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Retention in hydrophobic interaction chromatography and dissolution of nonpolar gases in water

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Abstract

The retention data of dansyl amino acids in hydrophobic interaction chromatography (HIC) is interpreted in terms of physicochemical properties of the eluites and solvent and the results are compared to data for the dissolution in water of nonpolar gases. Isothermodynamic temperatures, certain linear relationships between thermodynamic quantities and molecular structure as well as the hermeneutics of the solvophobic theory are employed for data analysis. van't Hoff plots of the retention data with dansyl amino acids are curved and intersect at the isoenergetic temperature, indicative of enthalpy-entropy compensation. The isoenthalpic and isoentropic temperatures are also evaluated and the three isothermodynamic temperatures are found to fall in the same narrow ranges as those for the dissolution of gases in water. Plots of thermodynamic quantities for the retention in HIC against the nonpolar molecular area are linear and thus allow the evaluation of enthalpy, entropy, Gibbs energy and heat capacity changes per unit nonpolar surface area of dansyl amino acids. By employing the solvophobic theory thermodynamic expressions are derived in terms of the nonpolar molecular area and interfacial tension. Using these expressions and n-heptane as model compound it was found that the above group molecular parameters are nearly identical for the retention in HIC and the dissolution of gases in water, thus confirming the mechanistic identity of the two processes.

Keywords: Hydrophobic effect; Isothermodynamic temperatures; Solvophobic theory; Mechanistic identity

1. Introduction

Hydrophobic interaction chromatography (HIC) is widely used for the separation and purification of proteins in their native state [1–3]. The technique employs mildly hydrophobic stationary phase and aqueous eluent with salt as the modulator. Protein retention is governed by the hydrophobic effect [4] and the effect of salt has been treated by an adaptation [5,6] of the solvophobic theory [7] and of Wyman's linked functions [8–10].

Recently, the effect of temperature in HIC from 5–50°C was investigated by HPLC using dansyl amino acids as model compounds [11]. Chromatographic retention data revealed large negative heat capacity and mostly positive entropy changes at 25°C [11] so that this approach parallels calorimetric studies on dissolution in water of nonpolar liquid [12], gaseous [13–15] and solid substances [16] as well as on protein folding [17,18].

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In this work the thermodynamic data [11] obtained with the retention of dansyl amino acids in HIC is examined first within the framework of enthalpy-entropy compensation, a diagnostic tool in mechanistic investigations of chemical reactions and equilibrium processes [19,20] such as chromatographic retention [21]. Chromatographic data is also used to examine the relationship between the thermodynamic quantities and the nonpolar water accessible surface area of the molecules of interest that has been found to be linear for hydrophobic interactions [22–24]. Lastly, the solvophobic theory [25] is employed to establish such a relationship also for retention in HIC by using cavity area and interfacial tensions. The results of the analysis are compared to those for dissolution in water of nonpolar gases in order to explicate further the role of hydrophobic interactions in HIC.

2. Evaluation of thermodynamic parameters from chromatographic data

A method for evaluation of thermodynamic quantities associated with the retention in HIC of hydrocarbonaceous eluites has been presented earlier [11]. In the following we briefly outline the key features of the method.

In the chromatographic literature, retention data is usually presented in terms of the retention factor, k', which is simply the ratio of the number of moles of the eluite that is adsorbed on the stationary phase to the number of moles in the mobile phase [26]. The retention factor is given by the product of the equilibrium constant, K', and the phase ratio, ϕ, of the column which is considered invariant with temperature. In our analysis the value of ϕ is set unity so that the retention factor simply represents the equilibrium constant of the process [11].

In this work we wish to compare the energetics of HIC retention with other processes based on the hydrophobic effect. For this reason, we present chromatographic data in a manner to reflect the transfer of hydrocarbonaccous eluites from the stationary phase to the mobile phase so that the standard Gibbs energy change is given by

$$\Delta G^o = -RT \ln K \qquad (1)$$

where K is the equilibrium constant, R is the gas constant and T is the temperature.

The temperature dependence of $\ln k'$ or $-\ln K$ is given by Haidacher et al. [11] assuming that the standard heat capacity change, ΔC_p^o, associated with the transfer of eluites from stationary to mobile phase is invariant with temperature as

$$\ln k' = -\ln K = -\frac{\Delta C_p^o}{R}\left(\frac{T_h}{T} - \ln\frac{T_s}{T} - 1\right) \qquad (2)$$

where T_h and T_s are the temperatures at which the enthalpy and entropy changes for the same transfer process are zero. By fitting the above equation to retention data measured on three different types of HIC columns [11] the thermodynamic parameters were evaluated for the transfer of eluites from sta-

Table 1
Thermodynamic parameters associated with the retention of dansyl derivatives of amino acids on three HIC columns as evaluated by fitting Eq. (2) to experimental data in [11]. The three parameters ΔC_p^o, T_h and T_s presented here can be used to evaluate the change in enthalpy by $\Delta H^o = \Delta C_p^o(T - T_h)$ and in entropy by $\Delta S^o = \Delta C_p^o \ln(T/T_s)$

Amino acid moiety	ΔC_p^o [cal mol^{-1} K^{-1}]	T_h [K]	T_s [K]
Column A (Spherogel™ HIC)			
Alanine	46.4	287.6	307.6
Valine	61.4	303.2	322.3
Phenylalanine	62.4	293.5	318.0
Leucine	73.7	313.3	331.5
Column B (SynChropak® Propyl)			
Glycine	15.2	124.2	215.1
Proline	26.2	260.3	298.3
Alanine	34.5	269.2	299.8
α-amino n-butyric acid	45.7	289.7	315.7
Valine	52.7	306.7	331.7
Norvaline	57.2	298.3	323.0
Leucine	65.8	314.2	337.0
Column C (TSK-GEL® Butyl-NPR)			
γ-amino n-butyric acid	70.0	271.0	285.6
Glycine	80.9	271.1	281.7
Alanine	97.7	293.4	301.0
α-amino n-butyric acid	103.2	302.0	311.2
Valine	116.5	307.0	316.3
Methionine	124.8	300.7	310.8
Norvaline	126.7	305.5	315.5
Leucine	135.5	313.3	323.1
Norleucine	140.5	306.3	318.5

tionary to mobile phase and they are listed in Table 1 for the retention of selected dansyl amino acids on columns Spherogel™ HIC (A), SynChropak® Propyl (B) and TSK-GEL® Butyl-NPR (C).

3. Isothermodynamic temperatures

Processes involving a group of structurally related substances exhibit compensation behavior when plots of ΔH^o versus ΔS^o are linear at a given temperature [20,21]. The slope represents the compensation temperature, T_C, that marks the common intersection point of linear van't Hoff plots and is a characteristic of the process, e.g., fundamentally related so called isoequilibrium processes have similar T_C values [19].

Classical enthalpy-entropy compensation (EEC) cannot occur with processes involving hydrophobic interactions because they are associated with significant heat capacity effects [27]. Compensation behavior in this case manifests a compensation temperature that is function of the experimental temperature and three other characteristic temperatures that are described as follows.

When ΔC_p^o is constant for a process involving structurally related substances, the entropy and enthalpy changes have been expressed as [18,28]

$$\Delta S^o = \Delta S^* + \Delta C_p^o \ln\left(\frac{T}{T_S^*}\right) \tag{3}$$

$$\Delta H^o = \Delta H^* + \Delta C_p^o (T - T_H^*) \tag{4}$$

where T_S^* and T_H^* are the isoentropic and isoenthalpic temperatures at which all participating substances attain a common value ΔS^* and ΔH^*, respectively. It is noted that T_S^* and T_H^* are different from T_S and T_h in Eq. (2). According to Eq. (3) and Eq. (4) a plot of ΔS^o or ΔH^o versus ΔC_p^o yields a straight line with $\ln((T/T_S^*))$ or $(T - T_H^*)$ and ΔS^* or ΔH^* as the slopes and intercepts, respectively. The significance of T_S^* and T_H^* has been recognized in studies on protein folding and Eq. (3) and Eq. (4) have been used for mechanistic interpretation of calorimetric data [17,18].

The isoenergetic temperature, T_G^*, is expressed in terms of T_S^* and T_H^* as [27]

$$T_G^* = \frac{(T_G^* - T_H^*)}{\ln \frac{T_G^*}{T_S^*}} \tag{5}$$

T_G^* is akin to T_S^* and T_H^* that mark common intersection points of the respective plots of entropy

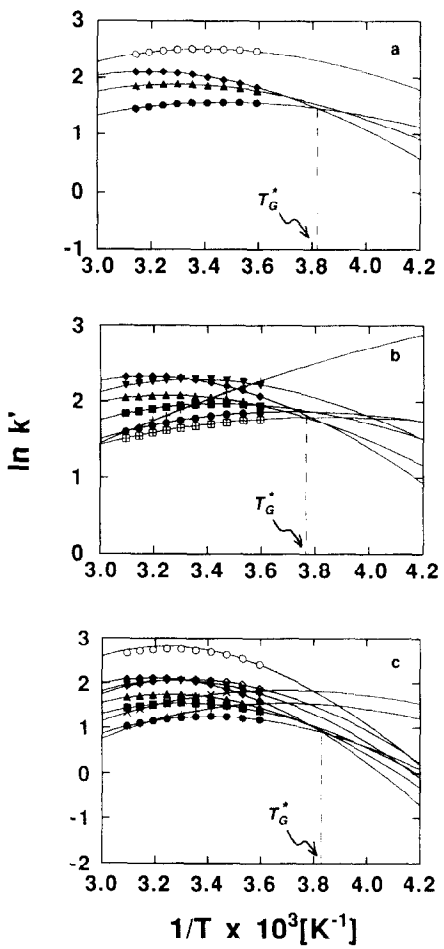

Fig. 1. Plots of the retention factor in HIC of dansyl amino acids against the reciprocal temperature. The data measured with (a) Column A, (b) Column B and (c) Column C is taken from Ref. [11]. Solid lines represent the best fits of the equation $\ln k' = -(\Delta C_p^o / R)(T_h / T) - \ln(T_s / T) - 1$. The isoenergetic temperature T_G^* marks the common intersection point of the van't Hoff plots. The symbols for dansyl amino acids are: (+) glycine, (●) alanine, (■) α-amino n-butyric acid, (▼) norvaline, (▲) valine, (◆) leucine, (×) γ-amino n-butyric acid, (⊙) phenylalanine, (⊞) proline, (○) norleucine, (◇) methionine.

and enthalpy versus temperature so that all three characteristic temperatures are collectively called isothermodynamic temperatures [27]. In another sense, T_G^* can be considered a generalized compensation temperature marking the intersection of nonlinear van't Hoff plots. Thus, T_C of classical EEC is a particular case of T_G^* in the absence of heat capacity effects and both T_C and T_G^* represent temperatures where all substances would have the same ΔG^o, e.g., they would coelute in HIC.

Retention in HIC with dansyl amino acids is examined here within the context of EEC. Fig. 1 illustrates van't Hoff plots for the retention of dansyl amino acids on columns A, B and C at temperatures 5–50°C [11]. The solid lines represent best fits of Eq. (2) to the experimental data. Upon extrapolation, three out of four plots in Fig. 1(a) show a common intersection point at a T_G^* value of −11°C at which dansyl alanine, valine and leucine would coelute. The retention of dansyl proline appears to be much higher than that of the other dansyl amino acids at the isoenergetic temperature and this may be ex-

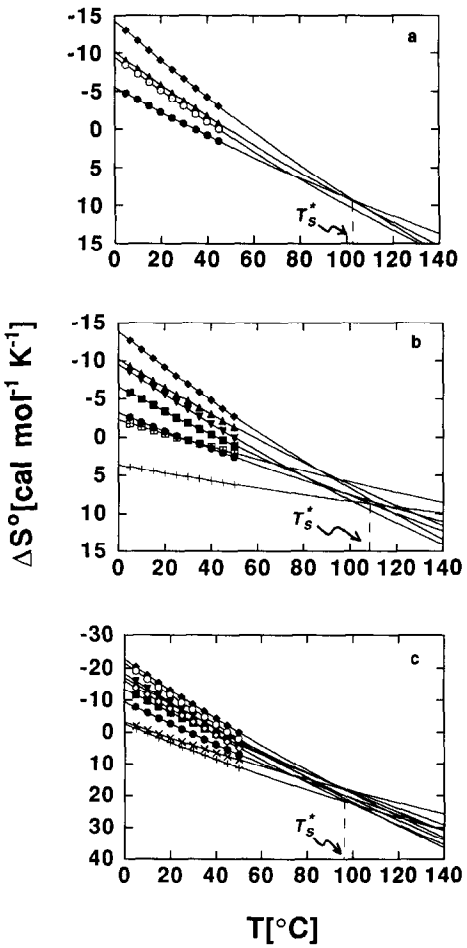

Fig. 2. Plots of entropy change against temperature for the HIC retention of dansyl amino acids on (a) Column A, (b) Column B and (c) Column C. The data is taken from Table 1. Solid lines represent the best fits of the equation $\Delta S^o = \Delta C_p^o \ln(T/T_s)$. The isoentropic temperature T_s^* marks the common intersection point of the plots. Symbols are the same as in Fig. 1.

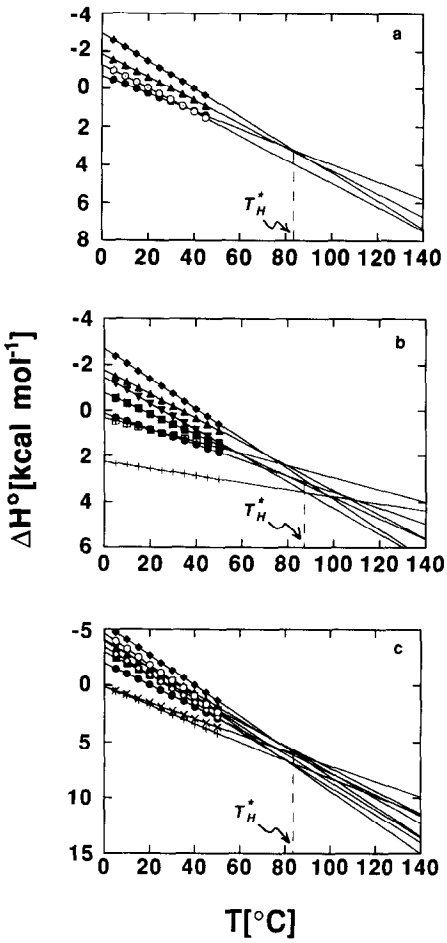

Fig. 3. Plots of enthalpy change against temperature for the HIC retention of dansyl amino acids on (a) Column A, (b) Column B and (c) Column C. The data is taken from Table 1. Solid lines represent the best fits of the equation $\Delta H^o = \Delta C_p^o(T\text{-}T_h)$. The isoenthalpic temperature T_H^* marks the common intersection point of the plots. Symbols are the same as in Fig. 1.

plained by a particularly strong interaction of the hydrophobic moiety of the stationary phase with the cyclic portion of proline. The retention data measured on columns B and C showed similar results as seen in Fig. 1(b) and 1(c), although the scatter in the intersection points of the plots is larger. Dansyl glycine is the most prominent outlier in both cases. This is probably due to its smaller size and greater polarity as compared to those of other dansyl amino acids. The T_G^* values for retention on columns B and C are obtained as -8°C and -12°C and they are close to those with dansyl amino acids on column A.

According to the isoenergetic temperatures in Fig. 1 the retention of dansyl amino acids exhibits compensation behavior on all three HIC columns. Therefore, T_S^* and T_H^* may also be evaluated from plots of entropy and enthalpy changes against temperature. Figs. 2 and 3 illustrate such entropy and enthalpy plots with the HIC retention data obtained on the three columns. Except with data presented in Fig. 2(a) and Fig. 3(a) (Column A) the intersection points of the plots scatter somewhat. However, there is a narrow temperature range where all eluites seem to attain about the same entropy and enthalpy values. The T_S^* and T_H^* values obtained from the plots shown in Figs. 2 and 3 are approximately 102°C, 109°C, 96°C and 84°C, 87°C, 84°C for columns A, B and C.

In the following we wish to calculate isothermodynamic temperatures for the dissolution of nonpolar gases in water and compare them to those obtained with HIC retention data. Since the calorimetric data for gas dissolution is available only at 25°C [13–15] we cannot obtain T_G^*, T_S^* and T_H^* values for gas dissolution in the same way as we did for HIC retention. Nevertheless, the use of Eqs. (3)–(5) offers an alternate means for the evaluation of isothermodynamic temperatures from thermodynamic data at a given temperature.

Fig. 4(a) illustrates virtually linear ΔS^o versus ΔC_p^o plots of data on three HIC columns and of calorimetric data for dissolution in water of nonpolar gases at 25°C. The isoentropic temperatures evaluated from the slopes of ΔS^o versus ΔC_p^o plots by using Eq. (3) are listed in Table 2. The T_S^* values from HIC retention on all three columns are about the same and very similar to those obtained from Fig. 2. Further, the T_S^* values for these apparently different processes that involve the interaction of

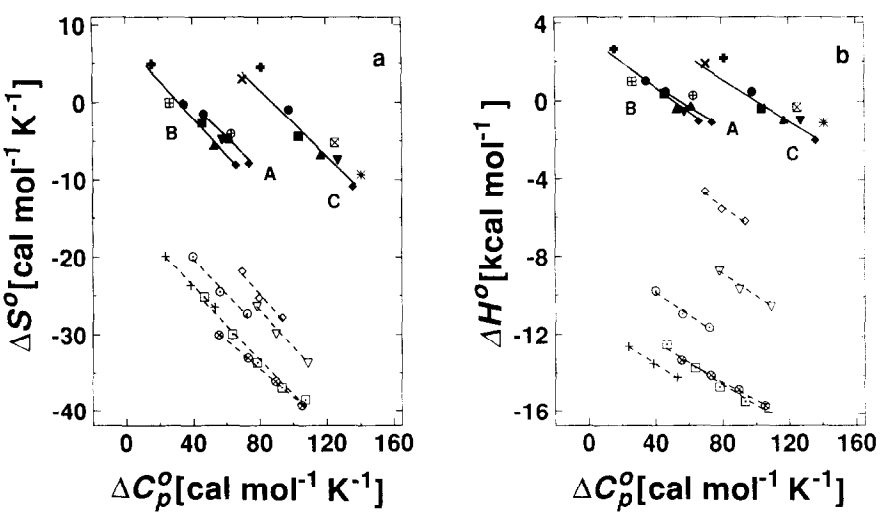

Fig. 4. Plots of the entropy (a) and enthalpy (b) against the heat capacity change at 25°C for retention in HIC and dissolution of gases in water. HIC retention data of dansyl amino acids on Columns A, B and C is indicated by the letter code of the column and the corresponding plots by solid lines. The symbols for dansyl amino acids are: (+) glycine, (●) alanine, (■) α-amino n-butyric acid, (▼) norvaline, (▲) valine, (◆) leucine, (×) γ-amino n-butyric acid, (⊕) phenylalanine, (⊞) proline, (*) norleucine, (⊠) methionine. Dashed lines are for the dissolution in water of the vapors of: (▽) alkyl benzenes, aliphatic (◇) hydrocarbons, (⊙) ketones, (□) alcohols, (+) acids and (⊗) amines. Sources of data are listed in Table 2.

Table 2
Isothermodynamic temperatures [°C] for the retention of dansyl amino acids in HIC and the dissolution of nonpolar gases in water. Isoentropic T_S^* and isoenthalpic T_H^* temperatures were derived from the linear dependence of the entropy and enthalpy on the heat capacity change at 25°C according to Eq. (3) and (4), respectively. The isoenergetic temperature T_G^* was evaluated by using Eq. (5). Standard states are ideal gas at 1 atm. and ideal aqueous solution at 1 M concentration. Errors shown are 1 SD of the best fit

Process	No.	Substances	T_S^*	T_H^*	T_G^*	Ref.
HIC on Column A	I	3 dansyl amino acids [a]	103 ± 3	83 ± 4	−13	[11]
HIC on Column B	II	7 dansyl amino acids [b]	103 ± 9	88 ± 9	2	[11]
HIC on Column C	III	9 dansyl amino acids [c]	95 ± 9	81 ± 8	−2	[11]
Dissolution of gaseous	IV	3 aliphatic hydrocarbons [d]	103 ± 13	85 ± 9	−7	[13–15]
substances in water	V	3 alkyl benzenes [e]	105 ± 10	84 ± 10	−14	[15]
"	VI	3 aliphatic ketones [f]	101 ± 13	83 ± 8	−9	[15]
"	VII	5 aliphatic alcohols [g]	99 ± 7	80 ± 5	−13	[15]
"	VIII	3 aliphatic acids [h]	100 ± 6	80 ± 3	−15	[15]
"	IX	4 aliphatic amines [i]	86 ± 2	73 ± 2	−6	[15]

[a] Dansyl alanine, valine and leucine. [b] Dansyl glycine, proline, alanine, α-amino n-butyric acid, valine, norvaline and leucine. [c] Dansyl γ-amino n-butyric acid, glycine, alanine, α-amino n-butyric acid, valine, methionine, norvaline, leucine and norleucine. [d] Ethane, n-propane, n-butane. [e] Methyl, ethyl and propyl benzene. [f] 2-Propanone, 2-butanone, 2-pentanone. [g] 1-Ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol. [h] Acetic, propanoic and butanoic acid. [i] Propyl, butyl, pentyl and hexylamine.

hydrophobic molecules or moieties in aqueous media fall in a very narrow range with a mean value of 99°C. The close resemblance of T_S^* is taken as a manifestation of the dominant role of water in determining the mechanism of hydrophobic interactions [18].

The same holds for the corresponding enthalpy plots depicted in Fig. 4(b), i.e., the dependence of ΔH^o on ΔC_p^o is also linear for both sets of processes and the plots have very similar slopes. The corresponding isoenthalpic temperatures calculated from the slopes by using Eq. (4) are listed in Table 2. Again, T_H^* values for HIC retention are very similar to those obtained from Fig. 3 and, along with the values for dissolution of nonpolar gases in water, fall into a narrow range with a mean value of 82°C.

We also calculated the errors in T_S^* and T_H^* values as 1 SD of the best fit to data in Fig. 4 and they are listed in Table 2 for the two processes based on the hydrophobic effect. It is seen that the errors obtained with HIC retention data are of the same order of magnitude as those obtained with calorimetric data for gas dissolution. This supports the validity of either method for the evaluation of isothermodynamic temperatures from chromatographic data.

The isoenergetic temperature, T_G^*, is evaluated by Eq. (5) using an iterative procedure. Since the isoentropic temperatures for HIC retention are higher than the corresponding isoenthalpic temperatures, we expect to observe two isoenergetic temperatures [27]. This is confirmed by the observation of another isoenergetic temperature at 225°C upon extrapolation to higher temperatures of HIC data presented in Fig. 1. In Table 2 we report only the lower T_G^* values because they are closer to the temperatures employed in the experiments. These compare well to the isoenergetic temperatures obtained from Fig. 1, lending further support to our approach for the evaluation of isothermodynamic temperatures from chromatographic data. The T_G^* values range from 2 to −15°C for the gas dissolution and HIC retention in Table 2, so that both processes are energetically similar and can be considered mechanistically identical. This prompted us to examine the energetics of the two processes at the molecular level by using molecular surface area correlations and the solvophobic theory.

4. Molecular area correlations

The Gibbs energy, entropy, enthalpy or heat capacity changes, ΔX^o, associated with hydrophobic interactions have been expressed as [28]

$$\Delta X^o = a_x \Delta A_{np} + b_x \qquad (6)$$

where ΔA_{np} is the change in nonpolar surface area exposed to water upon transfer. The group molecular

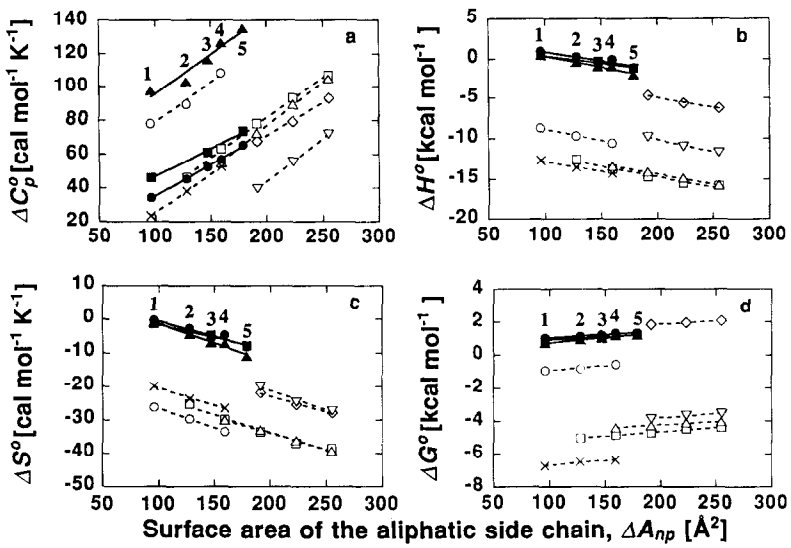

Fig. 5. Plots of (a) heat capacity, (b) enthalpy, (c) entropy and (d) free energy against ΔA_{np} at 25°C. Data for the retention of dansyl (1) alanine, (2) α-amino n-butyric acid, (3) valine, (4) norvaline and (5) leucine on (■) Column A, (●) Column B and (▲) Column C is shown by solid lines. Dashed lines are for the dissolution in water of the vapors of (○) alkyl benzenes and the following aliphatic substances: (◇) hydrocarbons, (▽) ketones, (□) alcohols, (×) acids and (△) amines. Sources of data are listed in Table 2 and the ΔA_{np} values are taken from [22].

parameters [27], a_x and b_x, are assumed to be the same for a set of substances under investigation so that Eq. (6) is likely to apply only to substances with identical polar moieties. The first and second terms on the rhs of Eq. (6) represent contributions by nonpolar and polar interactions, respectively. From the slopes and intercepts of linear plots of ΔX° at a given temperature against ΔA_{np}, the group molecu-

Table 3
Group molecular parameters a_g, a_h, a_s and a_c evaluated according to Eq. (6) from linear plots shown in Fig. 5. The sign of HIC data reflects transfer into aqueous phase

	Process	a_g cal mol^{-1} Å^{-2}	a_h cal mol^{-1} Å^{-2}	a_s cal mol^{-1} K^{-1} Å^{-2}	a_c cal mol^{-1} K^{-1} Å^{-2}
HIC on					
	Column A	3.6	−18.9	−0.08	0.33
	Column B	3.8	−23.0	−0.09	0.37
	Column C	6.0	−28.1	−0.12	0.48
	average value	4.5	−23.3	−0.10	0.39
Dissolution in water of gaseous					
	aliphatic hydrocarbons	3.9	−24.1	−0.09	0.40
	alkyl benzenes	5.6	−28.7	−0.12	0.48
	aliphatic ketones	5.1	−29.4	−0.12	0.51
	aliphatic alcohols	5.2	−26.3	−0.11	0.48
	aliphatic acids	5.5	−25.2	−0.10	0.46
	aliphatic amines	4.0	−24.9	−0.10	0.52
	average value	4.9	−26.4	−0.11	0.48

lar parameters, a_g, a_h, a_s, a_c and b_g, b_h, b_s, b_c, for the thermodynamic quantities can be evaluated.

In Fig. 5 the thermodynamic quantities associated with the retention in HIC of dansyl amino acids [11], which vary only in the length of the aliphatic side chain and thus have quantifiable structural differences, are plotted against the surface area, ΔA_{np}, of the side chain. For comparison, the thermodynamic data associated with the dissolution of nonpolar gases in water is also plotted against the surface area of the aliphatic chains. The plots are virtually linear and have quite similar slopes, from which the group molecular parameters, a_g, a_h, a_s and a_c, listed in Table 3, were calculated using Eq. (6). The average values of a_g, a_h, a_s and a_c from chromatographic data are $4.5\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$, $-23.3\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$, $-0.10\,\mathrm{cal\,mol^{-1}\,K^{-1}\,\AA^{-2}}$ and $0.39\,\mathrm{cal\,mol^{-1}\,K^{-1}\,\AA^{-2}}$, respectively. The corresponding average values obtained for the dissolution in water of various classes of substances are only slightly higher.

5. Solvophobic theory

In order to extend the scope of this study to the molecular aspects of hydrophobic interactions we employ the solvophobic theory [7,25]. This approach is broader than those aimed at the hydrophobic effect proper and applicable also to the effect of solvents other than water. Further, it employs only readily available physicochemical properties.

The standard Gibbs energy of molecular association, $A + B \rightleftharpoons AB$, occurring in the solvent S is expressed by the solvophobic theory as

$$\Delta G^{o}_{\mathrm{assoc.}} = \Delta G^{\mathrm{vac.}}_{\mathrm{assoc.}} + \Delta G_{\mathrm{solv.eff.}} \tag{7}$$

where $\Delta G^{\mathrm{vac.}}_{\mathrm{assoc.}}$ is the Gibbs energy for the association in vacuo or in the vapor phase and $\Delta G_{\mathrm{solv.eff.}}$ is the net Gibbs energy change due to solvent effects. The latter is expressed as

$$\Delta G_{\mathrm{solv.eff.}} = \Delta G_{\mathrm{cav.}} + \Delta G_{\mathrm{int.}} + \Delta G_{\mathrm{mix.}} + \Delta G_{\mathrm{red.}} + RT \ln \frac{RT}{\nu_s} \tag{8}$$

where $\Delta G_{\mathrm{cav.}}$ is the net Gibbs energy of cavity formation for A and B as well as for the complex AB in the solvent, $\Delta G_{\mathrm{int.}}$ is the net Gibbs energy of their interactions with the solvent molecules and $\Delta G_{\mathrm{mix.}}$ is the net Gibbs energy of mixing of molecules of different sizes. The $\Delta G_{\mathrm{red.}}$ term is the reduction of $\Delta G^{\mathrm{vac.}}_{\mathrm{assoc.}}$ by solvent so that it accounts for the change in the intrinsic Gibbs energy of the complex AB due to the solvent medium [7,25]. The last term which entails the molar volume of the solvent, ν_S, stands for the change in free volume. The standard states assumed are ideal gas at 1 atmospheric pressure and the hypothetical 1 M concentration of ideal aqueous solution.

In the following a simplified version of the theory is applied to dissolution in water and retention in HIC of hydrophobic substances.

5.1. Dissolution of nonpolar gases in water

The standard Gibbs energy change of dissolution, $\Delta G^{\mathrm{o}}_{\mathrm{g.diss.}}$, of a nonpolar solute A in water, is expressed as

$$\Delta G^{\mathrm{o}}_{\mathrm{g.diss.}} = \Delta G_{\mathrm{cav.}} + \Delta G_{\mathrm{int.}} + \Delta G_{\mathrm{mix.}} + RT \ln \frac{RT}{\nu_S} \tag{9}$$

The Gibbs energy of forming a cavity of surface area, ΔA, is

$$\Delta G_{\mathrm{cav.}} = \kappa^g_S \gamma_S \Delta A \tag{10}$$

and the interaction term is expressed as

$$\Delta G_{\mathrm{int.}} = (\kappa^g_{AS}\gamma_{AS} - \kappa^g_A\gamma_A - \kappa^g_S\gamma_S)\,\Delta A \tag{11}$$

where γ_S, γ_A and γ_{AS} are the surface tensions of water, solute and the solute/water interfacial tension, respectively, whereas κ^g_S, κ^g_A and κ^g_{AS} convert the respective surface or interfacial tensions to the microthermodynamic value appropriate for molecular dimensions [25,29]. In Eq. (11), the $\kappa^g_A\gamma_A\Delta A$ term represents the Gibbs energy change associated with cavity formation in liquid A when A is transferred from the gas phase to the liquid state (condensation of A). On the other hand, the $\kappa^g_S\gamma_S\Delta A$ term stands for the Gibbs energy of cavity formation associated with the transfer of solute A from the gas phase into the solvent S. In the first case the solute to solvent ratio of the molecular radii is unity so that κ^g_A is size invariant yet affected by the chemical nature of the substance, whereas in the second case

κ_A^g is dependent on the ratio of the solute to solvent molecular radii [25]. The $\kappa_{AS}^g \gamma_{AS} \Delta A$ term in Eq. (11) represents the interfacial Gibbs energy of a surface ΔA between the molecules of the liquids phases A and S [25]. Again, κ_{AS}^g depends on the ratio of solute to solvent molecular radii.

The Gibbs energy change of gas dissolution is given by Eqs. (9)–(11) as

$$\Delta G^{o}_{g,diss.} = (\kappa_{AS}^g \gamma_{AS} - \kappa_A^g \gamma_A)\Delta A + RT \ln \frac{RT}{\nu_S} + G_{mix.} \quad (12)$$

For aliphatic hydrocarbons $\Delta A = \Delta A_{np}$ and the γ_{AS} and γ_A values are rather similar [30] so that Eq. (12) predicts a linear dependence of $\Delta G^{o}_{g,diss.}$ on ΔA_{np} provided κ_{AS}^g and κ_A^g vary only slightly. Then, the Gibbs energy change per unit nonpolar surface area can be evaluated as

$$\frac{\partial \Delta G^{o}_{g,diss.}}{\partial \Delta A_{np}} = \kappa_{AS}^g \gamma_{AS} - \kappa_A^g \gamma_A \quad (13)$$

if the rhs of Eq. (13) is known. The entropy change of dissolution per unit nonpolar surface area can be obtained by differentiating Eq. (13) with respect to the temperature. In a manner similar to Sinanoğlu's analysis [25] we obtain that

$$\frac{\partial \Delta S^{o}_{g,diss.}}{\partial \Delta A_{np}} = \kappa_A^s \gamma_A \left(\frac{\partial \ln \gamma_A}{\partial T} + \frac{\partial \ln \Delta A_{np}}{\partial T} \right) - \kappa_{AS}^s \gamma_{AS} \left(\frac{\partial \ln \gamma_{AS}}{\partial T} + \frac{\partial \ln \Delta A_{np}}{\partial T} \right) \quad (14)$$

and the analogous expression for enthalpy is

$$\frac{\partial \Delta H^{o}_{g,diss.}}{\partial \Delta A_{np}} = \kappa_{AS}^h \gamma_{AS} \left(1 - \frac{\partial \ln \gamma_{AS}}{\partial \ln T} - \frac{\partial \ln \Delta A_{np}}{\partial \ln T} \right) - \kappa_A^h \gamma_A \left(1 - \frac{\partial \ln \gamma_A}{\partial \ln T} - \frac{\partial \ln \Delta A_{np}}{\partial \ln T} \right) \quad (15)$$

where κ^s and κ^h convert the surface tension to molecular dimensions in the expressions for entropy and enthalpy, respectively. Eq. (14) and Eq. (15) reflect that the spherical cavity area is a temperature dependent structural property and is simply related to the coefficient of thermal expansion of the solvent, α, by the following expression

$$\frac{\partial \ln \Delta A_{np}}{\partial T} = \frac{2}{3}\alpha \quad (16)$$

In a manner similar to the dissolution of gases in water, we adapt the solvophobic theory to express the Gibbs energy change for vaporization as

$$\Delta G^{o}_{vap.} = \kappa_A^g \gamma_A \Delta A - RT \ln \frac{RT}{\nu_A} \quad (17)$$

and the Gibbs energy change associated with the dissolution of liquids in water as

$$\Delta G^{o}_{l,diss.} = \kappa_{AS}^g \gamma_{AS} \Delta A + RT \ln \frac{\nu_A}{\nu_S} + G_{mix} \quad (18)$$

where ν_A is the molar volume of the solute. Expressions for other thermodynamic quantities may be easily derived from the above equations by taking the respective temperature derivatives. From the vaporization data κ_A^g values have been calculated and tabulated for a number of solvents along with κ values for the entropy and enthalpy [29]. The values of κ_{AS}^g, κ_{AS}^s and κ_{AS}^h were not available so that they were obtained from liquid dissolution data, by plotting the appropriate thermodynamic quantities for a set of hydrocarbons against their water accessible nonpolar surface areas, and from the relevant interfacial tensions.

For the dissolution of nonpolar gases in water the Gibbs energy, entropy and enthalpy changes, all per unit nonpolar surface area, were calculated with the γ_A, γ_{AS}, $(\partial \ln \gamma_A / \partial \ln T)$, $(\partial \ln \gamma_{AS} / \partial \ln T)$ and $(\partial \ln \Delta A_{np} / \partial \ln T)$ values from the literature and the κ values from vaporization and liquid dissolution data by using Eqs. (13)–(15).

5.2. Hydrophobic interaction chromatography

Salting out of proteins and HIC retention have been treated extensively in the absence of specific salt effects by an adaptation of the solvophobic theory [5,6] and the Gibbs energy was expressed by the surface tension of aqueous salt solutions. Experimental data for protein retention obtained with cosmotropic salts [31] that do not preferentially bind to the protein lends support to the theory.

The present treatment of HIC is somewhat different from the previous one [5] in that both the cavity and interaction Gibbs energy terms, c.f., Eq. (10) and Eq. (11), are lumped into the interfacial tension and molecular contact area. According to Eqs. (7), (8), (10) and (11) the Gibbs energy, ΔG^{o}_{HIC}, of reversible binding of a nonpolar eluite A at the hydrophobic ligate of the HIC stationary phase at relatively high salt concentration where electrostatic interactions are negligible is written as

$$\Delta G^{o}_{\mathrm{HIC}} = (\kappa^{g}_{AS}\gamma_{AS} - \kappa^{g}_{A}\gamma_{A}) \Delta A_{c} - RT \ln \frac{RT}{\nu_{S}} + \Delta G_{\mathrm{mix.}} + \Delta G_{\mathrm{red.}} + \Delta G^{\mathrm{vac.}}_{\mathrm{HIC}} \qquad (19)$$

where γ_{AS} is now the hydrocarbon/eluent interfacial tension, $\Delta G^{\mathrm{vac.}}_{\mathrm{HIC}}$ is the hypothetical retention Gibbs energy change in vacuo and ΔA_{c} is the contact area upon binding, i.e., the surface area of the eluite/ligate complex less the sum of solute and ligate surface areas, which has a negative value in the retention process. Eq. (19) is based on the assumption that the interfacial tensions between the solvent and the adsorbed eluite, the ligate or the unbound eluite are given by γ_{AS} and the free volume changes of the adsorbed eluite and of the ligate cancel each other. For the HIC retention of nonpolar eluites with similar γ and κ values, Eq. (19) predicts a linear relationship between $\Delta G^{o}_{\mathrm{HIC}}$ and ΔA_{c} at a given temperature.

Our analysis is based on the following two linear relationships. First, the contact area upon binding is linear in the nonpolar surface area of the molecules so that

$$\Delta A_{c} = \alpha \Delta A_{np} + \beta \qquad (20)$$

Second, the surface tension of the eluent, γ_{S}, depends on the molal salt concentration, m, as

$$\gamma_{S} = \gamma_{o} + \sigma m \qquad (21)$$

where γ_{o} is the surface tension of eluent in the absence of salt and σ is the molal surface tension increment of the salt [5]. If Eq. (21) holds also for the hydrocarbon/eluent interfacial tension, γ_{AS}, then the Gibbs energy per unit nonpolar surface area is obtained by evaluating the rhs of the following equation

$$\frac{\partial \Delta G^{o}_{\mathrm{HIC}}}{\partial \Delta A_{np}} = [\kappa^{g}_{AS}(\gamma_{AS,O} + \sigma m) - \kappa^{g}_{A}\gamma_{A}] \alpha \qquad (22)$$

where $\gamma_{AS,O}$ is the hydrocarbon/water interfacial tension. Analogous expressions for the entropy and the enthalpy may be written as

$$\frac{\partial \Delta S^{o}_{\mathrm{HIC}}}{\partial \Delta A_{np}} = \left[\kappa^{s}_{A}\gamma_{A}\left(\frac{\partial \ln \gamma_{A}}{\partial T} + \frac{\partial \ln \Delta A_{np}}{\partial T} \right) - \kappa^{s}_{AS}(\gamma_{AS,O} + \sigma m) \times \left(\frac{\partial \ln \gamma_{AS}}{\partial T} + \frac{\partial \ln \Delta A_{np}}{\partial T} \right) \right] \alpha \qquad (23)$$

$$\frac{\partial \Delta H^{o}_{\mathrm{HIC}}}{\partial \Delta A_{np}} = \left[\kappa^{h}_{AS}(\gamma_{AS,O} + \sigma m) \left(1 - \frac{\partial \ln \gamma_{AS}}{\partial \ln T} - \frac{\partial \ln \Delta A_{np}}{\partial \ln T} \right) - \kappa^{h}_{A}\gamma_{A}\left(1 - \frac{\partial \ln \gamma_{A}}{\partial \ln T} - \frac{\partial \ln \Delta A_{np}}{\partial \ln T} \right) \right] \alpha \qquad (24)$$

From Eqs. (13)–(15) and Eqs. (22)–(24) it follows that for both the dissolution in water of nonpolar gases and the retention in HIC, plots of thermodynamic parameters versus nonpolar surface area are straight lines and the slopes are nearly identical provided α is close to unity and the increment in hydrocarbon/eluent interfacial tension due to the presence of salt is negligible.

5.3. Comparison of retention in HIC and dissolution of nonpolar gases in water.

A way to the group molecular parameters is offered by the solvophobic theory and we shall calculate $(\partial \Delta G_{\mathrm{g.}}{}^{\mathrm{o}}{}_{\mathrm{diss.}}/\partial \Delta A_{np})$, $(\partial \Delta S_{\mathrm{g.}}{}^{\mathrm{o}}{}_{\mathrm{diss.}}/\partial \Delta A_{np})$ and $(\partial \Delta H_{\mathrm{g.}}{}^{\mathrm{o}}{}_{\mathrm{diss.}}/\partial \Delta A_{np})$ at 25°C using Eqs. (13)–(15) with n-heptane as model solute in order to test this approach. n-Heptane was chosen in our analysis because of the availability of surface tension and other physiochemical data. Its surface tension (γ_{A}) and the n-heptane/water interfacial tension (γ_{AS}) are taken as 28.3 [32] and 73.2 [33] cal mol^{-1} Å^{-2} whereas the κ^{g}_{A}, κ^{s}_{A} and κ^{h}_{A} values of 0.869, 0.542 and 0.687 as well as $(\partial \ln \gamma_{A}/\partial \ln T)$ and $(\partial \ln \Delta A_{np}/\partial \ln T)$ values of -1.505 and 0.248 are obtained from Ref. [25]. The microthermodynamic hydrocarbon/water interfacial tension, which is given by the product $\kappa^{g}_{AS}\gamma_{AS}$, is 30 cal mol^{-1} Å^{-2} [34,35] so that for κ^{g}_{AS} a value of 0.41 is obtained.

With data from the literature [17,22] for the dissolution of liquid aliphatic hydrocarbons in water at 25°C, plots of entropy and enthalpy against the molecular area are straight lines with slopes of $-0.08\,\mathrm{cal\,mol^{-1}\,K^{-1}\,\AA^{-2}}$ and $11.6\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$ which represent the second term of Eq. (14) and the first term of Eq. (15), respectively. With -0.56 for $(\partial \ln \gamma_{AS}/\partial \ln T)$ [33] the respective κ^s_{AS} and κ^h_{AS} values are obtained as -1.05 and 0.12. Eqs. (13)–(15) with these physical constants yield $5.4\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$, $-32.3\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$ and $-0.14\,\mathrm{cal\,mol^{-1}\,K^{-1}\,\AA^{-2}}$ for the respective molecular parameters a_g, a_h and a_s, in good agreement with those listed in Table 3 for various classes of substances.

We shall now estimate group molecular parameters associated with retention in HIC of dansyl amino acids by using the solvophobic theory. In most HIC experiments the eluting salt was $(NH_4)_2SO_4$ [11] that has a molal surface tension increment of 3.11 $\mathrm{cal\,kg\,mol^{-2}\,\AA^{-2}}$ [5]. The linear plots of the chromatographic data in Fig. 5 reveals that ΔA_c is directly proportional to ΔA_{np} for the eluites on each column so that Eq. (20) is applicable. The coefficient α is taken as $+0.7$ with the sign reflecting transfer to aqueous phase. This value was obtained in reversed phase chromatography [36,37] and is assumed to hold also in HIC with nonpolar sample components. Further, the physical properties of n-heptane were assumed to be representative for the hydrocarbonaceous moieties of the eluites in HIC. Since κ^g_A, κ^s_A and κ^h_A are defined for a unit ratio of the solute to solvent molecular radii, their values for n-heptane are not expected to be very different from those for dansyl amino acids. In contradistinction, the κ^g_{AS}, κ^s_{AS} and κ^h_{AS} values are dependent on the ratio of the solute to solvent molecular radii and, therefore, may be different for n-heptane and dansyl amino acids. As liquid dissolution data for dansyl amino acids were not available we used the κ^g_{AS}, κ^s_{AS} and κ^h_{AS} values of n-heptane instead. Since the ratio of molecular radii of dansyl amino acids under investigation to that of water is not very different from the radii ratio for n-heptane-water system this approach can be justified. With the data $(\partial \Delta G^o_{HIC}/\partial \Delta A_{np})$, $(\partial \Delta H^o_{HIC}/\partial \Delta A_{np})$ and $(\partial \Delta S^o_{HIC}/\partial \Delta A_{np})$ were evaluated according to Eqs. (22)–(24) and their respective values were $4.9\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$, $-22.2\,\mathrm{cal\,mol^{-1}\,\AA^{-2}}$ and $-0.10\,\mathrm{cal\,mol^{-1}\,K^{-1}\,\AA^{-2}}$ in excellent agreement with the corresponding average values listed in Table 3.

6. Conclusions

The scope of exothermodynamic relationships, such as enthalpy-entropy compensation and molecular area correlations, has been expanded by the use of the solvophobic theory. Employing readily available physicochemical data, the solvophobic theory establishes a link between thermodynamic quantities and the nonpolar molecular area for processes involving hydrophobic interactions. As shown here it can not only describe the thermodynamics of molecular interactions in solution and thus shed light on the role of solvent but can also account for the major factors determining the retention behavior in hydrophobic interaction chromatography (HIC).

The chromatographic data exhibits enthalpy-entropy compensation and the isothermodynamic temperatures are very similar to those for the dissolution of nonpolar gases in water. The availability of calorimetric data at 25°C for other processes driven by hydrophobic interactions, such as dissolution of solid cyclic dipeptides and liquid hydrocarbons in water as

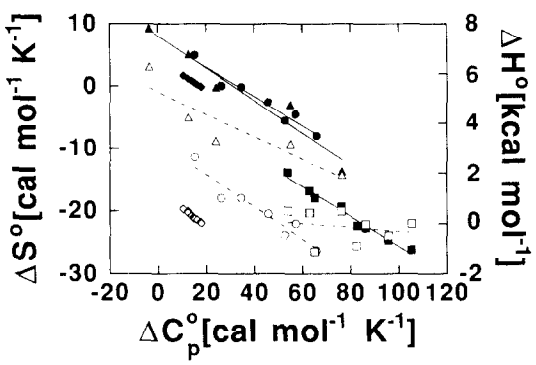

Fig. 6. Plots of the entropy (solid symbols and lines) and the enthalpy (open symbols and dashed lines) against the heat capacity change at 25°C for processes dominated by hydrophobic interactions. Data presented are for the (●, ○) HIC retention of dansyl amino acids on Column B; dissolution of nonpolar substances in water: (■, □) liquid hydrocarbons, and (▲, △) solid cyclic dipeptides; (◇, ◆) thermal denaturation data of 11 proteins calculated per mole of amino acid residue. Sources of data are listed in Table 4.

Table 4
Isothermodynamic temperatures [°C] for processes dominated by the hydrophobic effect. Isoentropic T_S^* and isoenthalpic T_H^* temperatures were derived from the linear dependence of the entropy and enthalpy on the heat capacity change at 25°C according to Eq. (3) and (4), respectively. The isoenergetic temperature T_G^* was evaluated by using Eq. (5). Standard states are ideal gas at 1 atm. and ideal aqueous solution at 1 M concentration unless stated otherwise. Errors shown are 1 SD of the best fit

Process	No.	Substances	T_S^*	T_H^*	T_G^*	Ref.
HIC on Column B	I	7 dansyl amino acids [a]	103 ± 9	88 ± 9	2	[11]
Dissolution of liquid hydrocarbons in water	II	8 alkanes and aromatics [b]	$104 + 6$	33 ± 14	-102	[17,38–40]
Dissolution of solids in water	III	5 hydrophobic cyclic dipeptides [c]	114 ± 15	70 ± 12	-55	[16]
Denaturation of proteins	IV	11 proteins [d]	112 ± 0	100 ± 5	20	[17]

[a] Those given in Fig. 1(b). [b] n-Propane, n-butane, n-pentane, n-hexane, benzene, toluene, ethylbenzene and cyclohexane. [c] c-Gly-gly, c-ala-gly, c-ala-ala, c-leu-gly and c-val-val where *c* indicates "cyclo". T_S^* and T_H^* values are different from those reported in the literature [18,41] because only corrected data for dipeptides containing aliphatic amino acids was used here [16]. Standard state: unit mole fraction. [d] Ribonuclease A, parvalbumin, egg-white lysozyme, fragment K4 of plasminogen, α-chymotrypsin, papain, staphylococcus nuclease, carbonic anhydrase, cytochrome c, pepsinogen and myoglobin.

well as denaturation of proteins, allows us further to extend the analysis to these processes. Fig. 6 illustrates plots of ΔS^o and ΔH^o against ΔC_p^o for HIC retention on column B and for the above mentioned processes involving hydrophobic interactions, whereas Table 4 lists the isothermodynamic temperatures evaluated from the plots by using Eqs. (3)–(5). It is seen that the T_S^* values are very close for these apparently disparate processes indicating that the processes are entropically similar. In contradistinction, the isoenthalpic and isoenergetic temperatures are quite different. For dissolution in water of solid cyclic dipeptides T_H^* is 70°C and this may be attributed to a contribution by the heat of sublimation to the enthalpy change. Further, for dissolution of liquid hydrocarbons in water T_H^* is 33°C that is explained by the high heat of vaporization included in the enthalpy change [28,40]. The isothermodynamic temperatures associated with the retention in HIC lie outside the experimental range employed so far, so that they are yet to be confirmed by experiments. HPLC at elevated [42] or sub-zero [43] temperatures with columns packed with micropellicular stationary phases [44] may offer a means to test the predictions by the isothermodynamic temperatures.

This work indicates that molecular chromatography, i.e. the evaluation of physicochemical parameters by precision chromatographic measurements, can be used to obtain and analyze thermodynamic quantities associated with hydrophobic interactions. The results obtained by comparing the chromatographic data from HIC to those from calorimetry for various processes involving hydrophobic interactions confirm the mechanistic identity of the processes at the molecular level. The common features of such processes are expected to facilitate the treatment of protein stability in terms of a hydrophobic and a nonhydrophobic contribution [18] as well as in terms of solvent effect. Indeed, values similar to those obtained in this study for the group molecular parameters have been employed to express the contribution of hydrophobic enthalpies [45], entropies [46] and Gibbs energies [46] to the energetics of protein folding.

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