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SIZE-EXCLUSION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF PEPTIDES

REQUIREMENT FOR PEPTIDE STANDARDS TO MONITOR COLUMN PERFORMANCE AND NON-IDEAL BEHAVIOUR

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SUMMARY

A series of five synthetic peptide polymers with the sequence Ac-(G-L-G-A-K-G-A-G-V-G)$_n$-amide, where n = 1–5, was employed to assess the resolving power of high-performance size-exclusion columns in peptide separations. The peptide standards showed great versatility in monitoring both ideal (no interactions of solutes with the column material) and non-ideal (hydrophobic and/or ionic interactions of solutes with the column material) size-exclusion behaviour in volatile and non-volatile mobile phases. The effectiveness of adding salts or organic solvents to overcome non-specific interactions of solutes with the column materials was well illustrated by the standards. In addition, the advantageous use of non-ideal size-exclusion behaviour was highlighted. The ability to predict the position and/or elution order of peptides during size-exclusion chromatography (SEC) requires peptides to be separated by a pure size-exclusion process. Although the peptide standards demonstrated similar ideal size-exclusion profiles in non-denaturing medium on all the columns studied, this study suggested that, if the conformational character of a peptide–protein mixture in a particular mobile phase is uncertain and ideal size-exclusion behaviour is required, SEC should be carried out under highly denaturing conditions.

INTRODUCTION

Despite the widespread application of high-performance size-exclusion chromatography (SEC) to the separation of proteins and polypeptides in recent years, relatively little attention has been paid to its potential for resolving peptides in the 200–5000 dalton range (2–50 residues). It would be extremely useful to extend SEC to peptide separations, possibly as the first step in the resolution of a complex peptide mixture. It is important in high-performance liquid chromatography (HPLC) to achieve good reproducibility of peptide separations. The need for standards to monitor column chromatography is well established, and protein standards are frequently used to demonstrate the resolving power of SEC columns or to calibrate columns for

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molecular weight determinations. The use of peptide standards designed specifically to monitor the peptide resolving capability of HPLC columns has only recently been addressed. Mant and Hodges[1] reported the great versatility of a series of five synthetic peptide standards in monitoring column performance for peptide separations during reversed-phase chromatography. Similarly, the availability of peptide standards designed for use on size-exclusion columns, would clearly be beneficial.

This paper describes the design and application of a series of synthetic peptide size-exclusion standards and attempts to demonstrate their versatility in monitoring SEC column performance for peptide separations. In addition, the utility of the standards in enabling rapid development of the optimal conditions for SEC of peptides is demonstrated.

EXPERIMENTAL

Materials

HPLC-grade water and acetonitrile were obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.). HPLC-grade trifluoroacetic acid (TFA) was obtained from Pierce (Rockford, IL, U.S.A.). A molecular-weight-marker kit of horse-heart myoglobin and its cyanogen bromide cleavage fragments was obtained from BDH (Poole, U.K.).

Peptide synthesis

The peptide standards described were synthesized on a Beckman Model 990 peptide synthesizer (Beckman Instruments, Berkeley, CA, U.S.A.) using the general procedure for solid-phase synthesis described by Parker and Hodges[2]. The size-exclusion standards are available from the Alberta Peptide Institute (Department of Biochemistry, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada).

Apparatus

The HPLC instrument consisted of a Varian Vista Series 5000 liquid chromatograph (Varian, Walnut Creek, CA, U.S.A.) coupled to a Hewlett-Packard (Avondale, PA, U.S.A.) HP 1040A detection system, HP85B computer, HP9121 disc drive, HP2225A Thinkjet printer and HP7470A plotter. Samples were injected with a 500-μl injection loop (Model 7125, Rheodyne, Cotati, CA, U.S.A.).

Columns

Peptide mixtures were separated on three size-exclusion columns: (1) SynChropak GPC60, 300 × 7.8 mm, I.D., 10-μm particle size, 60-Å pore size (SynChrom, Linden, IN, U.S.A.), (2) Altex Spherogel TSK G2000SW, 300 × 7.5 mm I.D., 10-μm, 130 Å (Beckman Instruments, Berkeley, CA, U.S.A.), and (3) Pharmacia Superose 12, 300 × 10 mm I.D., 10-μm (Pharmacia, Dorval, Canada). Total permeation volumes of the size-exclusion columns were determined by the elution time of TFA.

RESULTS AND DISCUSSION

Design of peptide standards

Separation of peptides by a mechanism based solely on peptide size (ideal SEC)

occurs only when there is no interaction between the solutes and the column matrix. Although high-performance size-exclusion columns are designed to minimize non-specific interactions, most modern SEC columns are weakly anionic (negatively charged) and slightly hydrophobic, resulting in deviations from ideal size-exclusion behaviour, *i.e.*, non-ideal SEC (nSEC)[3–7]. At the lower end of the fractionating range of a column, all small molecules should be eluted together under pure size-exclusion conditions. If they do not, the column is behaving in a non-ideal fashion. The volume of solvent required to elute small molecules in ideal SEC is the total permeation volume of the column. This column parameter is a combination of the void volume of the column (elution volume of a totally excluded species) and the pore volume of the size-exclusion matrix. By definition, under ideal size-exclusion conditions, no molecule will be retained beyond the total permeation volume of the column.

Peptide standards designed specifically for assessing the peptide resolving power of size-exclusion columns should be capable of monitoring both ideal and non-ideal chromatographic behaviour. To this end, a polymer series of five synthetic peptide standards was prepared, Ac-(G-L-G-A-K-G-A-G-V-G)$_n$-amide, where n = 1–5. The increasing size of the peptide standards (10, 20, 30, 40 and 50 residues; 800–4000 dalton) enables the accurate molecular-weight calibration of a column during ideal SEC; the increasingly basic character of the standards (1–5 positively charged residues) makes them sensitive to the anionic character of a size-exclusion column; the increasing hydrophobicity of the standards enables a determination of column hydrophobicity. In addition, the high glycine content of the standards minimizes or eliminates any tendency towards secondary structure. The basic character of the peptide standards also ensures good solubility in aqueous solvents.

Before using the standards to compare the separation properties of different SEC columns, it was important to verify that the synthetic polymer series could, indeed, exhibit ideal size-exclusion behaviour. The chromatographic profile of the five standards on a Spherogel TSK G2000SW silica-based column (Fig. 1), coupled with the linear character of the log MW *versus* peptide retention time plot, clearly demonstrates the ability of the polymer series to monitor pure size-exclusion behaviour on SEC columns.

Monitoring of non-ideal SEC with peptide standards

The ideal size-exclusion behaviour of the peptide standards illustrated in Fig. 1 was obtained with a volatile 0.1% aq. TFA eluent. The choice of a volatile mobile phase is certainly advantageous in peptide separations, since it enables lyophilization of peptide fractions prior to immediate analysis or their direct application to ion-exchange or reversed-phase columns. The results in Fig. 1 suggest that non-specific interactions of the standards with the TSK G2000SW packing were minimal in 0.1% aq. TFA. Similar results were reported by Lau *et al.*[8] and by Mant and Hodges[9] for TSK G3000SW packing. However, it should be noted that hydrophobic and/or electrostatic characteristics may be more pronounced with one size-exclusion column than with another[4] and the use of a simple, volatile mobile phase may be insufficient to suppress these interactions. For example, both the silica-based SynChropak GPC60 column and the agarose-based Superose 12 column exhibited non-ideal behaviour with 0.1% aq. TFA as eluent. Electrostatic effects between solutes and the column matrix may be minimized by the addition of salts to the eluent. Aqueous

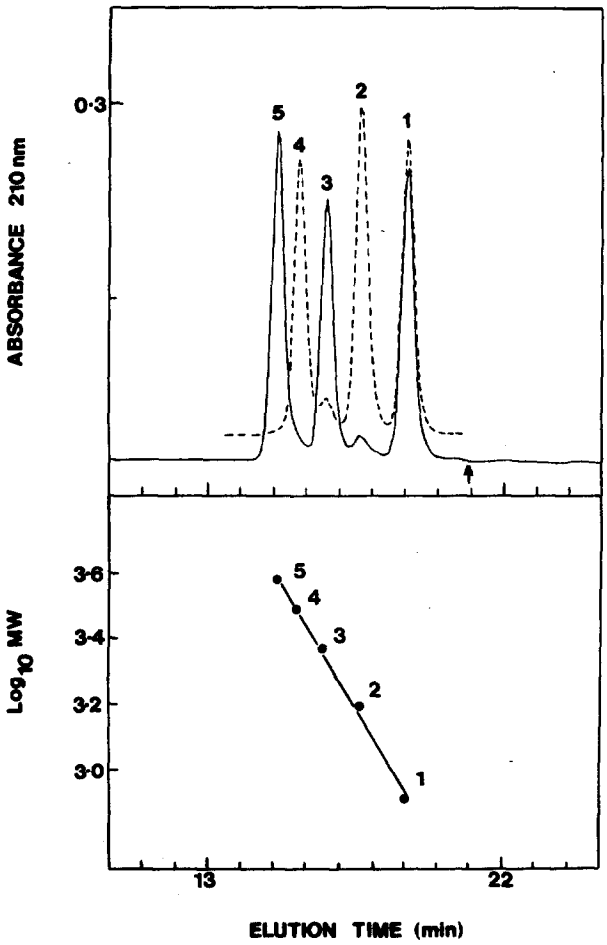

Fig. 1. SEC of a mixture of synthetic peptide standards. Top: elution profile of peptide standards obtained with an Altex Spherogel TSK G2000SW column (300 × 7.5 mm I.D.); mobile phase, 0.1% aq. TFA (pH 2.0); flow-rate, 0.5 ml/min; temperature, 26°C; absorbance at 210 nm. Bottom: plot of log molecular weight *versus* retention time of the peptide standards. The peptide mixture consisted of five peptides with the sequence Ac-(G-L-G-A-K-G-A-G-V-G)$_n$-amide, where n denotes the number of decapeptides (1–5). The arrow denotes the elution time for the total permeation volume of the column.

buffers containing 0.1 *M*–0.4 *M* salt are commonly employed as the mobile phase for SEC. Fig. 2 demonstrates the effect of increasing concentrations of potassium chloride in 0.1% aq. TFA on the elution profile of peptide standards 1, 2 and 5 on a SynChropak GPC60 column. This column is clearly exhibiting non-specific interactions between the peptides and column matrix at low potassium chloride concentrations, the smallest peptide (peptide standard 1, 10 residues) being eluted first and the largest peptide (peptide standard 5, 50 residues) being eluted last. In addition, all three peptides are being retained longer than the total permeation volume of the column (denoted by arrow). The column is, in fact, behaving like a cation-exchange column[3–7], the peptides being eluted in the order of increasing positive charge (peptide

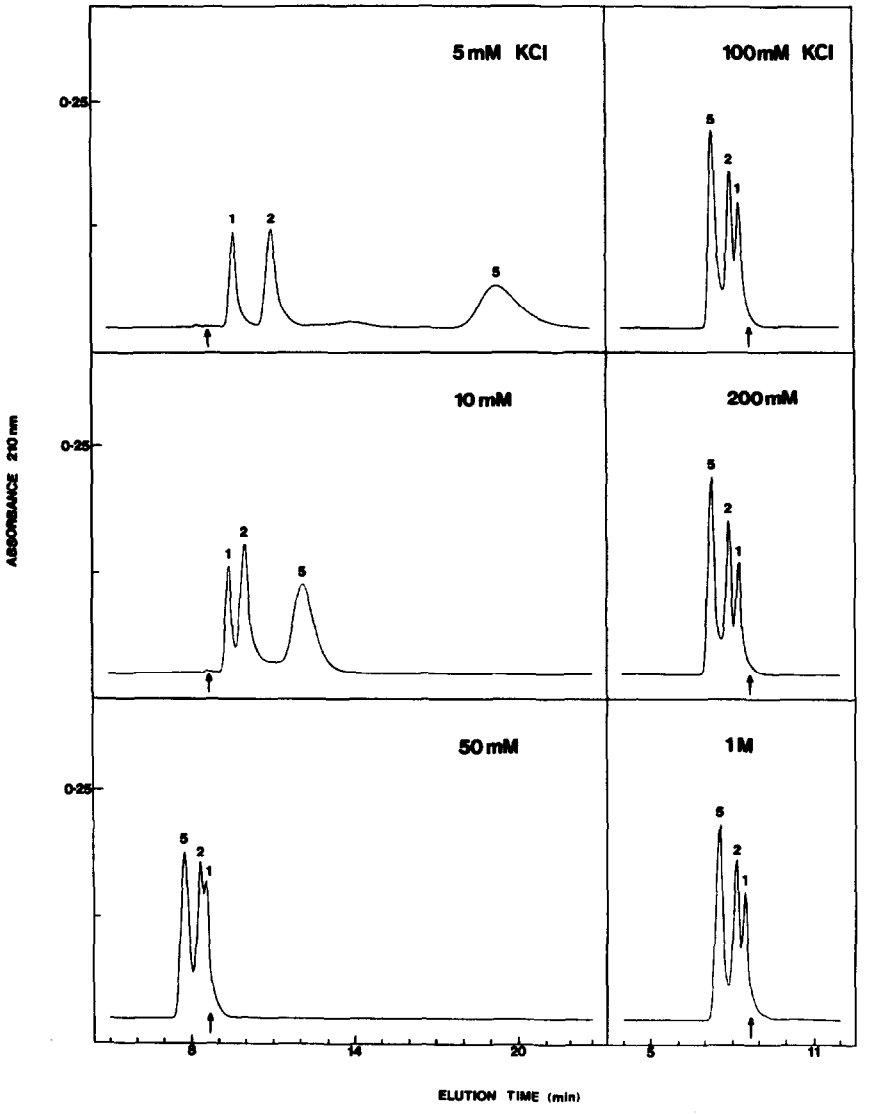

Fig. 2. Effect of salt on non-specific interactions in SEC at pH 2.0. Column, SynChropak GPC60 (300 × 7.8 mm I.D.); mobile phase, 0.1% aq. TFA (pH 2.0), containing 5 m*M*, 10 m*M*, 50 m*M*, 100 m*M*, 200 m*M*, or 1 *M* potassium chloride; flow-rate, 1 ml/min; temperature, 26°C; absorbance at 210 nm. Synthetic peptide standards 1, 2 and 5 contain 10, 20 and 50 residues, respectively (see Fig. 1). The arrows denote the elution time for the total permeation volume of the column.

standards 1, 2, 5 possess a +1, +2 and +5 net charge, respectively) instead of decreasing size. As the ionic strength of the mobile phase increases, these electrostatic effects are gradually overcome until an essentially ideal size-exclusion mechanism is apparent at a salt concentration of 200 m*M*. An increase in the potassium chloride concentration to 1 *M* does not improve the chromatographic profile of the standards.

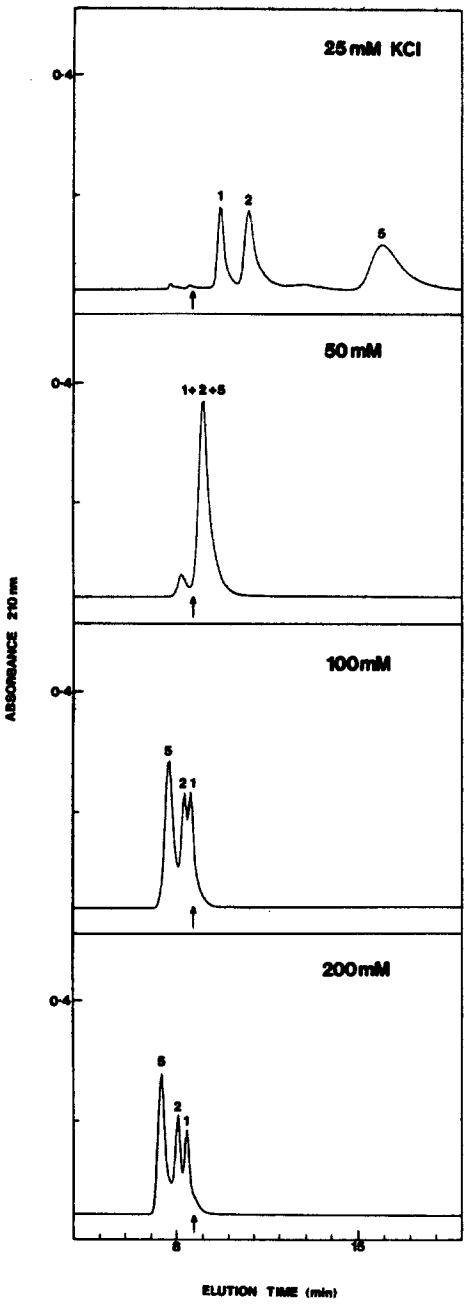

Fig. 3. Effect of salt on non-specific interactions in SEC at pH 6.5. Column, SynChropak GPC60 (300 × 7.8 mm I.D.); mobile phase, 5 m*M* KH_2PO_4 (pH 6.5), containing 25 m*M*, 50 m*M*, 100 m*M*, or 200 m*M* potassium chloride; flow-rate, 1 ml/min; temperature, 26°C; absorbance at 210 nm. Synthetic peptide standards 1, 2 and 5 contain 10, 20 and 50 residues, respectively (see Fig. 1). The arrows denote the elution time for the total permeation volume of the column.

In fact, there is a slight deterioration in peptide resolution and an increase in peptide retention times, possibly due to promotion of hydrophobic interactions at this high salt level. These results support the view that high-ionic-strength solvents (>0.6 *M*) should generally be avoided in SEC[3].

Different peptide mixtures may require eluents of markedly different pH values for optimal SEC separation. The effect of pH variations may not only affect the net charge of a particular peptide, but may also influence the non-specific interaction characteristics of the size-exclusion column matrix. Fig. 3 demonstrates the effect of

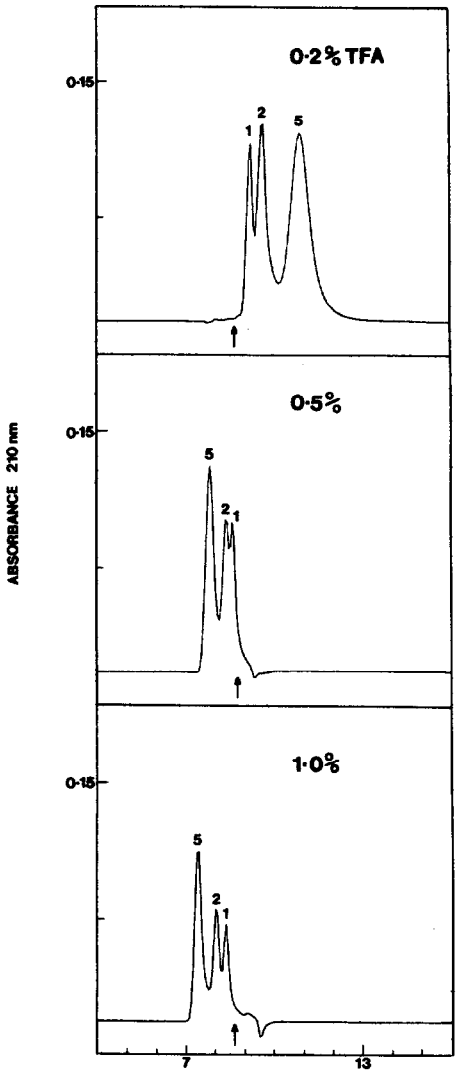

Fig. 4. Effect of increasing concentration of TFA on non-specific interactions in SEC. Column, SynChropak GPC60 (300 × 7.8 mm I.D.); mobile phase, 0.2, 0.5, or 1.0% aq. TFA; flow-rate, 1 ml/min; temperature, 26°C; absorbance at 210 nm. Synthetic peptide standards 1, 2 and 5 contain 10, 20 and 50 residues, respectively (see Fig. 1). The arrows denote the elution time for the total permeation volume of the column.

increasing concentrations of potassium chloride in 5 m*M* KH_2PO_4 buffer (pH 6.5) on the elution profiles of peptide standards 1, 2 and 5 for the GPC60 column. The results are similar to those illustrated in Fig. 2, where a more acidic mobile phase was used. However, a higher salt concentration was required at the higher pH to suppress the ion-exchange behaviour of the column. This is particularly well demonstrated by comparing the 50 m*M* potassium chloride profiles of Figs. 2 and 3. At pH 2.0 (Fig. 2), pure size-exclusion has almost been obtained, the three peptide standards being eluted in the correct order and at retention times less than the total permeation volume of the column. In contrast, at pH 6.5, all three peptides are eluted as a single peak and are still being retained slightly more than the total permeation volume.

Salts suppress non-specific ionic interactions of peptides with a SEC column matrix by competing with the matrix for interaction with the charged groups on the peptides. The results presented in Fig. 4 demonstrate that a similar effect may be obtained by increasing the concentration of aq. TFA in the mobile phase. The increasing TFA concentration parallels the effect of increasing potassium chloride concentration (Figs. 2 and 3) in suppressing non-ideal SEC behaviour of the GPC60 until essentially pure size-exclusion resolution of peptide standards 1, 2 and 5 is obtained at 1% aq. TFA. However, although 1% aq. TFA is an effective and volatile peptide solvent, the deleterious effect of high concentrations of acids would prohibit excessive use of this system with silica-based column materials[10].

Several researchers have demonstrated the utility of adding volatile organic solvents, such as acetonitrile or trifluoroethanol, to 0.1% aq. TFA for effective separations of peptides and proteins[8,11–14]. A volatile aqueous triethylammonium formate buffer (0.25 *M*; pH 3.0) containing 30% acetonitrile has also proved useful for the separation of peptides and proteins[15]. Apart from their UV transparency, these organic modifiers decreased non-specific hydrophobic adsorption of peptides on the SEC matrix and increased overall solubility of proteins. Fig. 5 demonstrates the effect of increasing acetonitrile concentrations in 0.1% aq. TFA on the elution profiles of the five peptide standards on the GPC60 column. As the concentration of the organic modifier increases, the retention times of all five peptides decrease to a minimum, presumably as hydrophobic interactions are overcome, and then proceed to increase again. The elution profiles of the peptides are, in fact, very similar at the 0% and 60% levels of acetonitrile. Plotting retention times of the peptide standards *versus* the level of organic modifiers in the eluent (Fig. 6) illustrates dramatically the effect of a wide range of acetonitrile concentrations on the non-ideal size-exclusion behaviour of the column. As the acetonitrile concentration in the eluent increases from 35% the peptides are bound more tightly to the SEC column until even very prolonged elution times (> 2 h) fail to remove the standards from the column. The results suggest that, following reduction of peptide elution times to a minimum (with *ca.* 35% acetonitrile), further increases in acetonitrile concentration are promoting ionic interactions of the standards with the column material. Figs. 5 and 6 also demonstrate that ideal size-exclusion behaviour of the peptide standards is never obtained on the GPC60 column with this volatile mobile phase. In contrast, the addition of salt to the 0.1% aq. TFA did produce pure size-exclusion behaviour of the peptide standards (Fig. 2). These results indicate that not only is the GPC60 exhibiting both electrostatic and hydrophobic interactions with the peptide standards, but also that electrostatic interactions are dominant. The inclusion of 35% (v/v) acetonitrile in 0.1% aq. TFA

containing 200 m*M* potassium chloride did not improve the pure size-exclusion profile of peptide standards 1, 2 and 5, demonstrated in Fig. 2. This suggests that the addition of salt is disrupting hydrophobic as well as electrostatic interactions.

Advantages of non-ideal SEC

The majority of reports in the literature concerning SEC of peptides and pro-

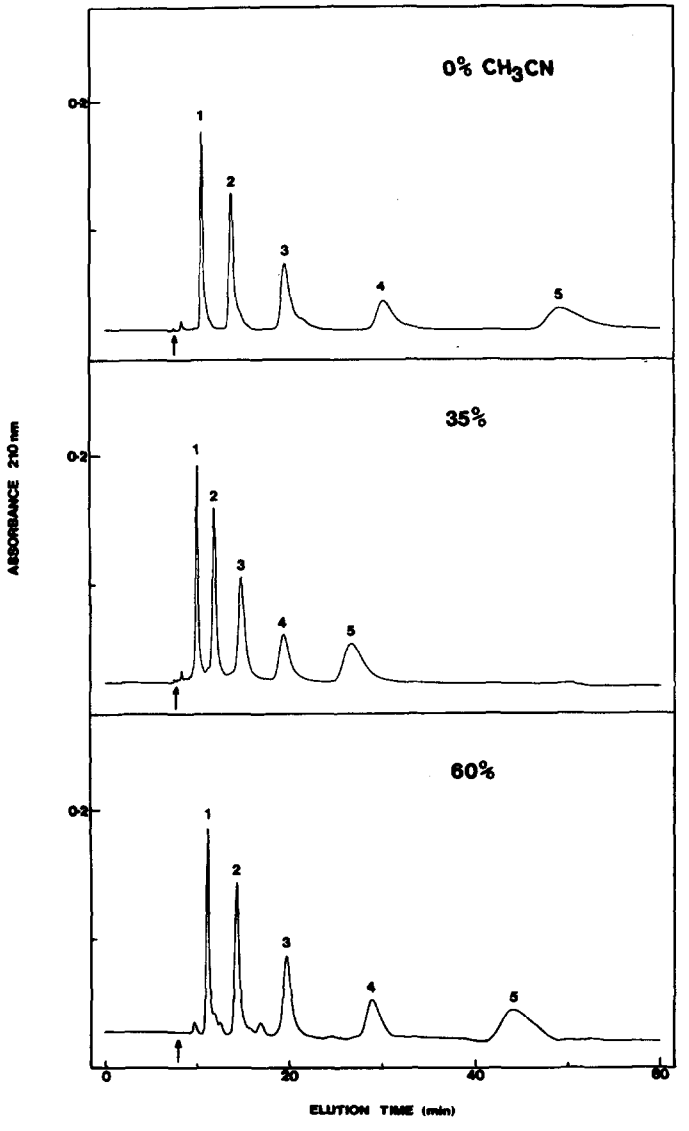

Fig. 5. Effect of increasing acetonitrile concentrations on non-specific interactions in SEC. Column, Syn-Chropak GPC60 (300 × 7.8 mm I.D.); mobile phase, 0.1% aq. TFA, containing 0, 35, or 60% acetonitrile; flow-rate, 1 ml/min; temperature, 26°C; absorbance at 210 nm. Synthetic peptide standards 1, 2, 3, 4 and 5 contain 10, 20, 30, 40 and 50 residues, respectively (see Fig. 1). The arrows denote the elution time for the total permeation volume of the column.

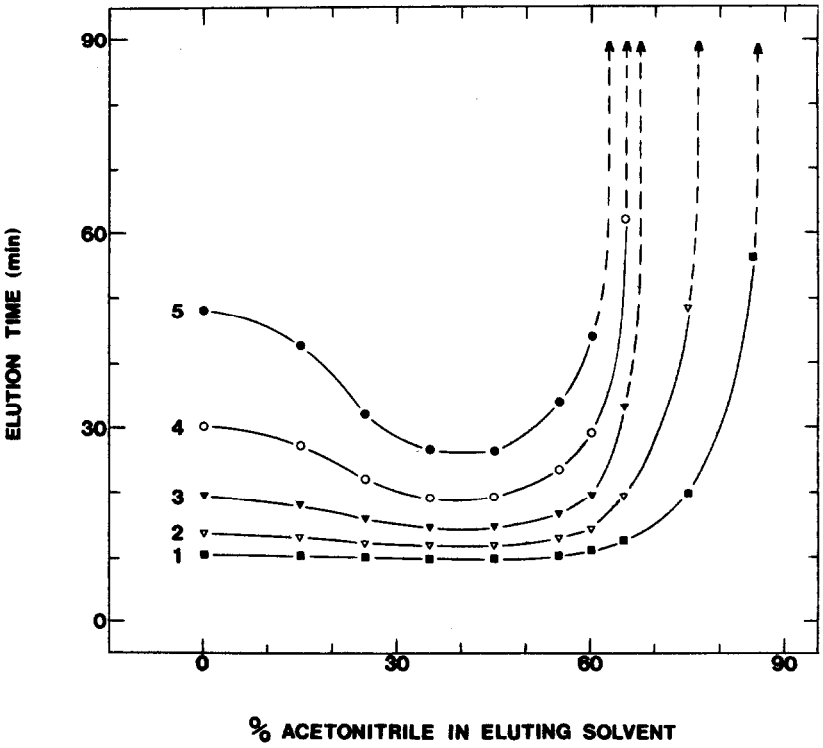

Fig. 6. Effect of acetonitrile concentration on peptide elution times in SEC. Column, SynChropak GPC60 (300 × 7.8 mm I.D.); mobile phase, 0.1% aq. TFA containing 0–85% acetonitrile; flow-rate, 1 ml/min; temperature, 26°C; absorbance at 210 nm. Synthetic peptide standards 1, 2, 3, 4 and 5 contain 10, 20, 30, 40 and 50 residues, respectively (see Fig. 1).

teins tend to describe chromatographic conditions designed to ensure a pure size-exclusion process. It is often overlooked that the non-ideal properties of size-exclusion columns can be advantageous in the separation of peptides[7]. From the results presented above (Figs. 2–6), it is clear that the separation of the peptide standards on the GPC60 column is markedly better when the column is acting as a cation-exchanger than under ideal size-exclusion conditions. The elution time required to elute all five peptide standards from the GPC60 with 0.1% aq. TFA (flow-rate, 1 ml/min) is rather excessive (48 min) (Fig. 7A). However, manipulation of the cation-exchange properties of the column by eluting the peptides with shallow TFA or salt gradients produces the excellent elution profiles demonstrated in Fig. 7B and C, respectively. The results of the salt gradient (Fig. 7C) make an interesting comparison with the elution profile of the peptide standards obtained on a Mono S polymer-based strong-cation-exchange column (Fig. 7D). The rate of increasing salt concentration employed to elute the standards from the ion-exchange column (5 m*M* potassium chloride/min) is five times the rate required to obtain a similar elution profile with the GPC60 (1 m*M* potassium chloride/min). The higher salt concentration required to elute the peptides from the Mono S within a similar time is due to a greater

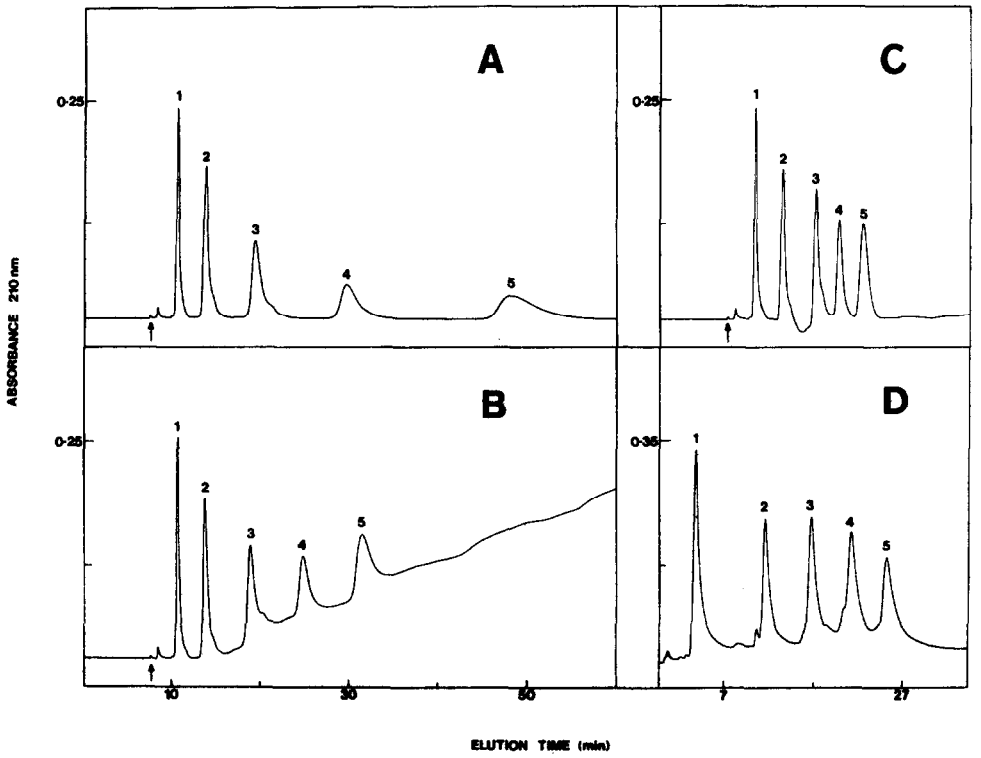

Fig. 7. Cation-exchange chromatography on a size-exclusion column. A–C: column, SynChropak GPC60 size-exclusion column (300 × 7.8 mm I.D.). Mobile phase: (A) 0.1% aq. TFA, (B) linear AB gradient, where solvent A is 0.1% aq. TFA and solvent B is 0.2% aq. TFA (100% A to 100% B in 50 min), and (C) linear AB gradient (1 m*M* potassium chloride/min), where solvent A is 0.1% aq. TFA and solvent B is 0.1% aq. TFA containing 30 m*M* potassium chloride; flow-rate, 1 ml/min; temperature, 26°C; absorbance at 210 min. D: column, Pharmacia Mono S HR 5/5 strong-cation-exchange column (50 × 5 mm I.D.); mobile phase, linear AB gradient (5 m*M* potassium chloride/min), where buffer A is 5 m*M* KH_2PO_4 (pH 6.5) and buffer B is 5 m*M* KH_2PO_4 containing 1 *M* potassium chloride; flow-rate, 1 ml/min; temperature, 26°C; absorbance at 210 nm. Synthetic peptide standards 1, 2, 3, 4 and 5 contain 10, 20, 30, 40 and 50 residues, respectively, and 1, 2, 3, 4 and 5 positively charged groups, respectively (see Fig. 1). Arrows denote the elution time for the total permeation volume of the size-exclusion column (Panels A–C).

number of negatively-charged functional groups on the Mono S ion-exchange column. Thus, the GPC60 has the potential to be used in either of two modes, depending on the eluent buffers.

Ideal SEC

The ability to predict the position and/or elution order of peptides during SEC of a peptide mixture would greatly simplify preliminary isolation of peptides from a chemical or proteolytic protein digest. Under conditions of ideal SEC, large peptide fragments, resulting from incomplete protein digestion, can be quickly identified and removed. The peptide standards enable an accurate comparison of the resolving power of different SEC columns under conditions which promote pure size-exclusion

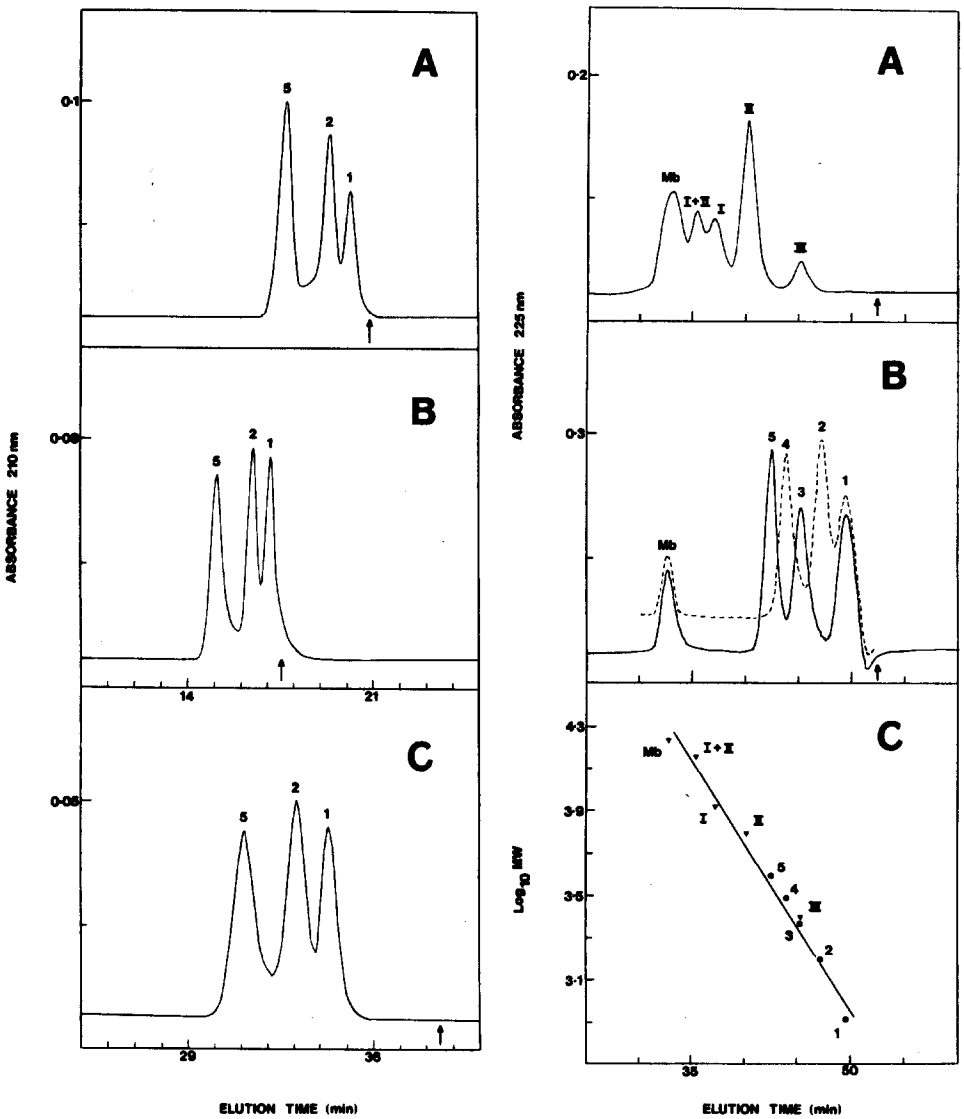

Fig. 8. Comparison of ideal SEC profiles of a mixture of synthetic peptide standards obtained with different size-exclusion columns. Columns: (A) Altex Spherogel TSK G2000SW (300 × 7.5 mm I.D.), (B) SynChropak GPC60 (300 × 7.8 mm I.D.), and (C) Pharmacia Superose 12 (300 × 10 mm I.D.). Mobile phase, 50 m*M* KH_2PO_4 (pH 6.5)/0.1 *M* potassium chloride; flow-rate, 0.5 ml/min; temperature, 26°C; absorbance at 210 nm. Peptide standards 1, 2 and 5 contain 10, 20 and 50 residues, respectively (see Fig. 1). The arrows denote the elution time for the total permeation volumes of the columns.

Fig. 9. Ideal SEC of protein fragments and a mixture of synthetic peptide standards. Column, Altex Spherogel TSK G2000SW (300 × 7.5 mm I.D.); mobile phase, 50 m*M* KH_2PO_4 (pH 6.5)–0.5 *M* potassium chloride–8 *M* urea; flow-rate, 0.2 ml/min; temperature, 26°C; absorbance at 225 nm. (A) Elution profile of horse-heart myoglobin (Mb) and its cyanogen bromide cleavage fragments (I, II, I + II, III); (B) elution profile of horse-heart Mb and synthetic peptide standards 1–5; (C) plot of log MW *versus* retention time of Mb, cyanogen bromide fragments of Mb, and the five synthetic peptide standards. Peptide standards 1, 2, 3, 4 and 5 contain 10, 20, 30, 40 and 50 residues, respectively (see Fig. 1). The arrows denote the elution time for the total permeation volume of the column (A and B).

behaviour. Fig. 8 compares the elution profiles of peptide standards 1, 2 and 5 obtained with a Spherogel TSK G2000SW column (Fig. 8A), a Synchropak GPC60 column (Fig. 8B), and a Superose 12 column (Fig. 8C), using a non-denaturing mobile-phase buffer [50 m*M* KH_2PO_4 (pH 6.5) + 0.1 *M* potassium chloride] and a flow-rate of 0.5 ml/min. All three columns exhibit similar peptide elution profiles under ideal size-exclusion conditions. The longer retention times and somewhat broader peptide peaks obtained with the Superose 12 are probably due to its significantly larger column volume compared to the two silica-based columns.

To achieve the full potential of peptide retention time prediction on SEC columns, it is important to obtain a linear relationship between log MW or ln MW over a wide molecular-weight range. Rivier[15] reported this linear relationship for peptides over a molecular-weight range of 1000–44000 dalton, using a Waters PAC I-125 column and an aq. triethylammonium buffer (0.25 *M*, pH 2.25) containing 15–30% acetonitrile. Similar results have been obtained using volatile solvents and a TSK G3000SW (900–8000 dalton)[8] or TSK G3000PW (300–134000 dalton[12] and 300–7000 dalton[11,14]) columns. These results and the pure size-exclusion profiles of the peptide standards demonstrated in Figs. 1 and 8 were obtained under non-denaturing conditions. However, under non-denaturing conditions, many proteins and large peptides may deviate from ideal size-exclusion behaviour due to conformational changes. Thus, the tendency of peptides or protein fragments to maintain or reform a particular conformation as opposed to a random coil configuration in non-denaturing media will complicate retention time prediction. In the present study, for instance, a linear log MW *versus* retention time relationship for horse-heart myoglobin and its cyanogen bromide fragments (2500–17000 dalton) for the TSK G2000SW, GPC60 and Superose 12 columns could only be obtained under highly denaturing conditions [50 m*M* KH_2PO_4 (pH 6.5)–0.5 *M* potassium chloride–8 *M* urea]. Fig. 9 demonstrates this linear relationship and elution profiles of myoglobin, its cyanogen bromide fragments and the five synthetic peptide standards for the Spherogel TSK G2000SW column. The peptide fragments produced by cyanogen bromide cleavage of myoglobin constitute substantial portions of the whole protein (fragment I, 8159 dalton; II, 6214 dalton; I + II [the result of incomplete cleavage], 14404 dalton; III, 2512 dalton) and maintenance of a folded structure or reassociation of the large fragments in non-denaturing media is most likely. In fact, molecular-weight determinations in the presence or absence of denaturants in SEC can provide a means of demonstrating tertiary or quaternary structures in peptides. Lau *et al.*[16] eloquently demonstrated the formation of two-stranded α-helical-coiled coils from 29- and 36-residue peptides. However, the results of the present study suggest that if the conformational character of a peptide-protein mixture in a particular mobile phase is uncertain and ideal size-exclusion behaviour is desired, SEC should always be carried out under highly denaturing conditions. It is interesting to note that the ideal size-exclusion profile of peptide standards 1, 2 and 5 on the TSK G2000SW column is somewhat improved when 0.1% aq. TFA is used as the mobile-phase solvent (Fig. 1), compared to the profiles obtained in non-denaturing (Fig. 8) or denaturing (Fig. 9) phosphate buffers. The presence of salt in the eluent apparently has an as yet unexplained deleterious effect on the resolution of peptides at the lower end of the fractionating range of the TSK G2000SW column.

The adoption of a common set of peptide standards by chromatographers

utilizing SEC could greatly facilitate an accurate comparison of results between different researchers. Indeed, for anyone involved in peptide separation, peptide standards are essential for comparing different columns and identical columns from different manufacturers. The polymer series of synthetic peptide standards described in the present study has proved extremely beneficial in enabling rapid development of the optimal conditions for SEC of peptides and for monitoring the resolving power of various size-exclusion columns. The results of this paper also suggest that the practical value of SEC columns in the pure size-exclusion mode is, at present, rather limited for resolution of peptides (up to 50 residues). Thus, although size-exclusion columns are of great potential value in the early stages of peptide purification, size-exclusion HPLC columns with the correct fractionation range are not yet available. The high cost and rapid deterioration of peptide separation on currently available columns, designed mainly for protein separations, makes their purchase prohibitive. A size-exclusion column designed specifically for peptides has yet to be produced; it will probably require pore-size diameters less than any currently available diameters.

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REFERENCES

1 C. T. Mant and R. S. Hodges, *LC. GC*, 4 (1986) 250.
2 J. M. R. Parker and R. S. Hodges, *J. Protein Chem.*, 3 (1985) 465.
3 F. E. Regnier, *Methods Enzymol.*, 91 (1983) 137.
4 E. Pfannkoch, K. C. Lu and F. E. Regnier, *J. Chromatogr. Sci.*, 18 (1980) 430.
5 H. Engelhardt and D. Mathes, *Chromatographia*, 14 (1981) 325.
6 H. Engelhardt, G. Ahr and M. T. W. Hearn, *J. Liq. Chromatogr.*, 4 (1981) 1361.
7 W. Kopaciewicz and F. E. Regnier, *Anal. Biochem.*, 126 (1982) 8.
8 S. Y. M. Lau, A. K. Taneja and R. S. Hodges, *J. Chromatogr.*, 317 (1984) 129.
9 C. T. Mant and R. S. Hodges, *J. Chromatogr.*, 326 (1985) 364.
10 D. Guo, C. T. Mant and R. S. Hodges, *J. Chromatogr.*, 386 (1987) 205.
11 G. D. Swergold, O. M. Rosen and C. S. Rubin, *J. Biol. Chem.*, 257 (1982) 4207.
12 G. D. Swergold and C. S. Rubin, *Anal. Biochem.*, 131 (1983) 295.
13 A. K. Taneja, S. Y. M. Lau and R. S. Hodges, *J. Chromatogr.*, 317 (1984) 1.
14 H. Sasaki, T. Matsuda, O. Ishikawa, T. Takamatsu, K. Tanaka, Y. Kato and T. Hashimoto, *Scientific Report of Toyo Soda Mfg. Co. Ltd.*, 29 (1985) 3.
15 J. E. Rivier, *J. Chromatogr.*, 202 (1980) 211.
16 S. Y. M. Lau, A. K. Taneja and R. S. Hodges, *J. Biol. Chem.*, 259 (1984) 13253.