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ADSORPTION OF AROMATIC MOLECULES ON HYDROPHILIC GEL CHROMATOGRAPHY MEDIA

A THERMODYNAMIC STUDY

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SUMMARY

The temperature dependence of the adsorption of three model compounds, tryptophan, adenosine 5'-monophosphate and ϵ-dinitrophenyllysine on two gel media, Sephadex G-25 and Bio-Gel P-2, was investigated. The adsorption was found to be temperature-dependent, increasing with decreasing temperature. Linear Van 't Hoff plots were obtained in all cases with the two gel systems employed. Thermodynamic parameters were calculated for the three compounds. From an analysis of the data obtained from this and other work [A. Sada, G. Di Pascale and M.G. Cacace, J. Chromatogr., 177 (1979) 353], it is likely that the adsorption is due to the contribution of at least two factors: (i) halophilic interaction with the water molecules tightly bound to the high-density gels and (ii) the presence of suitable groups apt to form charge-transfer complexes with the solute.

INTRODUCTION

Hydrophobic chromatography has become a widely used technique for the isolation of proteins and other biologically active macromolecules. At the time of their introduction [1—3], derivatized gels containing aliphatic chains of various lengths, provided apolar heads available for binding to hydrophobic domains in proteins. Under these conditions, however, binding was affected by ionic strength in different, sometimes opposite, ways.

Interestingly, unsubstituted Sepharose was shown to allow fractionation of transfer RNA with decreasing concentration gradients of ammonium sulphate [4]. Also, proteins from halophilic bacteria, functionally stable at high ionic strength, could be reversibly adsorbed on unmodified Sepharose [5]. Large-pore gel chromatography media can therefore exhibit toward biopolymers adsorption properties resembling those encountered in hydrophobic chromato-

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graphy, at least in the presence of a high salt concentration. In fact, since their introduction as gel permeation media [6], it is known that high-density gels, often of polysaccharidic nature, adsorb low-molecular-weight aromatic compounds even under low salt conditions. This effect is most evident, however, at higher ionic strengths [7, 8].

We have previously reported the effect of salts on the elution parameters of some aromatic molecules from Sephadex G-25 [9]. The linear dependence of the logarithm of the distribution coefficient, K_d, on ionic strength in a wide range of concentrations suggested that one of the causes responsible for the observed adsorption could be hydrophobic interaction.

To clarify the nature of this interaction further, we have investigated the thermodynamic parameters relative to the adsorption of some model compounds of different aromaticity, using two high-density matrices widely used in gel permeation chromatography: a polysaccharide containing O-ether groups in the cross-linking regions (Sephadex G-25) and a polyacrylamide gel with a homogeneous all-amide network (Bio-Gel P-2).

EXPERIMENTAL

Adenosine 5′-monophosphate (AMP), ϵ-dinitrophenyllysine (ϵ-DNP-lysine) and tryptophan were obtained from Sigma (St. Louis, MO, U.S.A.). Sephadex G-25 (fine, batch No. 6749) and Dextran Blue 2000 were from Pharmacia Fine Chemicals (Uppsala, Sweden). Bio-Gel P-2 was from Bio-Rad Labs. (Richmond, CA, U.S.A.).

Elution was carried out at the indicated temperature in 50 m*M* potassium phosphate buffer (pH 7.0). Temperature was controlled in water-jacketed columns with a Haake F-3C thermocryostat at ± 0.01°C sensitivity. Column bed dimensions were 38 × 1 cm I.D. Sample volume was always 200 μl. Column parameters were obtained as described elsewhere [9].

RESULTS

The effect of temperature on the elution pattern of the three aromatic compounds selected for this and previous work [9] is shown in Fig. 1. All compounds exhibited increasing retardation with decreasing temperatures; resolution improved at higher temperatures. Fig. 1 shows the pattern relative to Sephadex G-25. The chromatographic runs carried out using Bio-Gel P-2 showed a similar behaviour.

Table I gives the values of the distribution coefficient at the four temperatures used. The temperature dependence of K_d is given by the Van 't Hoff equation:

$$\ln K_d = -\Delta H/RT + I \quad (1)$$

where I is an integration constant.

A plot of the data obtained for the two gels at various temperatures is shown in Fig. 2.

ΔH values were calculated by the least-squares method and are summarized in Table II. From eqn. 2

$$\Delta G = -RT \ln K_d \quad (2)$$

and the Gibbs relationship

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

the full set of thermodynamic parameters can be obtained, which are shown in Table II.

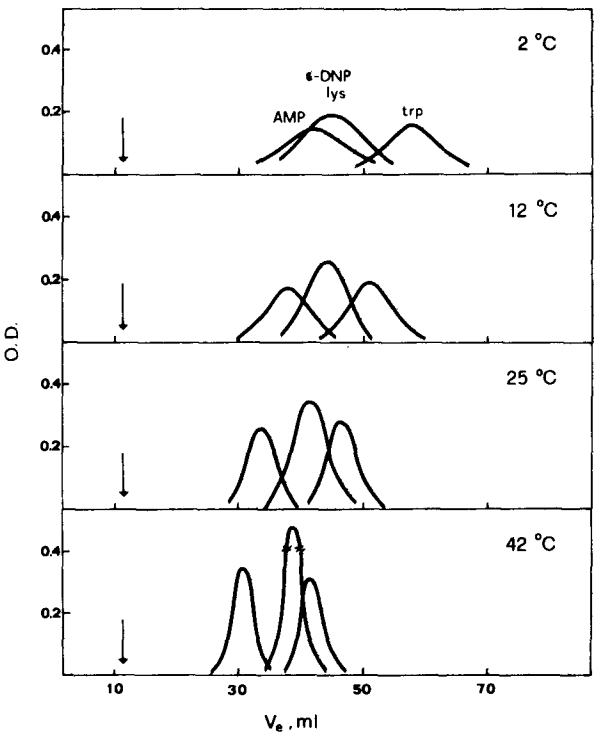

Fig. 1. Effect of temperature on elution patterns of various aromatic compounds on Sephadex G-25. Column dimensions: 38 × 1 cm I.D. Bed volume: 30 ml. Elution buffer: 50 m*M* phosphate buffer (pH 7.0). Samples (200 μl) contained each aromatic compound in 1% (w/v) Dextran Blue 2000, 1 *M* ammonium sulphate. The arrows indicate the elution position of Dextran Blue. The elution volume of ammonium sulphate ranged from 24.20 to 24.85 ml.

TABLE I

K_d VALUES OF THE THREE AROMATIC COMPOUNDS, AMP, TRYPTOPHAN AND ε-DNP-LYSINE, FOR THE TWO GEL SYSTEMS, SEPHADEX G-25 AND BIO-GEL P-2

Compound	K_d							
	Sephadex G-25				Bio-Gel P2			
	2° C	12° C	25° C	42° C	2° C	12° C	25° C	42° C
ε-DNP-lysine	2.52	2.54	2.25	2.18	5.13	4.49	3.98	3.11
AMP	2.33	2.06	1.65	1.54	1.24	1.15	1.08	0.96
Tryptophan	3.39	3.14	2.64	2.39	2.88	2.59	2.37	2.09

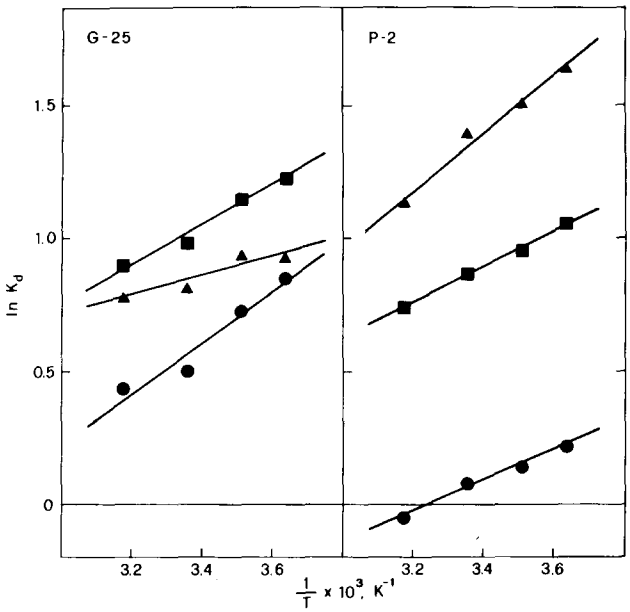

Fig. 2. Van 't Hoff plots relative to temperature dependence of elution parameters of the aromatic compounds using Sephadex G-25 and Bio-Gel P-2.

TABLE II

THERMODYNAMIC PARAMETERS RELATIVE TO THE ADSORPTION PROCESS OF THE AROMATIC COMPOUNDS TO THE TWO GEL MATRICES, SEPHADEX G-25 AND BIO-GEL P-2

Compound	Sephadex G-25			Bio-Gel P-2		
	ΔH	ΔG	ΔS	ΔH	ΔG	ΔS
ϵ-DNP-lysine	−710	−500	−0.73	−2100	−800	−4.40
AMP	−1860	−340	−5.10	−1090	−38	−3.54
Tryptophan	−1570	−593	−3.30	−1350	+510	+2.83

The temperature dependence of both elution position and resolution for the chosen compounds exhibited a similar behaviour with the two hydrophilic gels used, Sephadex G-25 and Bio-Gel P-2. A straight line was obtained in all cases when ln K_d values were plotted against $1/T$.

It can be noted that the behaviour of ϵ-DNP-lysine is peculiar: when chromatographed on Sephadex G-25, the temperature dependence of its K_d values is less pronounced than that of AMP and tryptophan. The opposite behaviour is observed with Bio-Gel P-2: temperature has the most pronounced effect and K_d values of ϵ-DNP-lysine are the highest among the three compounds.

DISCUSSION

The adsorption properties of highly cross-linked gels, which were introduced more than twenty years ago as molecular sieves, have since been reported to

occur with a number of molecules that possess a wide range of chemical and structural properties.

The focus has mainly been on aromatic and unsaturated compounds, in particular nucleotides, both in Sephadex G-10 [10, 11] and Bio-Gel P-2 [12] and P-4 [13], amino acids and peptides [5], indole derivatives [14], steroids [15] and isoflavones [16]. Adsorption effects, however, were also observed with a number of completely unrelated compounds, such as sodium dodecyl sulphate [17], aliphatic alcohols [18–20], cyclic dextrins [21], phenol and substituted phenols [22, 23] and inorganic ions [24–28]. The concomitant observation, in the case of Sephadex gels, that a higher degree of substitution by hydroxy-ether groups or, better, by hydroxyl alkylation of cross-linked dextrans (LH Sephadex) corresponded to a higher degree of "organophilicity", was interpreted as evidence that the groups introduced in the gel by these chemical manipulations were responsible for binding [22].

These ideas were further developed by Porath and co-workers [29–31] who prepared a series of derivatized gels containing immobilized ligands suitable for forming charge-transfer complexes with molecules of biological interest, such as nucleotides or peptides. In some cases, the complex formation within the gel was demonstrated by the appearance of colour in the course of the chromatographic run.

However, the following peculiarities in the observed adsorption phenomena, some of which are common to all systems investigated, may be due to an intrinsic property of all media: the occurrence of a highly hydrated network with a sufficiently high density of gel monomer in the lattice cell unit.

(i) Binding sites in the gel cannot be saturated by retarded compounds [22].

(ii) Swelling of the gels is dependent on temperature and ionic strength. All kinds of G-type Sephadex gels shrink when the temperature is raised in the presence of an aqueous solvent. The opposite effect is observed with Sephadex LH-20 in the presence of an organic solvent. On the other hand, polyacrylamide gel P-2 in aqueous media swells with increasing temperature [32].

(iii) Adsorption effects are reduced by the addition of methanol [15, 19, 20, 33].

(iv) Water-insoluble dyes "dissolve" in the internal water of swollen Sephadex gels [34]. This effect is more pronounced with increasing dextran-chain concentration and temperature.

(v) Different salts exert different effects on the chromatographic behaviour of aromatic substances, according to their position in the Hofmeister series [24, 27].

(vi) Aromatic adsorption is observed in a wide range of matrices possessing unrelated chemical structure (polyacrylamide, polystyrene, Nylon) or, in the case of polysaccharides (starch, cellulose, cross-linked dextran), with different physicochemical properties.

Besides the attempts directed toward the identification and characterization of the groups responsible for binding, studies on the molecular basis of the interaction of solutes with the chromatographic support, also focused on the analysis of the thermodynamic parameters, based on physical models of solute–stationary phase interactions.

This approach developed from the work of Sinanoglu and co-workers [35,

36] and from the subsequent comprehensive study by Horváth and co-workers [37, 38].

Interestingly, the temperature dependence of K_d observed in the present work is similar to that found in reversed-phase chromatography [39]. In the latter case, a linear relationship between the logarithm of the retention factors, k, and the reciprocal temperature was found and, in addition, a linear relationship is obtained when plotting ln k against solvent composition.

In the case of gel chromatography, most of the gel media employed have hydrophilic properties and some possess few apolar groups. The solute will therefore interact with a fairly strongly hydrated gel layer. A stationary phase can be envisaged, constituted by the water molecules of the first hydration layer of the gel, and by that part of the gel molecules to which bulk solvent has free access. The surface energy of the hydrated gel layer is sufficiently lower than that of water, thus allowing attachment of the solute molecules.

The negative ΔH values obtained in this work show that the process of binding for the three substances investigated is enthalpically favoured relative to leaving both the gel-lattice interacting unit and the aromatic molecule in their hydrated states.

The negative ΔS values for all three compounds (except for tryptophan with the polyacrylamide gel) indicate that the process is accompanied by a net ordering of the system (increase in water structure?).

It is worth mentioning at this point that Von Hippel et al. [27] described cases in which some ions (NaF) were eluted ahead of the water elution volume in polyacrylamide gel chromatography. The interpretation given in that case was that certain ions were excluded because of their relative inability to compete for and thus displace the water molecules directly bound to the amide dipole.

That biopolymers possess coordinated water was also shown by Lüscher-Mattli and Rüegg [40], who observed an anomaly in the desorption isotherms of proteins and DNA in the low-humidity range, corresponding to the hydration of the inner-sphere shell.

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