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Note

Comparison of phenyl- and octyl-Sepharose CL-4B in the hydrophobic interaction chromatography of simple aliphatic and aromatic compounds

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Previously we have published a series of papers[1–7] dealing with the hydrophobic interaction chromatography of simple aliphatic compounds on octyl-Sepharose CL-4B. We now report some results of analogous experiments with phenyl-Sepharose CL-4B, again using simple compounds, for this purpose aliphatic and aromatic in nature. For comparison, the same compounds were also chromatographed on octyl-Sepharose CL-4B.

Comparisons of these two hydrophobic adsorbents have been published before[8–12]. However, they were based on experiments with test compounds of more complicated structure, *i.e.*, proteins and polynucleotides, which obviously hampers the interpretation of the results. Nevertheless, certain peculiar effects, *e.g.*, reversal of the elution order of a particular protein or polynucleotide pair on the two materials, lead to speculations about a specific "aromatic" or "stacking" interaction mechanism[11,12] on phenyl-Sepharose. Such a detail should in principle be more readily discernible using aliphatic and aromatic compounds of much simpler structure.

EXPERIMENTAL

The experimental procedure has been fully described[1]. Test compounds (Aldrich, Brussels, Belgium; purity ⩾98%, used without further purification) were dissolved in the eluent at a concentration of 0.1–0.5 mg ml^{-1}. In some instances saturated solutions in the eluent were used. Aliquots of 1 ml of the sample solutions were applied to thermostatically controlled (25°C) columns of phenyl- and octyl-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) having bed volumes of about 120 ml and eluted at a flow-rate of 15–20 ml h^{-1}. The eluent was a 25 mM sodium phosphate buffer of pH 7.10 (analytical-reagent grade; Baker, Deventer, The Netherlands); detection was performed by measuring the UV absorbance at 206 or 254 nm (Uvicord S; LKB, Bromma, Sweden).

From the elution volume, the (partial molal) standard free energy change, ΔG°, for the partition of the test compound between the gel phase and the eluent was calculated using eqns. 3–6 in ref. 1. In these calculations the following values for the specific bed weight, β (milligrams of matrix material per millilitre of gel bed) and the

density, ρ, of the dry gel were used: $\beta_{os} = 36.3$ mg ml^{-1}, $\beta_{ps} = 38.0$ mg ml^{-1} and $\rho_{os} = \rho_{ps} = 1.6$ g ml^{-1}.

The degree of substitution of both gels is sufficiently high to permit the above treatment of the retention data in terms of a partition process[1,2].

RESULTS

In Fig. 1, $\Delta G°$ values for the partition on both gels are shown for three series of test compounds, *viz.*, carboxylic acids, $CH_3(CH_2)_{n-1}COOH$ ($n=6$–10), p-alkyl-benzoic acids, $CH_3(CH_2)_{n-1}C_6H_4COOHN$ ($n=0$–4), and ω-phenylcarboxylic acids, $C_6H_5(CH_2)_nCOOH$ ($n=0$–4). Except for p-isopropylbenzoic acid ($n=3$), the test compounds contained n-alkyl chains. Note that at the pH of the eluent the acids are more than 99% dissociated (their pK values range from 4.2 for benzoic acid to 5.0 for undecanoic acid).

The slopes, $-\Delta\Delta G°(CH_2)$, obtained by least-squares treatment of the data in Fig. 1 (only that for benzoic acid was omitted) are given in Table I.

DISCUSSION

We confine the discussion to the values of $-\Delta\Delta G°(CH_2)$ in Table I, which represent the contribution of a CH_2 group in the alkyl chains of the test compounds to the free energy change on hydrophobic interaction with octyl- and phenyl-Sepharose.
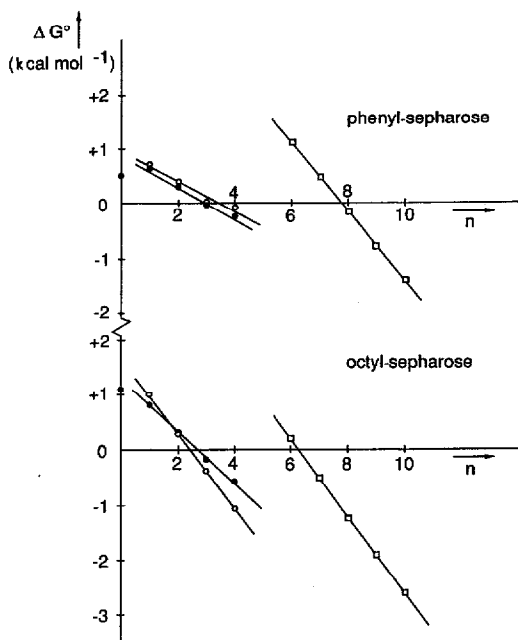

Fig. 1. Standard free energy change at 25°C *versus* alkyl chain length for ($\square$) carboxylic acids, ($\bigcirc$) p-alkylbenzoic acids and ($\bullet$) ω-phenylcarboxylic acids on phenyl- and octyl-Sepharose CL-4B at pH 7.10.

TABLE I

VALUES OF $-\Delta\Delta G^{\circ}(CH_2)$ FOR THE RETENTION OF THE THREE SERIES OF TEST COMPOUNDS AT 25°C ON PHENYL- AND OCTYL-SEPHAROSE CL-4B AT pH 7.10

Compounds	$-\Delta\Delta G^{\circ}(CH_2)$ (kcal mol^{-1})	
	Octyl-Sepharose	Phenyl-Sepharose
Carboxylic acids	0.70 ± 0.01	0.61 ± 0.02
p-Alkylbenzoic acids	0.69 ± 0.02	0.27 ± 0.04
ω-Phenylcarboxylic acids	0.47 ± 0.02	0.29 ± 0.03

Focusing first on the results with octyl-Sepharose, the value of 0.70 kcal mol^{-1} for the carboxylate ions is slightly smaller than that (0.74 kcal mol^{-1}) reported previously[1] for the corresponding uncharged acids at pH 3.52. Small effects of the charge were found previously[7] for the partition of carboxylic acids and alkylamines on octyl-Sepharose. Further, it was shown[7] that for both carboxylate and alkylammonium ions the $-\Delta\Delta G^{\circ}(CH_2)$ values tend to decrease with decreasing n. The cause of this effect is probably[1,7] an increasing influence of the charged head group on the solvation of CH_2 groups with decreasing n. This conclusion is corroborated by the results presented in this paper: for p-alkylbenzoate ions and carboxylate ions the $-\Delta\Delta G^{\circ}(CH_2)$ values are identical within experimental error (in both series the CH_2 group is far from the polar head), whereas for ω-phenylcarboxylate ions, where the CH_2 group is much closer to the charged head, $-\Delta\Delta G^{\circ}(CH_2)$ is much smaller (0.47 kcal mol^{-1}).

Turning now to the results with phenyl-Sepharose, it appears that $-\Delta\Delta G^{\circ}(CH_2)$ for aliphatic carboxylate ions is smaller than that on octyl-Sepharose (0.61 versus 0.70 kcal mol^{-1}). This reflects the lower hydrophobicity of phenyl compared with octyl groups. As only the hydrophobic interaction of a CH_2 group with the gels is expressed in these figures, we conclude that the ratio of hydrophobicities of the gels is phenyl/octyl = 61/70 = 0.87. We think that this is a more meaningful estimate of this ratio than those reported previously, which were based on interactions of proteins with the gels, often in the presence of high salt concentrations. The latter estimates, mostly expressed as a normal alkyl chain equivalence of a phenyl group (φ), are generally smaller than our estimate [compare, for instance, $\varphi \approx C_3$–C_4 (ref. 11) and $\varphi < C_5$ (ref. 9)]. The most peculiar results in Table I are the (within experimental error) identical and relatively small $-\Delta\Delta G^{\circ}(CH_2)$ values for the two series of aromatic carboxylate ions. A $-\Delta\Delta G^{\circ}(CH_2)$ value of 0.61 kcal mol^{-1} for p-alkylbenzoate ions is expected: as for aliphatic carboxylate ions, it represents the interaction of a CH_2 group, far from the charged head, with phenyl groups. The actual value is, however, less than half this. This might be explained by assuming that the interaction mechanism in this instance (and in that of the ω-phenylcarboxylate ions) is not a simple partition, but a bimolecular association of the test compounds with phenyl groups on the gel. The present results can then tentatively be interpreted as follows: the aromatic test compounds are held on the gel primarily by phenyl–phenyl bimolecular interaction. This phenyl–phenyl interaction fixes the orientation of the aromatic test compounds with respect to other phenyl groups, and to the glycidyl spacer arms connecting the

phenyl groups to the Sepharose chains. This orientation is probably not optimal for interaction of their alkyl chains with the phenyl-Sepharose, and thus a lower value of $-\Delta\Delta G^{\circ}(CH_2)$ results than is found for the aliphatic test compounds. Evidence for strong phenyl–phenyl interaction is afforded by the ΔG° value of benzoic acid on phenyl-Sepharose, which is lower than that on octyl-Sepharose (see Fig. 1), whereas the reverse holds for most other compounds.

In conclusion, we think that these results provide evidence for a special "aromatic" mechanism for the retention of aromatic compounds on phenyl-Sepharose. This mechanism differs from that of aliphatic compounds on phenyl-Sepharose and from that of aliphatic and aromatic compounds on octyl-Sepharose.

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