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Thermodynamic studies of pressure-induced retention of peptides in reversed-phase liquid chromatography

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Abstract

The pressure-induced retention of peptides on reversed-phase HPLC was studied by systematically changing organic solvent composition and temperature at both low (19 bar) and high (318 bar) pressures using a homologous series of hydrophobic poly-L-phenylalanine ($n = 2$–7) as the model compound. Based on van't Hoff plots under different organic solvent compositions and pressures, the enthalpy change for the solute (ΔH) was determined. Moreover, both the enthalpy and entropy change for each phenylalanine residue ($\Delta\Delta H$ and $\Delta\Delta S$), which corresponds to solute retention on a microenvironment along the depth of C18 chain, were also calculated by direct subtractions. Results indicate that under acetonitrile (ACN) compositions above 35%, the pressure caused $\Delta\Delta S$ value to change from a negative to a positive value and both ΔH and $\Delta\Delta H$ to change from a negative to a less negative value, all leading to a thermodynamic state closer to those under 35% acetonitrile composition. This implies that the pressure-induced retention observed in this study was an entropy-favored but enthalpy-unfavored process and was explained by pressure-induced desorption of solvent molecules that were associated with the stationary phase or with the peptide solute. Under 35% acetonitrile composition, however, it was found that neither $\Delta\Delta H$ nor $\Delta\Delta S$ value was significantly changed by the pressure. Whereas, both ΔH value and the intercept of van't Hoff plots under 35% acetonitrile composition were increased by pressure. This indicates that under low organic solvent composition, 35%, most of the acetonitrile molecules adsorbed on the surface of the stationary phase and only little solvent molecules were dissolved in the bulk stationary phase where the phenylalanine residues were partitioned. This study has provided new thermodynamic insights to the pressure-induced retention for peptides and proteins.

Keywords: Retention, pressure-induced; Thermodynamic parameters; Peptides

1. Introduction

Although liquid solution is normally considered to be incompressible, recent studies [1–9] indicate that, even in the LC pressure regime (<350 bar), solute retention in different modes of chromatographic methods can be significantly altered. These influences are achieved through shifts in many interaction equilibria that govern the solute retention such as ionization, complexation, and hydrophobic interactions [1–9] depending on the retention mode that is used in chromatographic separation. There is a review regarding pressure effects in HPLC, particularly for the influence of pressure and pressure changes on peak shape, base line, and retention volume in HPLC separation [10]. The primary mechanism determining the solute retention in reversed-phase liquid chromatography depends mainly on the hydrophobic interaction. For proteins, the retention process can be considered as adsorption of the solute at the hydrophobic stationary surface. The retardation is based on a hydrophobic association between the solute and the hydrophobic ligands of the surface and is described by the solvophobic theory, advocated by Vailaya and Horváth [11,12]. By increasing the organic solvent composition of the mobile phase in a gradient elution, the attraction force is weakened and the solute is eluted. At a constant temperature, the pH value of the mobile phase controls ionization of carboxylic acid and amine groups on the protein surface; the pairing agents such as trifluoroacetic acid (TFA) form ion pairs with the proteins to increase the solute retention on the hydrophobic stationary phase [13,14]. Therefore, protein retention is very sensitive with the variation of many chromatographic parameters [15,16]. On the other hand, because of the folding structure of proteins, conformational changes of proteins may be induced

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during the chromatographic separation and lead to a different retention mechanism [15–21]. For example, a non-polar environment, such as the hydrophobic stationary phase, may induce helical structures in potentially helical molecules [22]. If a molecule becomes helical on binding and contains a preferred binding domain, as in the case of an amphipathic α-helix, then obviously some residues may not be interacting with the reversed-phase sorbent [22]. Moreover, under a gradient elution, an organic solvent-induced conformational change may promote the elution of the adsorbed proteins from the hydrophobic stationary phase [21,23]. Pressure-induced denaturation of proteins, which is potentially useful for preparative scale of protein purification, has been reported to have a reaction volume on the order of minus tens to hundreds of ml/mol [24] under a high pressure regime (>1 kbar) [25,26]. In the medium pressure range for chromatographic separation, increasing the average column pressure has been found to be a costly but efficient way to increase the loading capacity of the column, hence the production rate in preparative chromatography [2].

Among the pressure studies of chromatographic separation of proteins, bovine insulin [3], lys-pro insulin, human insulin, porcine insulin [1,2], chicken egg white lysozyme [4], and poly-phenylalanine [4] have been found to show a pressure-induced retention under chromatographic conditions. Most of these studies have attributed the change to the decrease in solute molar volume (ΔV) on the range of −100 to −130 ml/mol for proteins and −50 to −70 ml/mol for peptides, which are much greater than that for typical model solutes such as the methylene homologues that ranges from −0.76 to −17 ml/mol per ethylene group [7]. The chromatographic retention process is governed by changes in the Gibbs free energy, which includes both enthalpy and entropy terms and the pressure effect on the volume change is explicitly expressed in the enthalpy term. Whereas, the pressure may implicitly affect the entropy term since the packing structure of the hydration layer is likely to be changed by the pressure. Results obtained from insulin variants on C8 bonded silica indicate that pressure and temperature affect the retention behavior of insulins in a different and separate way [1]. This implies that thermodynamic parameters, such as ΔH and ΔS, for different pressures should be obtainable from the temperature studies conducted under constant pressure. As long as the phase ratio can be de-convoluted from the capacity factor measurement, the thermodynamic properties under low and high pressures can be deduced to gain better understanding of the pressure-induced retention or adsorption on chromatographic surface. Therefore, we would like to first conduct such studies on a simplified model system, a homologous series of hydrophobic poly-aminoacids containing 2–7mers of L-phenylalanine. These solutes do not have secondary structures that could possibly be changed by the pressure. Understanding the peptide retention will lead to a better picture of protein adsorption since only part of the protein surface is in contact with the chromatographic surface and a peptide molecule is a mimic of the contact surface on the protein. In addition to pressure and temperature, this investigation will also include organic solvent composition. Organic solvent plays an important role in solute retention as well as in the hydration layer of the solute in the solution. The main objective of this work is to gain more insights into the present understanding regarding pressure-induced retention of biomolecules on reversed-phase liquid chromatography.

2. Experimental

2.1. Chemicals and reagents

The 2–7mers of L-phenylalanine were of the highest available grade purity purchased from Sigma and were used as received. HPLC grade of acetonitrile (ACN) and methanol (MeOH) were obtained from Labscan (Labscan Ltd., Ireland), phosphoric acid was from Fisher (Fisher Scientific, Japan), and TFA was from Lancaster (Lancaster, England). Water was deionized to 18 MΩ with a Barnstead NANO-ultrapure water system.

2.2. Chromatography and detection systems

The instrumentation is similar to that described before [1–6]. Briefly, a 57 cm fused silica tubing (200 μm i.d., 350 μm o.d.; Polymicro Technologies) was packed under 350 bar with a slurry of stationary phase in methanol. The stationary phase is polymeric bonded octadecysilica (Vydac 218, d_p = 5 μm) with 300 Å pore size, 8–9% carbon loading, and endcapped. The column temperature was kept at 25 ± 1 °C throughout the experiment. A high pressure pump (Model LC-10AD, Shimadzu, Japan) was used to deliver the mobile phase at a constant flow rate mode and the effluent was subsequently split (1:100) between the microcolumn and a splitting capillary, resulting in a nominal flow rate of 1.0 μl/min throughout the experiment. Sample injection was accomplished by means of a 60 nl injection valve (Valco Instruments Co., Houston, TX). The mixture of 2–7mers of L-phenylalanine was prepared by dissolving the poly-aminoacids in 0.1% TFA containing 50% ACN with a final concentration of 250 ppm for each mer. The mobile phase was composed of 0.1% TFA or phosphoric acid containing specified composition of organic solvent (ACN or MeOH) with a final pH value around 2.3. The experiments were designed to maintain a constant column flow rate while varying the absolute pressure on the column [4] by controlling the length of a restricting capillary attached to the column outlet. The UV detector (Model UV3000, Thermo Separation Products, San Jose, CA) at the wavelength of 214 nm was positioned on the packed bed at a distance of 34.5 cm from the column head. During the course of experiments, the lengths of the restricting and splitting capillaries were simultaneously decreased, so that the mobile phase velocity

and split ratio remained constant while the inlet pressure (P_{in}) was varied from 350 to 53 bar. Upon the change of the column pressure, no injection was performed until the system was stabilized. Assuming a linear pressure drop along the column (0.93 bar/cm), the absolute pressure (P_{abs}) on the detection window corresponding to P_{in} of 53 and 350 bar could be calculated to be 19 and 320 bar, respectively.

2.3. Calculations of capacity factor (k')

The capacity factor (k') was calculated based on the method of statistical moments because it requires no assumptions concerning the mathematical form of the zone profile. In this study, tyrosine served as the void marker and was proven to have no retention as long as the mobile phase contained more than 10% of ACN or 30% of MeOH at all pressures. Throughout the experiments, tyrosine was constantly injected in order to obtain the void time (t_0) values at each condition as well as to confirm the stability of the flow rate. The relative standard deviations of the k' values were within 3% based on the triplet injections under each condition.

3. Theory

The free energy (ΔG) corresponding to the thermodynamic equilibrium of liquid chromatography can be expressed as an enthalpy (ΔH) and an entropy (ΔS) term:

$$\Delta G = -RT\ln K = \Delta H - T\Delta S \quad (1)$$

where T and R are temperature and the gas constant, respectively. The effect of temperature, pressure (p), and organic solvent composition (%) on the equilibrium, K, may then be expressed implicitly as a function of ΔH and ΔS, respectively:

$$\ln K(T, p, \%) = -\frac{\Delta H(T, p, \%)}{RT} + \frac{\Delta S(T, p, \%)}{R} \quad (2)$$

The ΔH can be re-written as Eq. (3)

$$\Delta H(T, p, \%) = \Delta E(T, p, \%) + p\Delta V \quad (3)$$

where ΔE is the change of internal energy and ΔV is the volume difference between partial molar volume of the solute (peptides) in the stationary phase V_s and the mobile phase V_m. The measured capacity factor, k', can be further related to K and phase ratio (ϕ) which is defined as V_s/V_m:

$$k'(T, p, \%) = K(T, p, \%)\phi \quad (4)$$

In which ϕ is assumed to be independent of T, p, and %. Eq. (5) can be obtained by combining Eqs. (2)–(4):

$$\ln k'(T, p, \%) = -\frac{\Delta H(T, p, \%)}{RT} + \frac{\Delta S(T, p, \%)}{R} + \ln\phi \quad (5)$$

or

$$\ln k'(T, p, \%) = -\frac{\Delta E(T, p, \%) + p\Delta V}{RT} + \frac{\Delta S(T, p, \%)}{R} + \ln\phi \quad (6)$$

In previous studies (4), ΔE and ΔS were assumed to be independent of p under constant organic solvent composition (%) and temperature (T). Hence, the pressure-induced change of k' was derived by taking the first derivative of Eq. (6):

$$\left(\frac{\partial \ln k'}{\partial p}\right)_{T,\%} = \frac{\Delta V}{RT} = \frac{V_s - V_m}{RT} \quad (7)$$

Therefore, the pressure-induced retention was attributed to ΔV that can be influenced by many factors including the degree of solute solvation. In this study, we will measure k' at different temperatures under both constant pressure (p) and organic solvent composition (%):

$$\ln k'_{p,\%}(T) = -\frac{\Delta H_{p,\%}(T)}{RT} + \frac{\Delta S_{p,\%}(T)}{R} + \ln\phi \quad (8)$$

From the plot of $\ln k'_{p,\%}$ versus $1/T$ (van't Hoff plot), $\Delta H_{p,\%}(T)$ under constant p and % can be calculated from the first derivative of the plot, $-\Delta H_{p,\%}(T)/R$. If both ΔH and ΔS are independent of T, the plot will be linear. The first derivative of the plot will be its slope and its interception will be equal to $\Delta S_{p,\%}/R + \ln\phi$. By systematically changing p and %, the values of ΔH and $\Delta S_{p,\%}/R + \ln\phi$ corresponding to different p, and % can be determined.

For a homologous series of solutes, $\ln k'_{p,\%}$, $\Delta H_{p,\%}$, and $\Delta S_{p,\%}$ are usually additive, therefore, the enthalpy change ($\Delta\Delta H_{n,\%}$) for each phenylalanine residue (n = 3–7) along the peptide backbone can be determined by direct subtractions between adjacent solutes:

$$(\Delta\Delta H_{p,\%})_n = (\Delta H_{p,\%})_n - (\Delta H_{p,\%})_{n-1} \quad (9)$$

In the same manner, $\Delta\Delta S_{n,\%}$ for each phenylalanine residue (n = 3–7) along the peptide backbone can also be determined by subtracting the interception of the plot, ($\Delta S_{p,\%}/R + \ln\phi$), for solute $n-1$ from that for solute n without the knowledge of ϕ:

$$(\Delta\Delta S_{p,\%})_n = \left(\frac{\Delta S_{p,\%}}{R + \ln\phi}\right)_n - \left(\frac{\Delta S_{p,\%}}{R + \ln\phi}\right)_{n-1} \quad (10)$$

4. Results and discussion

4.1. Plots for a homologous series

A homologous series of hydrophobic poly-aminoacids containing 2–7mers of L-phenylalanine was judiciously chosen to assist the investigation for several reasons. First, these solutes contain homogeneous hydrophobic residues that do not have functional groups for hydrogen bonding. Therefore, a secondary structure is not expected for these solutes

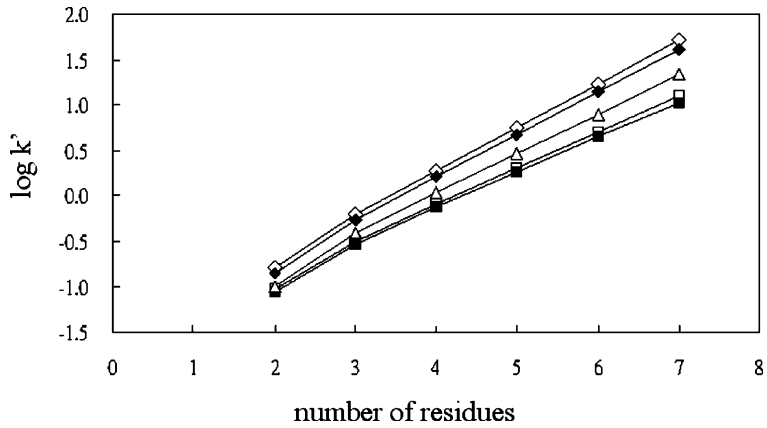

Fig. 1. Plots of k' vs. the number of residues under (■) 35% ACN, 25 °C, 19 bar; (□) 35% ACN, 25 °C, 318 bar; (△) 35% ACN, 60 °C, 19 bar; (◆) 40% ACN, 25 °C, 19 bar; and (◇) 40% ACN, 25 °C, 318 bar.

and their retention is mainly due to the hydrophobic interaction that is a function of the solute chain length. As shown in Fig. 1, the plots of $\ln k'$ versus the number of residues under different pressure, temperature, and organic solvent compositions all show good linearity, which confirms the above assumption. Second, these solutes serve as a convenient probe for the investigation of the microscopic environment along the depth of octadecylsilica stationary phase. This is based on the assumption that the solute is inserted into the space between C18 alkyl chains with its uncharged C-terminal (under pH < 2) immersed into the stationary phase and positively charged N-terminal suspended on the mobile phase. Therefore, the subtracted properties ($\Delta\Delta H$ or $\Delta\Delta S$) between adjacent solutes (n and $n-1$) will represent the local environment corresponding to "n-depth" of the stationary phase. A small negative deviation at low n number is noticed in Fig. 1 and this deviation could be due to a greater error associated with the measurement of small k' values (3%) or due to the oriental restriction associated with these short solutes as proposed in the literature [27,28]. Third, as described above, a direct subtraction can be used to compute the entropy, $\Delta\Delta S$, without the knowledge of ϕ.

4.2. van't Hoff plots

The van't Hoff plots for all solutes under different ACN compositions and pressures were constructed in order to calculate both the ΔH and ΔS parameters based on the slope and intercept of the plots according to Eqs. (8)–(10). As shown in Fig. 2, good linearity was observed for all plots of 5-phenylalanine under the examined temperature range, 25–80 °C, indicating that both ΔH and ΔS values were independent of temperature under this temperature range. Such typical plots were observed for all model solutes and only 5-phenylalanine was shown here. As seen in Fig. 2, both the slope and intercept of the plots vary under different pressures, indicating that the effect of the enthalpy and entropy term on the pressure-induced retention cannot be ignored. Fig. 2 also shows that the decrease of organic solvent composition led to an increase of capacity factors as a consequence of the change in slope and intercept of the plots.

The ΔH values determined from van't Hoff plots were listed in Table 1 and plotted against the number of residues shown in Fig. 3. The intercept determined from van't Hoff plots were also plotted against the number of residues

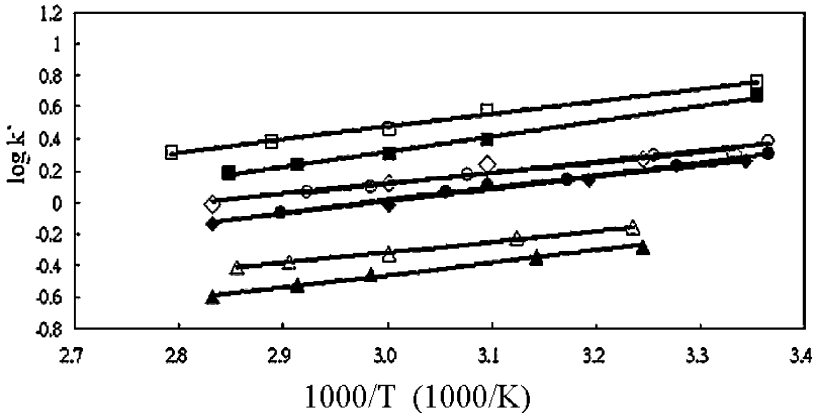

Fig. 2. Plots of $\log k'$ vs. $1/T$ for 5-phenylalanine under (▲) 45% ACN, 19 bar; (△) 45% ACN, 318 bar; (◆) 40% ACN, 19 bar; (◇) 40% ACN, 318 bar; (●) 38% ACN, 19 bar; (○) 38% ACN, 318 bar; (■) 35% ACN, 19 bar; (□) 35% ACN, 318 bar.

Table 1
The value of ΔH(kJ/mol) obtained from van't Hoff plots at pressure of 19 and 318 bar

	35% ACN		38% ACN		40% ACN		45% ACN	
	19 bar	318 bar	19 bar	318 bar	19 bar	318 bar	19 bar	318 bar
2-Phenylalanine	−5.495	−5.932	−2.621	−3.597	−2.236	−2.919	N/D	−2.854
3-Phenylalanine	−6.896	−5.390	−4.424	−5.321	−4.186	−4.441	−4.362	−4.932
4-Phenylalanine	−7.312	−5.871	−5.513	−5.418	−5.359	−4.823	−5.391	−4.998
5-Phenylalanine	−8.117	−6.681	−6.567	−5.981	−6.400	−5.423	−6.365	−5.580
6-Phenylalanine	−9.066	−7.439	−7.659	−6.633	−7.502	−6.048	−7.222	−6.142
7-Phenylalanine	−9.815	−8.389	−8.627	−7.536	−8.458	−6.827	−8.499	−6.776

N/D: not detected.

and shown in Fig. 4. As expected, all plots in both figures are approximately linear except for the data points of 2-phenylalanine. In Fig. 3, all ΔH values are negative, implying that the retention process of peptides under studied conditions were all enthalpy favored. However, the increase of pressure appears to increase (less negative) the ΔH value except for 2-phenylalanine (Fig. 3). The increase of ΔH for an exothermic equilibrium leads to a decrease of the excreted heat and therefore, is unfavored to the retention equilibrium. If the increase of k' is attributed to a negative ΔV, which is explicitly expressed in ΔH according to Eq. (3). A negative ΔV value, however, will lead to a more negative ΔH value upon pressurization, which is in contrast to results shown here. However, the contribution of ΔE cannot be ignored and more investigations are required to de-convolute the contribution of ΔH and ΔV on the effect of pressure-induced retention. In Fig. 4, the increase of pressure appears to increase the intercept that is associated with the ΔS and ϕ term under studied conditions. It is also noticeable that the pressure causes a vertical shift on both the ΔH (Fig. 3) and intercept plots (Fig. 4) corresponding to 35% ACN without changing their slopes. The pressure, however, has significantly changed the slope for both the ΔH (Fig. 3) and intercept plots (Fig. 4) corresponding to 40% ACN, particularly that the slope for the intercept plot is changed from a negative to a positive value. Since the number of residue (n) represents n-depth of the stationary phase, the cause of this result could be related to extensive modifications of the stationary phase by organic solvents. Further explorations of ΔS and ΔH values for each residue ($\Delta\Delta S$ and $\Delta\Delta H$) will provide deeper insights into these effects.

The $\Delta\Delta H$ and $\Delta\Delta S$ values were calculated from direct subtraction described in Eqs. (9) and (10). Figs. 5 and 6 are results of $\Delta\Delta H$ and $\Delta\Delta S$ values deduced from the subtraction between $n = 5$ and 4 against the organic solvent composition. It was found that results deduced from all n values were quantitatively similar except for that deduced from the subtraction involving $n = 2$, which shows linear deviation in Figs. 2–4. This indicates that the bulk property of the stationary phase could be uniform in terms of its $\Delta\Delta H$ and $\Delta\Delta S$ values as long as the depth $n \geq 3$. Therefore, Figs. 5 and 6 are representative of the solute retention in bulk stationary phase. It is noticeable that both $\Delta\Delta H$ and $\Delta\Delta S$ values are negative under high ACN compositions (45, 40, and 38%) with low pressure (19 bar), indicating that the peptide retention on C18 surface under such conditions is an enthalpy-driven process. Whereas, $\Delta\Delta S$ values are positive under either low ACN composition (35%) or high compositions with high pressure (318 bar), indicating that the entropy effect becomes important under these conditions. It appears that under ACN compositions above 35%, the pressure causes the $\Delta\Delta S$ value to change from a negative to a positive value and the $\Delta\Delta H$ to change from a negative to a less negative value, both leading to a thermodynamic state closer to that under 35% ACN composition.

The above results imply that the pressure-induced retention observed in this study is an entropy-favored but

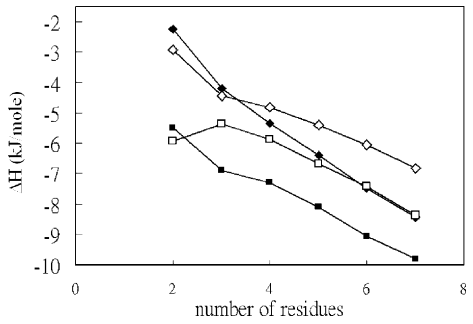

Fig. 3. Plots of ΔH vs. the length of the solute (number of residues) under (◆) ACN, 19 bar; (◇) % ACN, 318 bar; (■) 35% ACN, 19 bar; (□) 35% ACN, 318 bar.

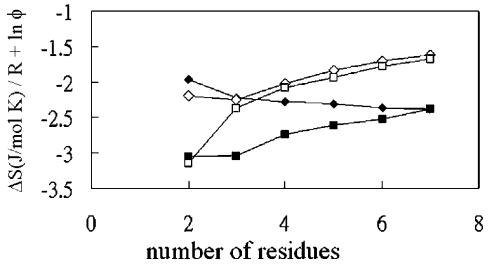

Fig. 4. Plots of $\Delta S/R + \log\phi$ (intercepts of van't Hoff plots) vs. the length of the solute (number of residues) under (◆) 40% ACN, 19 bar; (◇) 40% ACN, 318 bar; (■) 35% ACN, 19 bar; (□) 35% ACN, 318 bar.

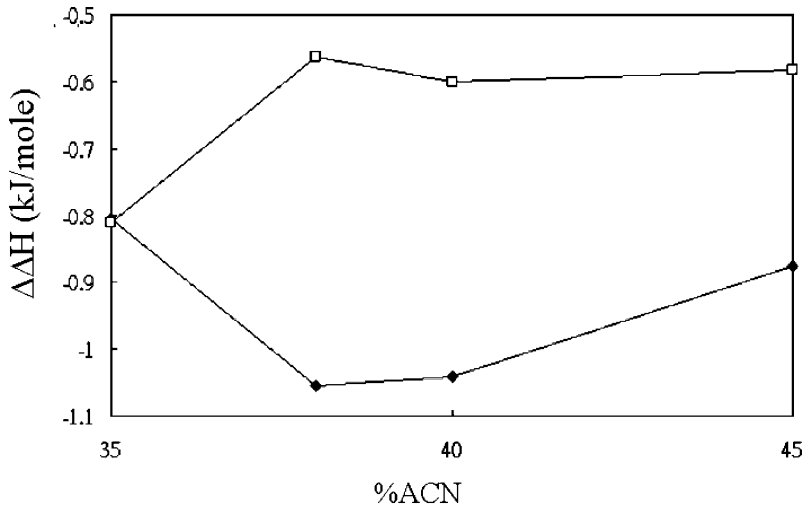

Fig. 5. The $-\Delta\Delta H$ values of 5-phenylalanine at different ACN compositions under (▲) 19 bar and (□) 318 bar.

enthalpy-unfavored process, which is, however, in contract with the general believing that adsorption of solute molecules on the stationary phase decreases the mobility of the solute and should lead to a decrease of the entropy. These phenomena could be explained as that pressure forces desorption of solvent molecules that are associated with the stationary phase or with the peptide molecule. The decrease of organic solvent molecules associated with the stationary phase will reduce the rigidity of the packing structure surrounding the solute in the stationary phase since the organic solvent is relatively more polar than the non-directional hydrophobic C18; the decrease of organic solvent molecules associated with the solute will raise the rigidity of the hydration layer of the solute in the aqueous mobile phase since the organic solvent is relatively less polar than the water molecule. Thus, the overall rigidity of the solute was decreased as seen in this study that both ΔS and $\Delta\Delta S$ values increased upon pressurization under high ACN compositions. Under 35% ACN composition, however, fewer organic solvent molecules were expected to absorb in the bulk stationary phase and thus, little pressure-induced changes in $\Delta\Delta H$ or $\Delta\Delta S$ value were observed. The pressure-induced retention under 35% ACN composition could be mainly associated with the surface of the stationary phase or with the change of ϕ. It should also be noted that the pressure-induced desorption of organic solvent proposed here could be negligible for the retention of hydrophilic peptides since these peptides have fewer organic solvent molecules surrounded. In a separate study, we have proved that the pressure-induced retention was barely detected for hydrophilic peptides. Nevertheless, this study has suggested the importance of entropic change, which is likely to be due to pressure-induced desorption of organic solvents, with regard to the observed pressure-induced retention of hydrophobic peptides. Detailed investigations on a microscopic scale will further shed light on the mechanism of protein/peptide retention.

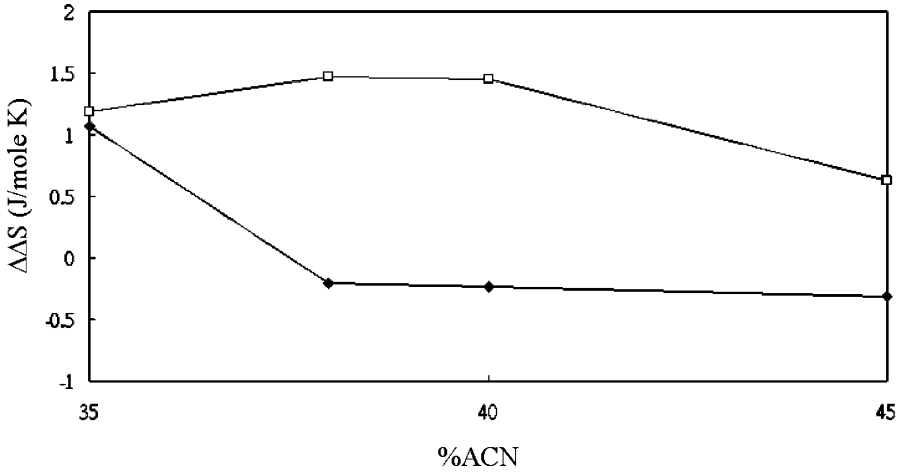

Fig. 6. The $\Delta\Delta S$ values of 5-phenylalanine at different ACN compositions under (▲) 19 bar and (□) 318 bar.

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References

[1] P. Szabelski, A. Cavazzini, K. Kaczmarski, X. Liu, J.V. Horn, G. Guichon, J. Chromatogr. A 950 (2002) 41.
[2] X. Liu, P. Szabelski, D. Zhou, G. Guichon, J. Chromatogr. A 988 (2003) 205.
[3] A. Bylina, M. Ulanowicz, Chem. Anal. (Warsaw) 43 (1998) 955.
[4] S.H. Chen, C.T. Ho, K.Y. Hsiao, J.M. Chen, J. Chromatogr. A 891/2 (2000) 207.
[5] M.C. Ringo, C.E. Evans, Anal. Chem. 69 (1997) 643.
[6] M.C. Ringo, C.E. Evans, Anal. Chem. 69 (1997) 4964.
[7] V.L. McGuffin, C.E. Evans, S.H. Chen, J. Microcolumn Sep. 5 (1993) 10.
[8] V.L. McGuffin, S.H. Chen, J. Chromatogr. A 762 (1997) 35.
[9] V.L. McGuffin, S.H. Chen, Anal. Chem. 69 (1997) 930.
[10] T. Macko, D. Berek, J. Liq. Chromatogr. Rel. Technol. 24 (9) (2001) 1275.
[11] A. Vailaya, Cs. Horváth, J. Chromatogr. A 828 (1998) 469.
[12] A. Vailaya, Cs. Horváth, J. Phys. Chem. B 102 (1998) 701.
[13] H.P.J. Bennett, in: C.T. Mant, R.S. Hodges (Eds.,) High-Performance Liquid Chromatography of Peptides and Proteins: Separation, Analysis, and Conformation, CRC Press, Boca Raton, 1991, p. 319.
[14] B.S. Welinder, in: C.T. Mant, R.S. Hodges (Eds.), CRC Press, High-Performance Liquid Chromatography of Peptides and Proteins: Separation, Analysis, and Conformation, Boca Raton, 1991, p. 343.
[15] F.E. Regnier, Science 238 (1987) 319.
[16] X.M. Lu, A. Figueroa, B.L. Karger, J. Am. Chem. Soc. 110 (1988) 1978.
[17] S. Wick, M.G. Mulkerrin, G. Bathory, L.H. Khundkar, B.L. Karger, Anal. Chem. 66 (1994) 3908.
[18] M. Hanson, K.K. Unger, J. Schmid, K. Albert, E. Bayer, Anal. Chem. 65 (1993) 2249.
[19] M.T.W. Hearn, A.N. Hodder, M.-I. Aguilar, J. Chromatogr. 327 (1985) 47.
[20] S.A. Cohen, K.P. Benedek, S. Dong, Y. Tapuhi, B.L. Karger, Anal. Chem. 56 (1984) 217.
[21] P. Oroszlan, S. Wicar, G. Teshima, S.-L. Wu, W.S. Hancock, B.L. Karger, Anal. Chem. 64 (1992) 1623.
[22] N.E. Zhou, P.D. Semchuk, C.M. Kay, R.S. Hodges, in: C.T. Mant, R.S. Hodges (Eds.) High-Performance Liquid Chromatography of Peptides and Proteins: Separation, Analysis, and Conformation, CRC Press, Boca Raton, 1991, p. 643.
[23] V.E. Turula, J.A. de Haseth, Anal. Chem. 68 (1996) 629.
[24] T. Gross, R. Jaenicke, Eur. J. Biochem. 221 (1994) 617.
[25] V.V. Mozhaev, K. Heremans, J. Frank, P. Masson, C. Balny, Proteins: Structure, Function and Genetics 24 (1996) 81.
[26] S.D. Samarasinghe, D.M. Campbell, A. Jonas, J. Jonas, Biochemistry 31 (1992) 7773.
[27] A. Tchapla, H. Colin, G. Guichon, Anal. Chem. 56 (1984) 621.
[28] P.W. Carr, L.C. Tan, J.H. Park, J. Chromatogr. A 724 (1996) 1.