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PREPARATION OF LIPOPHILIC ANION EXCHANGERS FROM CHLOROHYDROXYPROPYLATED SEPHADEX AND CELLULOSE

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SUMMARY

Chlorohydroxypropyl derivatives of Sephadex and cellulose were reacted with ammonia and primary, secondary or tertiary amines to prepare a wide range of lipophilic anion exchangers. These can be used for chromatography in organic solvents, exemplified by the separation of a phospholipid mixture using a dibutylaminohydroxypropyl derivative of Sephadex LH-20.

INTRODUCTION

In a previous communication, the synthesis of chlorohydroxypropyl Sephadex and cellulose from the corresponding hydroxypropyl derivatives was described[1]. It was shown that these lipophilic chlorine-containing derivatives are highly versatile, in that the halogen atom makes further substitution reactions possible. Thus, derivatives with primary, secondary and tertiary amines or quaternary ammonium ions attached to the matrix can be prepared by reacting with ammonia and primary, secondary or tertiary amines. Since this type of reaction represents a new and simple way of preparing a wide range of Sephadex and cellulose ion exchangers suitable for chromatography in organic solvents, it was further investigated.

MATERIALS AND METHODS

Reagents

Hydroxypropyl Sephadex (Sephadex LH-20) was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Fibrous cellulose (Whatman CF 11), amines (purum, Kebo, Stockholm), methanol, chloroform, methylene chloride, ethylene chloride (p.a., Merck), propylene oxide (technical quality, Kebo), epichlorohydrin (purum, Kebo), sodium and potassium hydroxide (p.a., Eka, Sweden) and boron trifluoride ethyl etherate (47% BF_3, Kebo) were used as supplied.

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Solvent regain values

Solvent regain values (g of solvent imbibed in the gel beads per g of dry Sephadex or cellulose derivative) were determined in a stoppered glass tube with sintered glass bottom, essentially as described by HELFFERICH[2].

Elemental analyses

Elemental analyses were kindly carried out by Løvens AS, Ballerup, Denmark.

Preparation of hydroxypropyl cellulose (HP-cellulose)

Fibrous cellulose powder (100 g) was suspended for 1 h in 30% (w/v) aqueous sodium hydroxide, and the excess liquid was then removed by filtration on a Büchner funnel, equipped with a fine-mesh polyethylene filter cloth. The damp cellulose (516 g) was transferred into a 5-l round-bottom glass flask, equipped with a stirrer passing through a bulb condenser. Propylene oxide (2000 ml) and epichlorohydrin (400 ml) were added, and the mixture was refluxed for 12 h with stirring, 800 ml of ethylene chloride being added after the first 2 h. The product (190 g) was collected on a Büchner funnel, washed carefully with water, ethanol and chloroform, and dried at 60°.

Preparation of chlorohydroxypropyl Sephadex LH-20

Sephadex LH-20 (75.7 g) was suspended in methylene chloride (200 ml) in a 1-l round-bottom glass flask, equipped with a stirring rod (a magnetic stirrer will destroy the gel particles) and a separatory funnel. Boron trifluoride ethyl etherate (19 ml) was added after 30 min. 15 min later, a mixture of epichlorohydrin in methylene chloride (35%, v/v; 50 ml) was added slowly via the separatory funnel (1–2 ml/min), and the reaction was allowed to proceed for another 30 min. The product (98.2 g, 8.57% Cl) was collected on a Büchner funnel, washed with ethanol and chloroform and dried at 50°.

Preparation of chlorohydroxypropyl cellulose

This reaction was carried out in a manner similar to that for the preparation of chlorohydroxypropyl Sephadex LH-20. HP-cellulose (10.0 g) was suspended in methylene chloride (25 ml) and reacted with boron trifluoride ethyl etherate (0.6 ml) and epichlorohydrin in methylene chloride (35%, v/v; 34 ml). The product (19.7 g) had a chlorine content of 17.8%.

Preparation of dibutylaminohydroxypropyl Sephadex LH-20

Chlorohydroxypropyl Sephadex LH-20 (13.6 g, 8.57% Cl) was suspended for 30 min in a 15-fold molar excess of dibutylamine (0.51 moles, 86 ml). A solution of potassium hydroxide (2.87 g) in 118 ml of methanol was added to make the final concentration of potassium hydroxide 0.22 *M* and the final volume (in ml) 15 times the weight (in g) of the starting material. After heating to 55° for 3 h with occasional shaking of the reaction vessel, the product was collected on a Büchner funnel and washed with ethanol (1 l), potassium hydroxide (0.1 *M*) in ethanol–water (9:1) (2 l) and ethanol until the eluate was neutral to indicator paper. The product (16.0 g) was dried at 50° for 6 h. Elemental analysis of N was 2.71%. In order to check for absence of chlorine, the product was further washed with 1 *M* aqueous potassium

hydroxide, whereupon the eluate was acidified. No precipitate was obtained upon the addition of silver nitrate. Derivatives using other amines and/or chlorohydroxypropyl cellulose as starting materials were prepared in a similar way.

Column chromatography

10 g of dibutylaminohydroxypropyl Sephadex LH-20 (free base form) were converted to the acetate form by washing on a Büchner funnel with 1000 ml of ethanol–acetic acid (5:1, v/v), followed by ethanol–water (9:1, v/v), until the eluate was neutral to indicator paper, and finally ethanol. The material was dried at 50°. Glass chromatography columns (4–10 mm I.D.), equipped with a porous Teflon or fritted glass end-piece and a solvent reservoir, were packed with a slurry of the Sephadex derivative (acetate form) in chloroform–methanol–water (20:65:35, v/v). Phospholipid samples were applied in a small volume of the eluting solvent. The phospholipid content of each fraction was analyzed by thin-layer chromatography on Silica Gel S using the solvent system chloroform–methanol–acetic acid–water (85:15:10:4.5, v/v). Radioactivity was determined with a gas-flow counter.

RESULTS

Preparation of Sephadex and cellulose ion exchangers

To find the optimal reaction conditions, chlorohydroxypropyl Sephadex LH-20 was reacted with dibutylamine in a number of experiments. Products with a high degree of substitution were obtained if the reactions were carried out in methanol at 50°, using a 15–20-fold molar excess of amine as calculated on the amount of halogen in the chlorohydroxypropyl derivative (Fig. 1A). Maximum substitution was obtained when the reaction mixture was 0.21 *M* with respect to potassium hydroxide (Fig. 1B). Under these conditions, most chlorine groups were replaced by amine within 4 h, after which time no further substitution took place. Several derivatives of Sephadex and cellulose were prepared under these or similar reaction

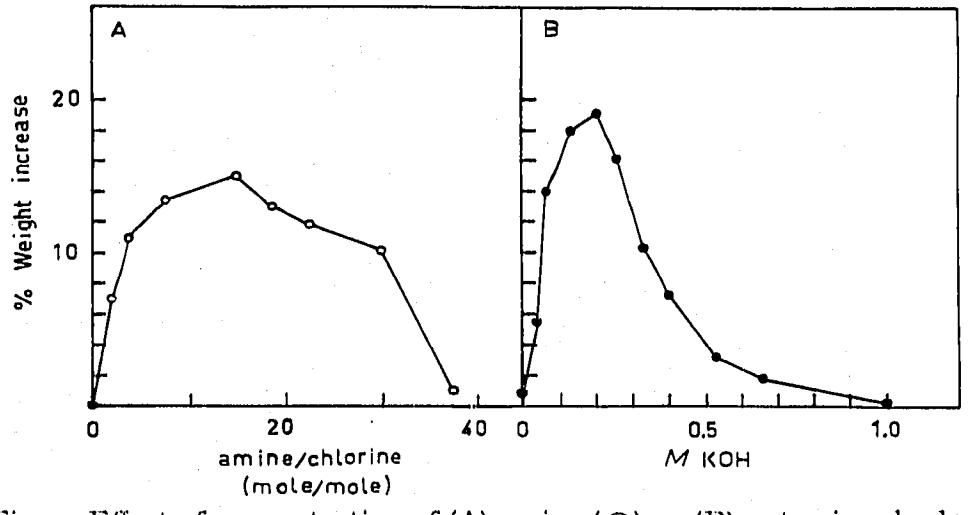

Fig. 1. Effect of concentration of (A) amine (○) or (B) potassium hydroxide (●) on the degree of substitution of chlorohydroxypropyl Sephadex LH-20. The optimal concentration of amine was determined in 0.21 *M* potassium hydroxide. When the influence of base concentration was studied, the Sephadex derivative was reacted with a 15-fold molar excess of dibutylamine.

TABLE I

REACTION OF CHLOROHYDROXYPROPYL SEPHADEX LH-20 WITH DIFFERENT AMINES

Chlorohydroxypropyl Sephadex LH-20 (8.57% Cl = 2.37 mequiv./g) was reacted in methanol with a 15-fold molar excess of amine, the reaction mixture being 0.21 *M* with respect to potassium hydroxide. *b* = free base, *c* = chloride.

Amine	*Weight increase (%)*	*Nitrogen content (%)*	*Capacity (mequiv./g)*[a]	*Solvent regain*									
				Water		*Ethanol*		*Ethylene chloride*		*Benzene*		*Heptane*	
				b	*c*	*b*	*c*	*b*	*c*	*b*	*c*	*b*	*c*
Ammonia	−2	1.57	1.12	0.7	2.0	1.1	1.2	0.9	0.8	0.6	0.4	0.3	0.2
Ethylamine	0.2	2.84	2.03	1.3	3.9	1.5	1.8	1.4	1.1	0.8	0.5	0.2	0.3
Butylamine	8.7	1.95	1.39	0.9		1.5	1.4	1.5	1.0	1.1	0.7	0.2	0.2
Cyclohexylamine	12.6	2.04	1.46	1.1	3.8	1.4	1.7	1.4	1.1	0.8	0.5	0.3	0.3
Ethanolamine	4.7	2.70	1.93	2.5	3.6	1.5	1.5	1.1	0.8	0.9	0.3	0.3	0.2
Aniline	2.0	2.26	1.61										
Diethylamine	5.6	2.27	1.62	1.1	1.8	1.4	1.9	2.1	1.0	0.6	0.5	0.3	0.3
Dibutylamine	15.2	2.71	1.94	0.7	1.5	1.7	1.9	2.4	1.8	1.7	1.2	0.8	0.8
Diethanolamine	14.7	2.24	1.60	1.0	3.3	1.3	1.5	1.0	0.7	0.7	0.3	0.2	0.3
Dimethylcyclohexylamine	5.3	1.27	0.91										
Tributylamine	0.4	0.81	0.58	0.9	0.9	1.5	0.7	1.9	1.2	1.3	0.3	0.4	0.1

[a] As calculated from the nitrogen content.

TABLE II

REACTION OF CHLOROHYDROXYPROPYL CELLULOSE WITH DIFFERENT AMINES

Chlorohydroxypropyl cellulose (18.59% Cl = 5.0 mequiv./g) was reacted in methanol with a 15-fold molar excess of amine, the reaction mixture being 0.21 *M* with respect to potassium hydroxide. b = free base; c = chloride.

Amine	*Weight increase (%)*	*Nitrogen content (%)*	*Capacity (mequiv./g)*[a]	*Solvent regain*									
				Water		*Ethanol*		*Ethylene chloride*		*Benzene*		*Heptane*	
				b	*c*	*b*	*c*	*b*	*c*	*b*	*c*	*b*	*c*
Butylamine	3.9	2.63	1.88	1.4	0.8	0.5	0.4	0.7	0.7	0.8	0.5	0.3	0.3
Cyclohexylamine	8.8	2.49	1.78	1.4	0.7	0.4	0.6	0.6	0.5	0.4	0.3	0.3	0.3
Diethylamine	5.2	2.79	1.50	1.7	0.6	0.7	0.9	0.8	0.6	0.6	0.6	0.4	0.3
Dibutylamine	11.1	1.49	1.01	1.3	0.8	0.4	0.6	0.8	0.8	0.8	0.6	0.5	0.4
Diethanolamine	11.9	2.94	2.10	1.7	0.8	0.5	0.5	1.1	0.9	0.6	0.4	0.3	0.3

[a] As calculated from the nitrogen content.

conditions. The reaction mixtures were worked up quantitatively and the weight increase was noted. The products were further characterized by nitrogen determination, solvent regain measurement and, in some cases, potentiometric titration. The results are summarized in Tables I and II. It is seen that ammonia and primary and secondary amines react readily with the chlorohydroxypropyl derivative, whereas the tertiary amines show low reactivity.

Titrations

The exchange capacities of some dibutylamine derivatives (free base form) were determined by direct potentiometric titration in methanol with 0.1 *M* methanolic HCl. Three derivatives with nitrogen contents corresponding to 2.39, 1.93 and 1.80 mequiv/g had the actual capacities of 2.1, 1.9 and 1.7 mequiv/g, respectively. The results indicate that over 90% of the amine groups are accessible for ion exchange in organic solvents.

Swelling of the products

In this paper, the swelling of the derivatives has been measured as solvent regain, *i.e.* the amount of solvent imbibed in the gel beads per g of dry derivative has been measured after removal of the interstitial fluid by centrifugation. Values below approximately 1.0 (Sephadex derivatives) and 0.6 (cellulose derivatives) indicate poor swelling. The starting material in the synthesis, a halogenated derivative of Sephadex LH-20 or HP-cellulose, is lipophilic and swells in water, alcohols and chlorinated hydrocarbons, but swells poorly in aromatic and aliphatic hydrocarbons. As is seen in the Tables I and II, the ion exchangers swell in the same solvents. In addition, the derivatives prepared from mono- and dibutylamine swell in aromatic hydrocarbons, indicating the importance of the alkyl part of the amine. As expected, the chloride form of the ion exchangers swells best in water[3], whereas the free base form is better swollen in less polar solvents. The same tendency is also seen with the quaternary ammonium derivative prepared from tributylamine, where the

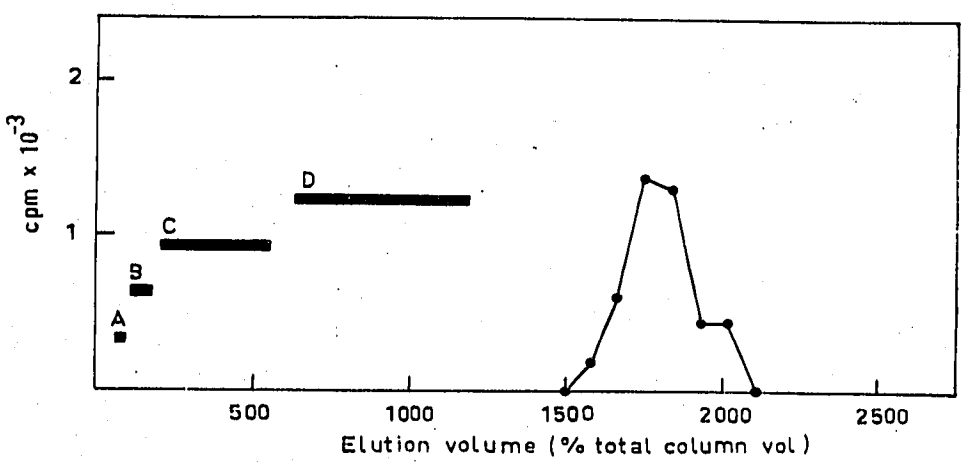

Fig. 2. Separation of egg phospholipids (3 mg) and [4-^{14}C]cholesterol. Heavy bars indicate presence (TLC determination) of: A = lysolecithin, B = lysophosphatidylethanolamine, C = lecithin and D = phosphatidylethanolamine. Column: 225 × 4.4 mm containing 1.0 g of dibutylaminohydroxypropyl Sephadex LH-20 in the acetate form (nitrogen content: 2.71%, w/w). Solvent: Chloroform–methanol–water (20:65:35, v/v). Flow rate: 1.8 ml/h.

hydroxide form (see under "free base", Table I) *e.g.* swells in benzene in contrast to the chloride form.

Applications

In a preliminary experiment, a mixture of egg phospholipids was applied to a column of dibutylaminohydroxypropyl Sephadex LH-20 in its acetate form. The solvent was chloroform–methanol–water (20:65:35, v/v). As is seen in Fig. 2, lysolecithin, lysophosphatidylethanolamine, lecithin, phosphatidylethanolamine and cholesterol are eluted as separate peaks.

DISCUSSION

Synthesis procedure

The chlorohydroxypropyl Sephadex LH-20 starting material contains 8.57% Cl. Although higher chlorine contents can be easily obtained[1], this content was considered suitable, since it corresponds approximately to one chlorine atom per glucose unit. In the subsequent amination procedure, between 50 and 100% of the chlorine atoms are utilized, with the exception of the reaction with tertiary amines, which gives a fairly low yield. The highest degree of substitution is obtained in the reaction with dibutylamine, in which virtually all the chlorine atoms are utilized. Since the optimal reaction conditions were determined using this amine (Fig. 1), somewhat different conditions might be more favourable for the reaction of the chlorohydroxypropyl derivative with other amines. Since every chlorine atom is replaced by an amine group in the dibutylamine reaction, no crosslinking should occur. This is probably true for the other amination reactions too, where the general reaction conditions are identical. The main side reaction is likely to be the hydrolysis of the chlorohydroxypropyl derivative to form the 2,3-dihydroxypropyl derivative.

Applications

Several papers have described the use of cellulose anion exchangers in the separation of phospholipids. However, in solvents of low polarity, such columns are difficult to pack and mechanical problems like channelling frequently disturb the chromatography. Evidently it would be of advantage to use lipophilic ion exchangers[4,5]. The derivatives described in this paper show excellent chromatographic properties in relatively non-polar solvent mixtures. The recovery is good, as determined by chromatography of trace amounts of phospholipids and labelled substances. Further investigations of the use of lipophilic Sephadex and cellulose ion exchangers for the separation of phospholipids and bile acids are currently carried out in this laboratory.

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