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SEPARATION OF 1-DIMETHYLAMINO-NAPHTHALENE-5-SULPHONYLAMIDES BY GEL CHROMATOGRAPHY

N. SEILER AND H. H. SCHNEIDER

Max-Planck-Institut für Hirnforschung, Arbeitsgruppe Neurochemie, Frankfurt/M-Niederrad (G.F.R.)*

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SUMMARY

For the determination of biogenic amines as their 1-dimethylamino-naphthalene-5-sulphonyl derivatives in tissue samples it is often necessary to remove 1-dimethylamino-naphthalene-5-sulphonylamides and the 1-dimethylamino-naphthalene-5-sulphonyl derivatives of some other amines present in large amounts in the tissue. Moreover a pre-separation of the complex mixture of amines is desirable. We recommend the pre-separation of dansylated tissue extracts by chromatography on molecular sieves (Sephadex LH-20). The method is universal in its applicability, simple and has a number of advantages over other pre-separation methods.

INTRODUCTION

The identification of biogenic amines in the form of their 1-dimethylamino-naphthalene-5-sulphonyl (DANS) derivatives is normally performed after their separation by thin-layer chromatography (TLC)[1–4]. The concentrations of biogenic amines in tissues are generally very low, and the amounts vary over a wide range. Ammonia and the polyamines spermidine and spermine, are often present in much greater amounts than the other amines, so that they cause trouble in the TLC, particularly, when large amounts of tissue have to be worked up for the determination of certain amines. The high concentration of γ-aminobutyric acid (GABA) can also give overlapping spots, if dansylated brain or retina extracts are separated on thin-layer chromatograms. The DANS derivative of GABA moves together with the DANS-amides[5].

Dansylation also gives rise to side reactions. One of the chief side reaction products is DANS-dimethylamide[4], which is produced in a fairly high concentration, if excessive amounts of DANS-Cl are used for completion of the dansylation reaction. Owing to this it is necessary, in many cases, to apply pre-separation methods before certain amines can be determined in tissues, in spite of the sensitivity of the dansyl method and the effectiveness of TLC on Silica Gel G for the separation of DANS derivatives.

In principle the whole spectrum of chromatographic and electrophoretic techniques is applicable for the pre-separation of the tissue extracts before dansylation.

* Head of Unit: Prof. Dr. G. WERNER.

However, in some cases certain dansyl derivatives can be concentrated from a dansylated tissue extract on account of their specific properties; *e.g.* in the determination of GABA and related compounds[6,7].

On the basis of our experience with the dansylation method it seemed desirable to develop a generally applicable pre-separation method for dansylated tissue extracts. Gel column chromatography recommended itself for this purpose, since separations roughly according to molecular weight could be expected. The molecular weights of the DANS-derivatives of biogenic amines are in the range of 250 (DANS-NH_2) to 1134 (tetra-DANS-spermine).

In the present paper, the chromatographic behaviour of some DANS derivatives on Sephadex LH-20 gel columns is described and the applicability of gel column chromatography to the determination of biogenic amines from tissues, in form of their DANS derivatives, is discussed.

MATERIALS AND METHODS

DANS-amides were prepared in our laboratory on a preparative scale. They moved on thin-layer chromatograms as single spots in many different solvent systems and were further characterized by elemental analysis[3,8] and mass spectrometry[9].

Sephadex LH-20 was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Solvents were obtained from E. Merck, Darmstadt, G.F.R., and were of analytical grade.

Sephadex LH-20 was allowed to swell in an excess of solvent for at least 3 h with occasional stirring with a glass rod. The suspension of the gel in solvents with a low specific gravity were poured into ordinary chromatographic glass columns (0.9 × 120 cm) in the usual manner (see the instructions by Pharmacia for the preparation of gel columns). The height of the gel bed was: 100 cm; the flow rate was 10 ml/h. Gel suspensions in chloroform were poured into glass columns (2 × 100 cm) with special PTFE end assemblies with porous polyethylene support discs, which enabled us to use the column for ascending or descending chromatography. Height of the gel bed was: 70 cm; flow rate, 30 ml/h.

Both 1.5- and 2.5-ml fractions of the eluates were collected in test tubes by means of an automatic fraction collector (FRS 63/E, Serva, Heidelberg, G.F.R.).

For the identification of the DANS derivatives, aliquots of the fractions were applied to Silica Gel G plates (layer thickness 200 μ). The chromatograms were developed by ascending chromatography in normal solvent vapour saturated tanks. The solvents were: trichloroethylene–methanol (95:5); diethyl ether–cyclohexane (90:10); benzene–cyclohexane–methanol (85:15:2)[3]. The fluorescent spots were observed under a UV lamp (365 nm).

RESULTS

Preliminary experiments demonstrated that the separation of DANS-NH_2 from most of the DANS derivatives of the natural amines is possible on gel columns filled with Sephadex G-10, if alcohol–water mixtures were used for swelling the gel. However, the separation of the different DANS-amines from each other was rather poor in this system. Better separations were obtained with LH-20 in ethyl acetate, methanol

and acetone. But really satisfactory separations were only observed when dioxane was used as solvent. The only important disadvantage of using dioxane is the formation of peroxides, which can destroy the DANS-amides. Table I shows the elution volumes obtained on elution of DANS-amides from a Sephadex LH-20 column with dioxane.

TABLE I

MOLECULAR WEIGHTS OF DANS-AMIDES AND -PHENOL ESTERS AND THEIR ELUTION VOLUMES ON SEPHADEX LH-20 COLUMNS

Column A: 0.9 × 120 cm; gel bed volume, 63 ml; flow rate, 10 ml/h; solvent, dioxane. Column B: 2 × 100 cm; gel bed volume, 220 ml; flow rate, 30 ml/h; solvent, chloroform.

Compound	*Mol. wt.*	*Elution volumes (ml)*	
		Column A	*Column B*
Tetra-DANS-spermine	1134	47	90
Tri-DANS-adrenaline	882	51	106
Tri-DANS-spermidine	845	51	106
Bis-DANS-5-hydroxy-tryptamine (serotonin)	642		200
Bis-DANS-pyridoxamine	634	66	118
Bis-DANS-cysteamine	618	56	
Bis-DANS-tyramine	603	59	114
Bis-DANS-histamine	577	58	114
Bis-DANS-putrescine	554	59	145
Bis-DANS-N-methyl-trimethylenediamine	554		110
Bis-DANS-trimethylenediamine	540	59	
Mono-DANS-glucosamine	412	100	> 1000
Mono-DANS-hordenine	398	62	119
Mono-DANS-tryptamine	393	69	223
Mono-DANS-β-hydroxy-β-phenylethylamine	370	69	
Mono-DANS-adenine	368	56	280
Mono-DANS-β-phenylethylamine	354	64	132
Mono-DANS-phenol	327	61	
Mono-DANS-piperidine	318	62	
Mono-DANS-pyrrolidone[a]	318	70	121
Mono-DANS-imidazole	301	69	
Mono-DANS-ethanolamine	294	77	400
Mono-DANS-dimethylamine	278	66	121
Mono-DANS-methylamine	264	72	
DANS-NH_2	250	94	600

[a] Reaction product of GABA with excess DANS-Cl.

On account of drawbacks due to peroxide formation in dioxane we looked for other useful solvents. Chloroform was found to give the best separations. Since, however, chloroform has a higher specific gravity than the gel in the swollen state, it was necessary to use chromatographic columns with adjustable end assemblies. Table I shows the elution volumes obtained for some DANS-amides in LH-20–chloroform.

DISCUSSION

The elution volumes of the DANS derivatives obtained by Sephadex LH-20 gel chromatography do not, however, correspond to their molecular weights. There are significant interactions between the DANS derivatives and the gel matrix, which influence the elution volumes considerably. These interactions are obviously de-

pendent on the polarity of the solvent. In methanol for instance, DANS-dimethylamide is eluted from the column before even tetra-DANS-spermine and tri-DANS-spermidine, because the latter molecules are reversibly adsorbed on the matrix.

A decrease in the dielectric constant of the solvent generally improves the separation of the DANS derivatives on LH-20, provided that the gel swells sufficiently. Dielectric constants and gel volumes per g dry gel after swelling in the solvents, we have studied, are summarized in Table II. The ratios between gel volume and dielectric constant are maximal in the case of dioxane and chloroform, *i.e.* for those solvents which effected the best separations. It seems that the magnitude of the gel volume/dielectric constant ratio is to some extent a measure for the efficacy of the separating system for DANS derivatives.

TABLE II

DIELECTRIC CONSTANTS[10] OF THE SOLVENTS AND GEL VOLUME PER G DRY GEL OF SEPHADEX LH-20 IN DIFFERENT SOLVENTS

Solvent	*Dielectric constant, D*	*Gel volume per g dry gel (ml/g)*	*Ratio of gel volume per g dry gel/dielectric constant (ml/g · D)*
Dioxane	3.0	3.6	1.20
Diisopropyl ether	3.9	1.5	0.38
Chloroform	5.1	4.2	0.82
Ethyl acetate	6.1	2.4	0.39
Butanone-2	10.0	2.5	0.14
Cyclohexanone	18.2	2.9	0.16
Acetone	21.5	2.9	0.13
Propanol-2	26.0	3.8	0.15
Methanol	31.2	4.5	0.14

With non-polar solvents DANS-NH_2, DANS-ethanolamine and other hydroxyl-group containing compounds are strongly retained by the gel column (see Table I). This effect of non-polar solvents together with the rapid elution of tetra-DANS-spermine and tri-DANS-spermidine is of great importance in the application of the gel column to the separation of dansylated tissue extracts, since the main sources of interference with the TLC can thus be eliminated very simply. It is possible to remove DANS-NH_2 nearly completely by chromatography of a dansylated extract of 1–2 g tissue even with a gel column 1 × 15 cm. However, the separation of DANS-dimethylamide from some of the other significant components is not possible by gel chromatography, as can be seen from the values in Table I. This compound overlaps, among other compounds, the DANS derivative of GABA, piperidine, hordenine and partially overlaps β-phenylethylamine as well.

The use of our pre-separation method for DANS derivatives has a number of advantages in comparison to other pre-separation methods, provided that no specific method for the concentration of a particular amine is available. They are:

(1) Large amounts of tissue can be worked up without difficulty, since the capacity of the gel column is high. This is especially of interest for the identification of amines by mass spectrometry, since larger samples are necessary for this method than for the quantitation of the DANS derivatives by fluorometry[8,9].

(2) Dansylation prevents certain compounds from autoxidation, *viz.* catecholamines. However, auto-oxidation of these compounds has to be prevented during the dansylation by suitable precautions.

(3) The quantitative extraction of many amines and phenols in form of their DANS derivatives is in many cases simpler than the isolation of the free amines, since DANS-amides and -phenol esters are soluble in non-polar solvents.

(4) The by-products of the dansylation reactions accumulate only in certain fractions of the gel column chromatogram. Because of this the thin-layer chromatograms prepared from gel column chromatography fractions are generally very clean, and therefore they are especially suited for quantitative evaluation. By combination of gel chromatography with suitable TLC separations, quantitative methods can be worked out for practically all those amines and phenols in a tissue sample, which react in a reproducible way with DANS-Cl.

DANS-amino acids are strongly retained by the gel under the conditions described.

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