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Hydrophobic interaction chromatography of proteins II. Solution thermodynamic properties as a determinant of retention

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Abstract

A general thermodynamic relation was derived to correlate protein solubility to retention in hydrophobic interaction chromatography (HIC). This relation is built on a thermodynamic formulation presented previously by Melander, Horváth and co-workers in the context of the solvophobic theory, but the final result is independent of this model framework. The relation reflects an increase in protein retention in HIC under conditions that promote precipitation or crystallization, consistent with early descriptions of HIC. To examine the contribution of protein solubility to retention in HIC, isocratic elution experiments were performed with four different commercially available agarose media and four model proteins (ribonuclease A (RNA), lysozyme (LYS), myoglobin (MYO), and ovalbumin (OVA)). A wide variety of retention trends were observed as a function of protein, adsorbent type, salt type and concentration, and pH. In general, however, the results show that solubility, or its surrogate, the second osmotic virial coefficient, which reflects solution thermodynamic properties, correlates well with HIC retention in many cases; this includes correctly predicting reverse Hofmeister effects, which cannot be explained by retention models based on the solvophobic theory and preferential interaction theory. However, solution properties could not explain retention behavior under some conditions. In those cases, effects such as protein–surface interactions or conformational change could be important determinants of protein adsorption.

Keywords: HIC adsorbents; Octyl; Butyl; Phenyl; Second osmotic virial coefficient; Protein solubility; Adiabatic compressibility; Retention factor

1. Introduction

Hydrophobic interaction chromatography (HIC) is a useful chromatographic technique for purification of biologics. Protein adsorption in HIC is induced by high salt concentrations such as those used in precipitation, and kosmotropic salts with greater "salting-out" ability, such as ammonium sulfate (AS), generally increase protein retention in HIC [1]. Indeed, in early studies HIC was sometimes referred to as salting-out chromatography [2], solubility chromatography [3], salt-induced protein chromatography [4] or salt-mediated hydrophobic chromatography [5]. HIC has been used as a rapid screening method to determine the optimal conditions for protein precipitation [6] and protein partitioning in aqueous two-phase systems [7]. In addition, analysis of retention has been guided by that of protein solubility, with the protein–surface interactions in HIC modeled analogously to the protein–protein interactions relevant to solubility. However, these analyses have typically been developed in parallel, and the thermodynamics of the protein in solution have often been neglected entirely in HIC retention models in favor of modeling only the protein–surface interactions.

The modeling approaches most commonly used are the solvophobic theory [8–13] and preferential interaction theory [14–19]. The solvophobic model is based on interfacial tension arguments that suggest that salts with higher molal surface tension increments promote retention. A corollary of this is that the trend of effectiveness of different salts in promoting salting-out or HIC retention should be the same for all proteins. The preferential interaction model is based on the exclusion of salts from the protein and ligand surfaces, allowing protein interactions with other proteins or with ligands. In addition to the solvophobic theory and preferential interaction theory, other retention models have also been developed [20,21], although they have been less widely used. The model developed by Staby and Mollerup [21] differs from the solvophobic and preferential interaction models in explicitly accounting for the solution properties, specifically

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in suggesting that salts that increase the activity coefficient of the protein in the mobile phase improve retention. This emphasizes the important general point that protein hydrophobicity and the resulting interaction with hydrophobic ligands is not the only factor affecting retention in HIC. The uncertain role of surface hydrophobicity is also reflected in results showing that ribonuclease S is more strongly retained on a butyl surface than other ribonuclease variants although they all have similar average surface hydrophobicities [22]. The authors suggested that higher structural flexibility of ribonuclease S could be responsible for its stronger retention. This is consistent with our conclusions in a companion paper, which showed that proteins with higher adiabatic compressibilities have higher retention factors independent of the base matrix of the adsorbents [23]. Jones and Fernandez [24] and Wu et al. [19] suggested that conformational change could affect protein retention behavior on HIC media.

An alternative correlative analysis is developed here, based on the observation that protein retention in HIC is driven by some of the same effects that determine solubility [2,8,17,25]. These parallels are present in the thermodynamic relations of Horváth and co-workers [8,9] that were used, along with the solvophobic theory [26,27], to obtain functional forms for k' in HIC and for S as a function of the molal surface tension increment. However, the two sets of phenomena, viz. HIC retention and solubility, were considered independently in the earlier work, and were similar only in invoking the same mechanisms to describe protein–ligand and protein–protein interactions in the respective processes. Therefore, the model as presented previously assumes that protein retention in HIC is driven solely by protein–ligand interactions. The approach employed here provides a novel and more general interpretation of the mechanism that drives protein retention in HIC by relating the two sets of phenomena directly via the thermodynamic properties of the protein in solution. This allows us to explore the relation of protein solubilities to HIC retention and to determine if a direct correlation exists between retention in HIC and the solubility, where the latter is in turn correlated with the solution thermodynamic properties.

2. Theory

To derive a correlation between protein retention and solubility, we follow an approach similar to those presented by Melander, Horváth and co-workers [8,9,28]. Protein in solution is simultaneously at equilibrium with protein adsorbed on the surface and protein in the solid phase; these two equilibria are treated separately, with 1 mg protein/g solvent dissolved at the solution conditions of interest (pH 7, 25 °C, 1 atm) taken as the standard state.

For HIC retention, the retention factor, k', under linear retention conditions can be expressed as

$$k' = K_{eq}\phi \tag{1}$$

where ϕ is the phase ratio of the adsorbent and K_{eq} is the adsorption equilibrium constant. For the adsorption equilibrium, the equilibrium constant of a solute on a planar and uniform surface, K_{eq}, can be expressed as [29,30]

$$K_{eq} = \int_{z_0}^{\infty} (e^{-\Delta G_{PL}/RT} - 1)\, dz \tag{2}$$

where ΔG_{PL} is the change in free energy due to the protein–surface interaction, R the gas constant, T the absolute temperature, z_0 the cutoff distance incorporated to account for the steric hindrance between the solute and the surface and z is the distance of the solute from the adsorption surface. It should be noted that K_{eq} in Eq. (2) has units of length. In HIC, it is generally believed that the forces involved in protein–surface interaction are short-ranged. An approximation is to describe them as a square well with depth ΔG_{PL} and width given by the range of the interaction Δz; other formulations could be used instead, with less mathematical convenience but the same general functional dependence. Assuming that $|\Delta G_{PL}|$ is significantly greater than RT, Eq. (2) can be simplified to

$$\ln K_{eq} = -\frac{\Delta G_{PL}}{RT} + \ln(\Delta z) \tag{3}$$

The change in molal free energy due to protein adsorption on a HIC surface at standard conditions, ΔG_{PL}, is [28]

$$\Delta G_{PL} = G_{PL} - G_P - G_L \tag{4}$$

where the subscripts PL, P, and L denote protein–ligand complex, protein and ligand, respectively. With this formulation, Eq. (1) can be rewritten as

$$\ln k' = \frac{-(G_{PL} - G_L)}{RT} + \frac{G_P}{RT} + \ln(\Delta z\phi) \tag{5}$$

For the equilibrium of protein in solution with the solid, the partial molar Gibbs free energy of the protein in dilute solution, G_P^D, can be written as [8,31]

$$G_P^D = G_P + RT\ln(x) \tag{6}$$

where G_P is the molar free energy of protein in solution at the standard state and x is the protein concentration in milligram per gram solvent. The omission of an activity coefficient from this expression reflects primarily the use of a standard state based on the solvent of interest, in this case, the appropriate salt concentration, but it also implies a negligible dependence of the activity coefficient on protein concentration in the dilute solutions of interest. Specialization of Eq. (6) for a saturated protein solution in equilibrium with solid protein, where the molar free energy of protein in solution is equal to that the molar free energy of protein in the solid, G_S, introduces the protein solubility, S, in milligram per gram solvent via

$$G_S = G_P + RT\ln(S) \tag{7}$$

Substituting Eq. (7) into Eq. (5) gives

$$\ln k' = -\ln(S) + \ln(\Delta z\phi) + \frac{G_S}{RT} - \frac{G_{PL} - G_L}{RT} \tag{8}$$

where the term in $\Delta z\phi$ is salt-independent. Eq. (8) suggests that retention in HIC should increase with decreasing protein solubility. However, the terms in $G_{PL} - G_L$ and G_S, which represent,