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Note

Salt effects on adsorption of aromatic compounds in Sephadex G-25 chromatography

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Aromatic and unsaturated compounds exhibit an unusual retardation when chromatographed on highly cross-linked dextran gels. These compounds show an enhanced adsorption in the presence of salts and commonly used buffers[1]. Determann and Walter[2] have suggested that this retardation is caused by interaction with ether groups arising from the cross-linking of the dextran gels. To clarify the mechanism of interaction of these compounds with the dextran gel, we have investigated the effect of salts on the elution parameters of some molecules with various extents of aromatic character using Sephadex G-25 gel as chromatographic support.

EXPERIMENTAL

Adenosine 5'-monophosphate (AMP), ε-dinitrophenyl (DNP)-lysine and tryptophan were obtained from Sigma (St. Louis, Mo., U.S.A.). Sephadex G-25 (Superfine, batch No. 6277) and Dextran Blue 2000 were from Pharmacia (Uppsala, Sweden).

Elution was carried out at 25 ± 1° in potassium phosphate buffer, pH 7.0, at the given concentration. A 39 × 1.5 cm column was used. The sample volume was 100 μl. Fractions of 1.1 ml were collected with a Gilson TDC-80 fraction collector. The peak positions for AMP, tryptophan and ε-DNP-lysine in the eluate were determined spectrophotometrically at 260 nm, 280 nm and 340 nm, respectively. Sulphate was determined nephelometrically as its barium salt at 500 nm[3]. Special care was taken to measure accurately the elution parameters of the column. The exact position of each peak was determined graphically and the volume of elution carefully calculated by weighing the fractions in a Sartorius 2254 balance (sensitivity, = 0.01 g).

RESULTS

In all cases the void volume (V_0) was determined with Dextran Blue and was found to be independent of the salt concentration. The inner volume (V_i) was determined by using ammonium sulphate which does not interact with the gel. In all the experiments performed at various buffer concentrations, the elution volume of the ammonium sulphate peak was constant. The true inner volume (V_i) of the column was measured both by determining the elution volume of ^3H_2O at various buffer

concentrations[4] and by determining the water regain (W_r) of bed material. Potassium phosphate buffer was used to obtain the desired ionic strength. This salt enabled us to investigate a wide concentration range (up to 2.0 *M*) and avoided the use of an accessory buffer. The elution profiles of AMP, ε-DNP-lysine and tryptophan at different salt concentrations are shown in Fig. 1. It can be seen that the adsorption increases with the ionic strength; however, the effect of salt concentration is different for the three substances. The adsorption of AMP is the least effected by salt, whereas the elution position of ε-DNP-lysine, which at 100 m*M* phosphate is intermediate between AMP and tryptophan, at 2.0 *M* phosphate lies after that of tryptophan.

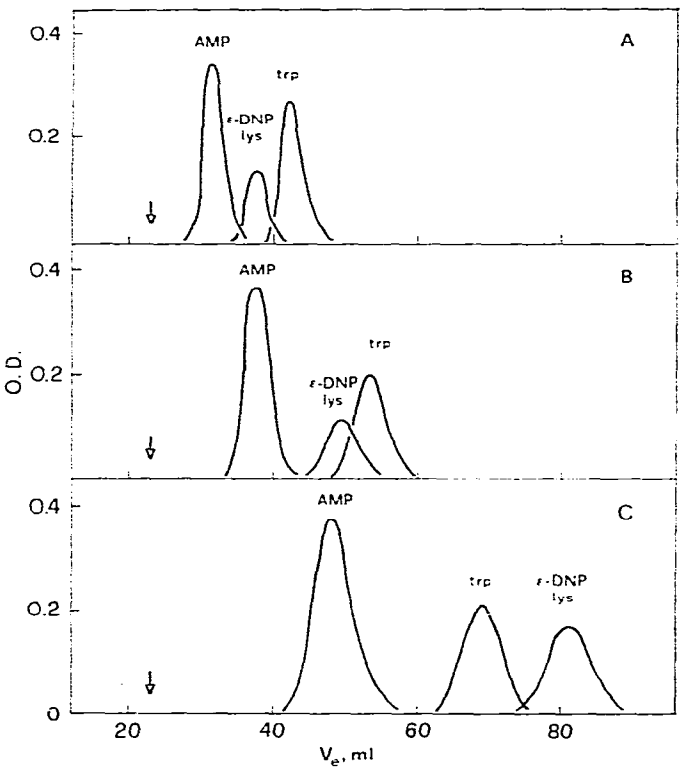

Fig. 1. Elution patterns of some aromatic compounds on a Sephadex G-25 column at different salt concentrations. Experimental conditions as described in the text. Potassium phosphate buffer, pH 7, was used as eluent at the following concentrations: 0.1 *M* (A), 1 *M* (B) and 2 *M* (C). The arrow indicates the elution position of ammonium sulphate. V_e = Elution volume.

Fig. 2 gives the salt effects on the distribution coefficient, K_d, of each substance. As proposed by Melander and Horváth[5], a molal scale has been used in preference to other concentration scales. A linear plot was obtained in all cases throughout the concentration range explored. As shown, the largest effect is observed for ε-DNP-lysine.

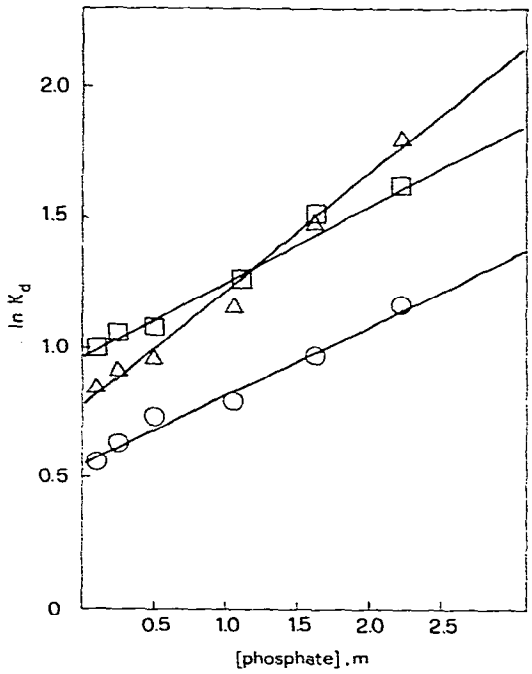

Fig. 2. Dependence of ln K_d of the tested substances on the salt molality. ○, AMP; △, ε-DNP-lysine; □, tryptophan.

CONCLUSIONS

We believe that two effects make different contributions to the observed affinity to the tested compounds for the Sephadex gel, *i.e.*, (a) complex formation with a suitable binding group in the matrix, and (b) hydrophobic interaction.

The direct proportionality of the experimental K_d values to the ionic strength up to exceptionally high salt concentrations indicates that the hydrophobic interaction of the compounds tested is more predominant with unsubstituted highly cross-linked Sephadex gels. It is worth mentioning that the position of the salt employed in Hofmeister's lyotropic scale is such that the salt can be supposed to enhance hydrophobic interaction. However, there have been several reports on the interaction of highly cross-linked Sephadex with various non-aromatic substances such as thiourea and *n*-alcohols[6], cyclic dextrins[7] and some inorganic ions, such as halides, thiocyanate and alkaline-earth metals[8]. It is unlikely that the adsorption of these substances is due to a unique mechanism, and the different experimental conditions (mainly the use of water or buffers as eluents) render difficult an analysis of these results in terms of our approach.

Recently, new substituted gels have been prepared by coupling π-electron acceptor groups capable of forming charge-transfer complexes, thus increasing the resolving power for aromatic substances[9]. However, a similar pattern of elution of the tested substances, even though much less resolved, was obtained on unsubstituted Sephadex G-25 under identical operating conditions. It cannot be excluded that uncharacterized π-electron acceptor groups are present in Sephadex G-25 and G-10,

probably derived from the chemical manipulations used for the cross-linking, even if these groups are not the hydroxy-ether linkages suggested by Determann and Walter[2]. Studies on the temperature dependence of the K_d values of these substances may clarify the relative contributions of the hydrophobic and charge-transfer complex interactions to the overall observed retardation effects.

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