of the mobile phase and to observe the effects on the selectivity of four anion-exchange columns for the components of four different protein mixtures. The weak anion exchangers, SynChropak AX300 and AX1000, were polyamine bonded phases on 300 Å and 1000 Å supports, respectively. The strong anion exchangers, SynChropak Q300 and Q1000, were made by quaternizing the weak anion exchangers. The proteins used for evaluation, with the exception of lactate dehydrogenase, were of low purity to provide more peaks, and therefore, more information as to selectivity during purification.

Ovalbumin

The ovalbumin sample used in this study was composed of a major component with several minor peaks which were eluted earlier. Fig. 1 shows that decreased retention times of the major peak were seen on the weak anion exchangers at high pH, while on the strong anion exchangers, short retention times occurred at low pH. These phenomena resulted in similar chromatograms on both columns at pH 7 and almost identical chromatograms when the profile at pH 6 on Q300 is compared with that at pH 8 on AX300 (Fig. 2). More resolution was seen on the 300-Å columns than on the 1000 Å, as would be expected for a protein with a molecular weight of 45 000.

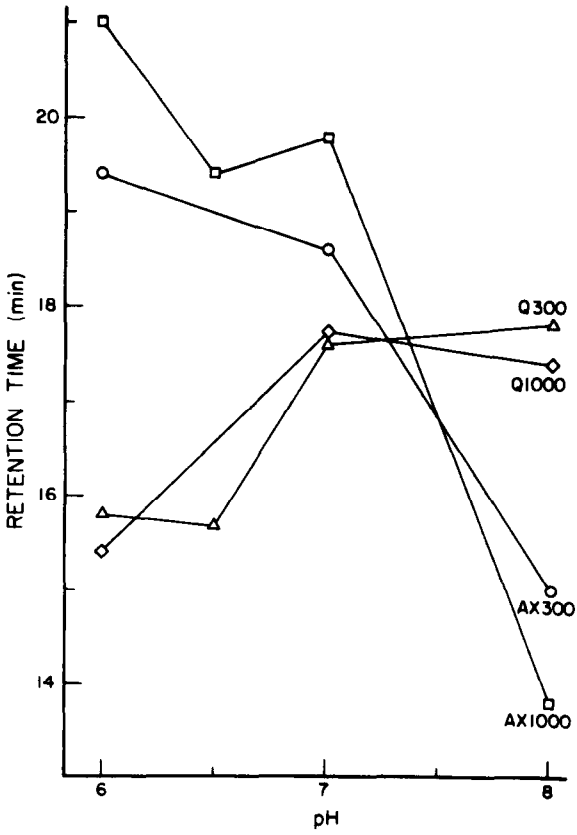

Fig. 1. Effect of pH on the retention of ovalbumin. Column, Synchropak AX300, 250 × 4.1 mm I.D.; flow-rate, 1 ml/min; pressure, 80 atm; buffer, 0.02 *M* Tris, 30 min gradient from 0–1 *M* sodium acetate.

Bovine serum albumin

Commercial BSA samples contain mixtures of BSA and its dimer, tetramer, etc. There was little change in the retention time of the major BSA peak on either the weak or the strong anion-exchange columns when the pH was varied from 6–8; however, the selectivity for separating BSA from its dimeric and tetrameric forms was greatest on all columns when the pH was less than 7 (Fig. 3). 300-Å pores showed better resolution than 1000-Å pores for both weak and strong anion exchangers.

Lactate dehydrogenase

LDH is an enzyme with five major isoenzymes and several minor ones. As the pH of the mobile phase was increased from 7 to 8, the resolution of the LDH isoenzymes improved; this was contrary to the effect seen for BSA. The improved resolution at pH 8 was mainly for the later-eluted peaks, and the improvements were not as significant as those seen for BSA. Fig. 4 illustrates that the weak and strong anion exchangers exhibit similar resolution of LDH isoenzymes at pH 8, but each has some specific selectivity in a different section of the gradient, as indicated by the arrows.

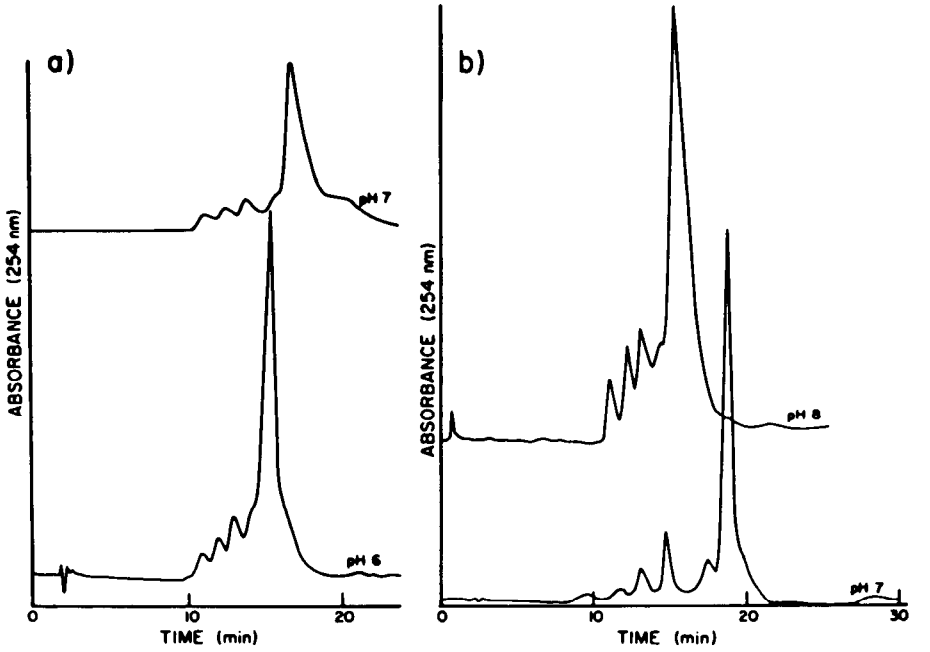

Fig. 2. (a) Analysis of ovalbumin by strong anion-exchange chromatography. Column, SynChropak Q300, 250 × 4.1 mm I.D. Conditions as in Fig. 1. (b) Analysis of ovalbumin by weak anion-exchange chromatography. Column, SynChropak AX300, 250 × 4.1 mm I.D. Conditions as in Fig. 1.

The pH affected the retention times of LDH isoenzymes in a manner similar to that of ovalbumin on both weak and strong anion exchangers.

Because the molecular weight of LDH is 140 000, it was expected that a larger pore might improve the resolution of its isoenzymes. However, Fig. 5 shows that the resolution on the 1000-Å columns is slightly less than that on the 300-Å materials. The difference is so slight, that it may be due to the fact that the 1000-Å supports are 10 μm and the 300-Å supports are 6.5 μm.

Catalase

The chromatographic profile of the catalase used in this study was very complex, with many poorly resolved peaks. Because catalase has a molecular weight of 230 000, it exhibited improved resolution when it was purified on a column material with 1000-Å pores. There was a drastic, but similar, loss in resolution on both the weak and strong anion exchangers as the pH was lowered from 8 to 6. Fig. 6 illustrates this pH effect on a 1000-Å strong anion-exchange column. This decrease in the retention times of some of the catalase components was observed on all the columns when the pH was decreased.

CONCLUSIONS

Similar, but not identical, selectivity could be obtained on weak and strong anion exchangers when they were used for the purification of four different protein

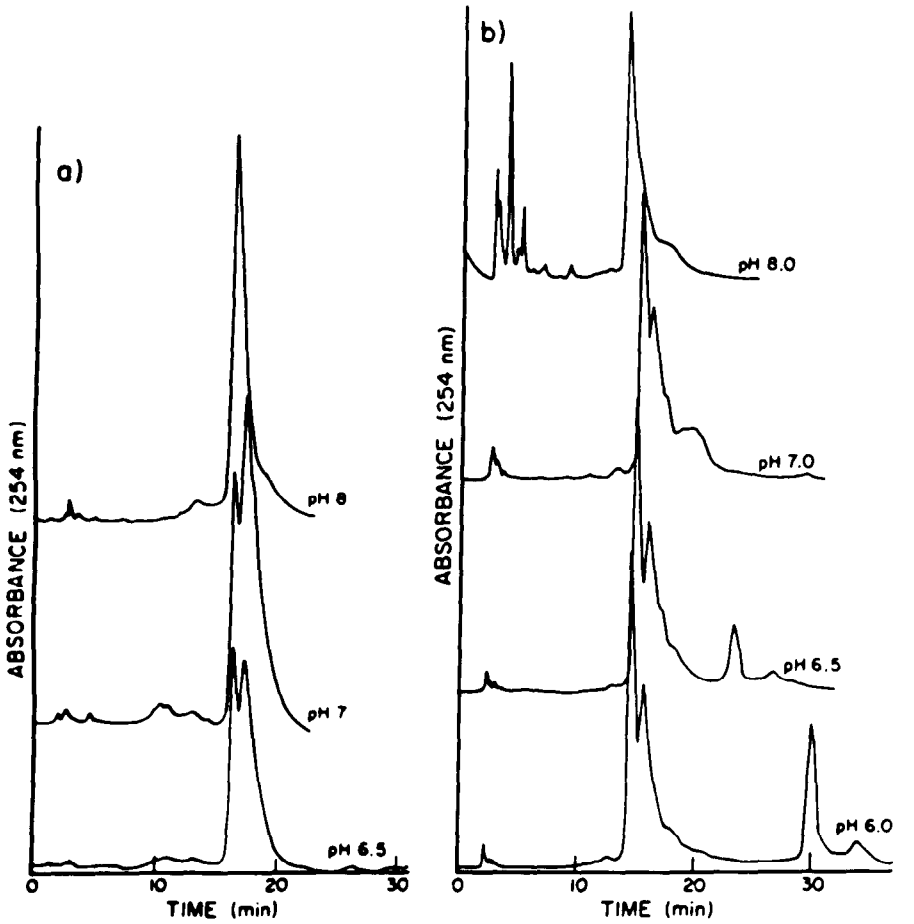

Fig. 3. (a) Analysis of BSA by strong anion-exchange chromatography. Column, SynChropak Q300, 250 × 4.1 mm I.D. Conditions as in Fig. 1. (b) Analysis of BSA by weak anion-exchange chromatography. Column, SynChropak AX300, 250 × 4.1 mm I.D. Conditions as in Fig. 1.

mixtures. The quaternized supports yielded decreased or unchanged retention times with decreasing pH. The weak anion-exchange materials, on the other hand, exhibited increased retention times as mobile phases with lower pH were used for the analyses of ovalbumin and LDH. Similar retention characteristics for ovalbumin on a weak anion exchanger were seen previously by Kopaciewicz and Regnier[3]. These phenomena can best be explained by examining the physical characteristics of the two types of supports.

The weak anion exchangers used in this study (SynChropak AX300 and AX1000) are composed of a cross-linked polyethyleneimine polymer which is adsorbed to the surface of porous silica. Chromatographically, this polymer behaves as if it is composed of tertiary amines, but it also contains a small number of primary and secondary amines. The titration curves are broad, with no inflection points between pH 9 and pH 2; therefore, the ionization of the support increases with a de-

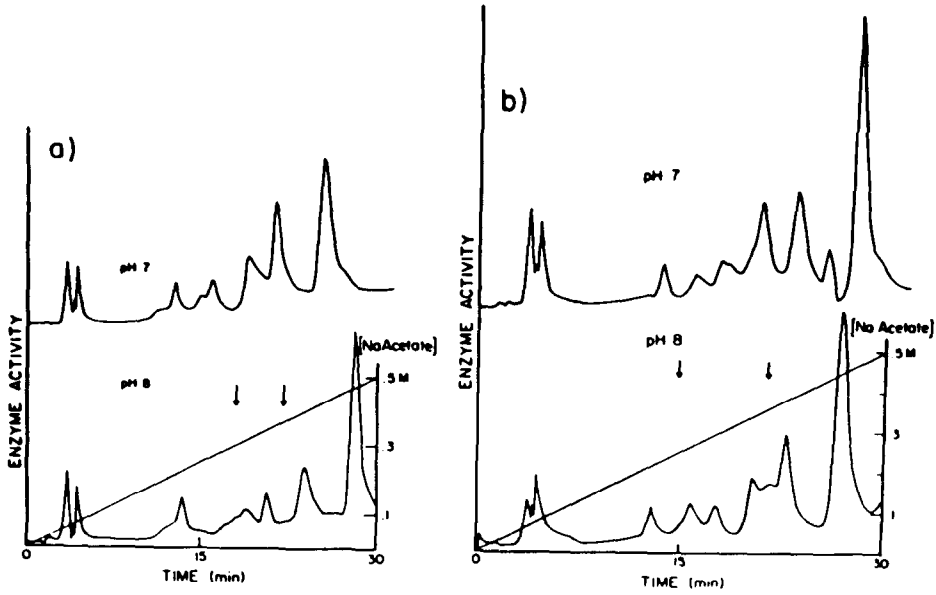

Fig. 4. (a) Analysis of LDH isoenzymes by strong anion-exchange chromatography. Column, SynChropak Q300, 250 × 4.1 mm I.D.; buffer, 0.02 *M* Tris, 30 min gradient from 0–0.5 *M* sodium acetate, flow-rate, 1 ml/min; pressure, 100 atm. Detection by post-column reaction as described under Experimental. (b) Analysis of LDH isoenzymes by weak anion-exchange chromatography. Column, SynChropak AX300, 250 × 4.1 mm I.D. Conditions as in a.

crease in pH. Some of the amines are inaccessible to proteins and other solutes due to the adsorbed nature of the coating[10].

The corresponding strong anion exchangers (SynChropak Q300 and Q1000) are synthesized by exhaustively methylating the weak anion exchangers. Because there is a sharp inflection in the titration curve between pH 8 and pH 3, the decreased

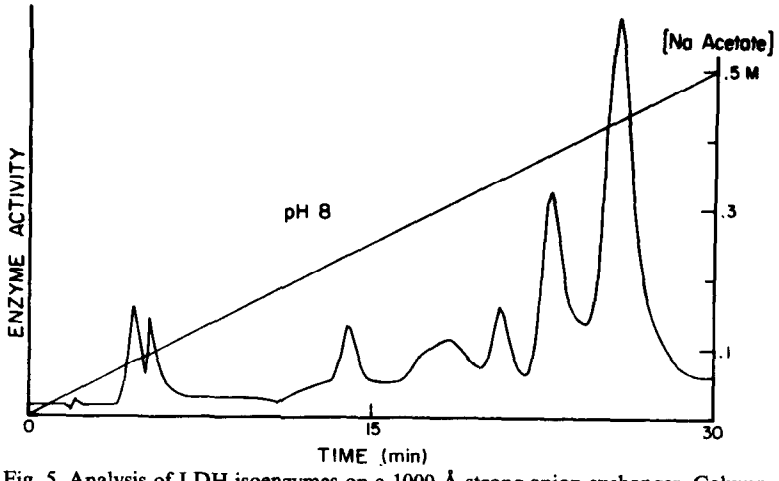

Fig. 5. Analysis of LDH isoenzymes on a 1000-Å strong anion exchanger. Column, SynChropak Q1000, 250 × 4.6 mm I.D. Conditions as in Fig. 4a.

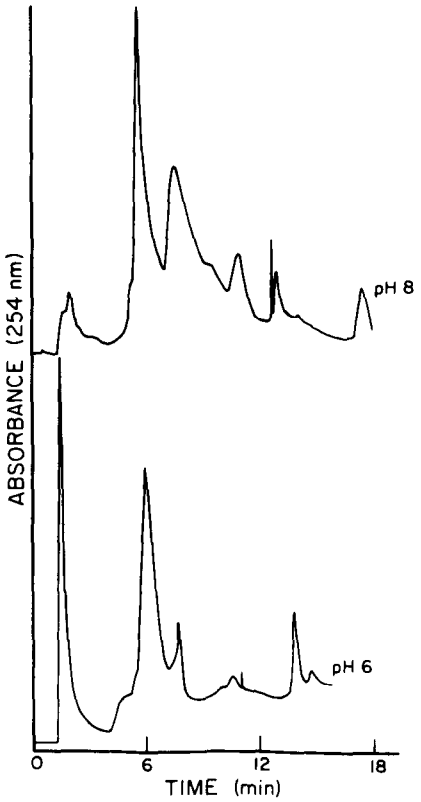

Fig. 6. Analysis of catalase by strong anion-exchange chromatography. Column, SynChropak Q1000, 250 × 4.6 mm I.D. Conditions as in Fig. 1.

retention times that were seen with decreased mobile phase pH would be due to the ionization of the protein, not to changes in the polymeric bonded phase.

These two polymeric layers are quite different in both spatial arrangement and chemical properties such as the adsorption to the silica and the relationship of neighboring amine groups. The quaternized amines would be more sterically hindered than the protonated tertiary amines; therefore, the weak anion exchanger would have stronger attraction for anions[8]. The polyamine that is not quaternized would exhibit differential hydration of the amines that would be dependent on environment, including the solvent composition[8]. This polymer becomes more hydrophilic as the salt concentration is increased or the pH is lowered. This change in the conformation of the layer was seen in the different titration curves that were obtained in 1 *M* salt and in water[4]. The nature of the associated water and ionic layers would also differ for the two types of polymer. The net effect would be that more of the backbone of the weak anion exchanger would be exposed, giving it a slightly hydrophobic character. Evidence of this slight hydrophobicity has also been seen by Kopaciewicz *et al.*[7]. This increased retention and selectivity could be exploited, especially for analyses at neutral pH. Improved resolution of BSA and catalase on all four columns at a specific pH appears to be purely a function of the physical characteristics of each protein and not of the chemical nature of the columns.

The effect of pore diameter was consistent with the effective available surface area, which is a function of protein volume, pore size, and surface area. 300-Å Pores gave superior resolution for proteins smaller than 100 000 in molecular weight. A protein with a molecular weight of 140 000 showed equivalent resolution on 300-Å and 1000-Å materials. The 1000-Å pore size was definitely better for the protein with a molecular weight of 230 000 daltons. This pore diameter effect is consistent with that seen in previous research[9]. Because loading capacity is also higher for proteins smaller than 100 000 daltons on 300-Å ion exchangers, they would be preferred over 1000-Å materials for analysis of proteins with molecular weights smaller than 150 000 (ref. 11).

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