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Short Communication

Charge-transfer adsorption chromatography

JERKER PORATH and KARIN DAHLGREN CALDWELL
Institute of Biochemistry, University of Uppsala, Box 576, S-751 23 Uppsala (Sweden)
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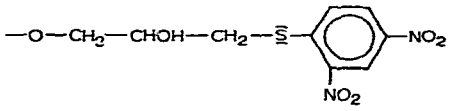
As a step in our development work on analytical and preparative fractionation methods based on gel chromatography, we have now synthesized gel adsorbents with ligands capable of forming charge-transfer complexes[1,2]. We describe here a π-electron acceptor gel that can be used for the separation of electron-donor substances, as exemplified with some neurophysiologically important compounds.

SYNTHESIS OF CHARGE-TRANSFER ADSORBENTS

We selected cross-linked dextran (Sephadex G-25) and agarose (Sepharose 6B) as suitable gel matrices. The gels were "activated" by the introduction of thiol groups according to a procedure similar to that of Axén *et al.*[3]. Dinitrophenyl groups were attached to the thiolated gel to give the following charge-transfer ligand:



The final coupling step for the Sephadex-based gel was as follows. A 100-ml volume of thiolated Sephadex G-25 containing 250 μmole of sulphur per gram of dry gel was transferred into a 500-ml round-bottomed flask and 100 ml of a 0.1 *M* solution of sodium hydrogen carbonate in 50% ethanol containing 1 m*M* EDTA was added. Then 1.5 g of 2,4-dinitrochlorobenzene was added and the reaction was allowed to proceed with stirring for 1.5 h at room temperature. The gel was washed with ethanol and water on a glass filter. The product contained 143 μmole of dinitrophenyl groups per gram of dry gel.

DNP-S-Sepharose 6B was prepared in an analogous manner and contained 375 μmole of DNP-S groups per gram of dry gel.

CHARGE-TRANSFER ADSORPTION

A 1 × 25 cm bed of DNP-S-Sepharose 6B in the swollen form was prepared in 0.1 *M* ammonium formate buffer, pH 3.2. A number of test substances, each dissolved in 0.5 ml of eluent solution, were run separately on the column at room temperature (25°) at a flow-rate of 4 ml/h. The absorbance of the eluate at 254 nm was

TABLE I

RESULTS OF EXPERIMENTS ON DNP-S-SEPHAROSE 6B

Temperature, 25°; pH 3.2.

Test substance	V_E/V_T	*Test substance*	V_E/V_T
Trp	1.29	Tyr–Tyr	1.41
Trp–Trp	5.27	Tyr–Tyr–Tyr	2.08
Trp–Gly	1.43	His–Tyr	1.29
Gly–Trp	1.51	His	1.16
Tryptamine–HCl	1.81	Phe	1.13
Indoleacetic acid	1.81	L-Dopa	1.16
Tyr	1.11	3-Iodotyrosine	1.34
Tyramine	1.25	3,5-Diiodotyrosine	1.94
Dopamine	1.31	3,5-Dibromotyrosine	1.25
Adrenaline	1.31	3,5-Dinitrotyrosine	1.11
Noradrenaline	1.29		
Mescaline	1.28	Acetophenazine	13.75
Gly–Tyr	1.21	Fluphenazine	9.82
Tyr–Gly–Gly	1.18	Chlorpromazine	15.25

recorded with a Uvicord spectrophotometer and the reduced elution volumes (V_E/V_T) were calculated, V_E and V_T being the elution volume and the total bed volume, respectively. The results are given in Table I.

Similar series of experiments were performed with DNP-S-Sephadex G-25 at 25° and 4°. Control experiments were also carried out at 25° and 4° with unsubstituted Sephadex G-25. The results are given in Table II.

Fig. 1 shows a chromatogram obtained at 4° on DNP-S-Sephadex G-25 with substances that were identified by their UV spectra.

The retention of the test substances in the gel bed may be caused by molecular sieving[4], ionic adsorption[5], hydrogen-bond formation[6], hydrophobic interaction[7,8], Van der Waals–London forces[9] or electronic charge transfer. Probably several of these factors act together to give the adsorption effects observed in the chromatograms. The fact that V_E/V_T exceeds unity in every instance indicates that at least one of the last five factors must be operative in addition to molecular sieving. Ionic adsorption, however, is not likely to be important.

TABLE II

RESULTS OF EXPERIMENTS ON DNP-S-SEPHADEX G-25

Test substance	V_E/V_T		
	DNP-S-Sephadex G-25		*Sephadex G-25 (25°)*
	25°	*4°*	
Phenylalanine	1.06	—	—
Mescaline	1.10	—	—
Dopamine	1.29	1.48	1.10
Tryptamine	1.99	2.60	1.45
Tryptophan	2.07	—	1.65
Serotonin	2.69	3.85	1.74
Indoleacetic acid	3.65	5.18	2.03

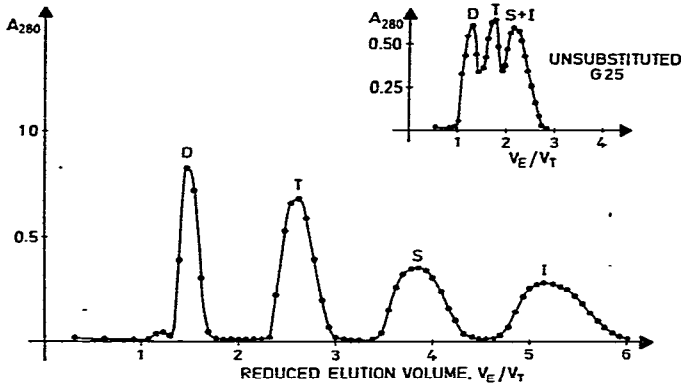


Fig. 1. Chromatographic resolution of a mixture of dopamine (D), tyramine (T), serotonin (S) and indoleacetic acid (I) on DNP-S-Sephadex G-25 at 4°. Elution buffer: 0.1 *M* ammonium formate, pH 3.2. Insert: pattern obtained on unsubstituted Sephadex G-25 under identical operating conditions.

The introduction of dinitrophenylthioether substituents strengthens the affinity of the gels for aromatic substances. The results given here, together with the results of exploratory studies on riboflavin-containing gels, provided convincing evidence that adsorption is due mainly to the π-electron acceptor capacity of the ligands. The increase in V_E/V_T values reflects the strength of the charge-transfer complexes formed with the dinitrophenylthioether groups and the solute. A simultaneous increase in hydrophobic interaction as a result of the substitution cannot be excluded and might explain the enhanced adsorption that accompanies an increase in the ionic strength of the eluent.

The inclusion of 40% ethylene glycol in the elution medium decreases the adsorption only slightly. This finding also supports the view that the solutes interact with the gels by a combined effect of hydrophobic attraction and electron-transfer adsorption. These two factors tend to counteract one another upon decreasing the temperature and it is therefore possible to evaluate their relative importance from the V_E/V_T values obtained at different temperatures. The results given in Table II indicate that electron charge transfer is the more important of the two proposed mechanisms.

Adsorption seems to parallel the electron-donor properties of the solutes. Tyrosine and tyrosine peptides are thus less strongly adsorbed than are the comparable tryptophan peptides. The strong retardation of iodinated tyrosines indicates that the iodine also partakes directly in the electron transfer[10], probably to form complexes of the σ,π and/or n,σ types[11]. The large incremental increase in V_E/V_T accompanying an increase in the number of donor groups per molecule as, for example, in Tyr–Tyr and Trp–Trp–Trp, might be due to cooperative interaction between the ligand and some other group in the matrix or between matrix-bound water on the one hand and solute on the other. Another explanation can be formulated in terms of the increased probability of local re-adsorption immediately following the desorption step.

A strong enhancement of adsorption resulting from an increase in the number of electron-donating groups per molecule explains our observation that nucleic acids are strongly adsorbed to riboflavin gels, while the simple nucleotides are not. The strong adsorption of phenazine and phenothiazine derivatives (see Table I) to the

electron-accepting DNP-S groups can readily be explained through formation of π,π electron-transfer complexes. We expect that these strong affinities will provide a basis for analytical procedures suitable for neuropharmaceuticals in urine and blood.

It should be possible to enhance the efficiency of the charge-transfer adsorbents further by increasing the ligand concentration and by the use of more effective ligands.

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