Gel permeation chromatography of neutral hydroxy lipids on Sephadex LH-20

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SUMMARY Gel-permeation chromatography on Sephadex LH-20, using ethanol as eluent, permits the resolution of neutral hydroxy lipids according to molecular size. The influence of molecular shape, functional groups, chain lengths, and degree of unsaturation, as well as the effect of the eluent on the elution pattern are discussed. The usefulness of the method for the separation of classes of hydroxy lipids which cannot be resolved by other chromatographic procedures, is demonstrated. Examples include the separations of 1,2- and 1,3-diglycerides from long-chain alcohols, and of alkyl ethanediol monoethers from cholesterol.

SUPPLEMENTARY KEY WORDS gel filtration · lipophilic Sephadex · diglycerides · alcohols · cholesterol · dialkyl glycerol ethers · alkyl ethanediol ethers · diol lipids

In gel-permeation chromatography molecular size is a decisive parameter in determining the chromatographic behavior of the compounds to be separated. The sequence of elution of neutral lipids from lipophilic dextran gels (1, 2) is generally in the order of decreasing molecular weights, although other criteria, such as polarity of the lipid or eluent, may cause different patterns of fractionation (3–5). Nonpolar lipids which migrate in adsorption chromatography ahead of triglycerides can be readily fractionated according to molecular size on columns of Sephadex LH-20 with chloroform as eluent (1, 5). However, lipids containing hydroxy or carboxyl groups can cause severe solute–gel interaction unless more polar solvents are used. Despite the fact that such effects may be used advantageously, as was reported for the separation of isomeric diglycerides (5) or for the segregation of steroids (6), "pure molecular sieving" is usually desirable for the resolution of complex lipid mixtures.

The present investigation was undertaken to study the influence of functional groups, chain lengths, and degree and type of unsaturation on the behavior of some hydroxy lipids in gel-permeation chromatography on Sephadex LH-20 using ethanol as eluent. Model compounds were also selected in view of the possible application of the procedure to the separation of closely related hydroxy lipids which cannot be resolved by conventional chromatographic methods.

Special care was taken in preparing the dextran slurry and in packing the column. Sephadex LH-20 (Pharmacia Fine Chemicals, Inc., New Market, N.J.), 110 g, was suspended in approximately 600 ml of ethanol which had been redistilled over Drierite. Some of the solvent was removed under reduced pressure on a rotary evaporator. More ethanol was added and again evaporated under reduced pressure in order to remove air from the slurry. The gel was allowed to equilibrate for 4–5 hr at room temperature and then was poured carefully into a solvent-resistant Sephadex column, SR-25/100 (Pharmacia Fine Chemicals, Inc.), while a continuous flow of solvent was maintained through the column. The column was conditioned for 48 hr. Lipid samples, up to 100 mg and dissolved in 1 ml of ethanol, were applied to the top of the column and were eluted at room temperature at a flow rate of 0.5 ml/min. Fractions of 1 ml were collected, and the amount of lipid material in each fraction was determined gravimetrically. The fractions were also analyzed by adsorption chromatography on layers of Silica Gel G (7) and (or) by gas chromatography after acetylation (8).

The effect of chain length and unsaturation on the elution pattern of hydroxy lipids was studied by subjecting a mixture of equal amounts of alkyl ethanediol monoethers, having chain lengths from C_{14} to C_{20} and up to two double bonds (9), to chromatography on Sephadex LH-20. As depicted in Fig. 1, the total weight curve does not indicate any fractionation. However, when the collected fractions were acetylated and then analyzed by gas chromatography, some subfractionation according to chain lengths as well as to number and configuration of double bonds became evident. The individual elution curves *A–F* (Fig. 1) demonstrate that eicosyl ethanediol ether (*A*) was eluted ahead of the octadecyl ether (*B*) which was followed by the hexadecyl (*D*) and tetradecyl ethers (*F*). Broadening of curve *C*, which represents both *cis*- and *trans*-9-octadecenyl ethanediol ethers, is due to the fact that the acetates of the two compounds were not resolved by gas chromatography under the conditions employed. Analysis of the fractions, collected from the Sephadex column, by argentation chromatography (developing solvent hexane–diethyl ether 85:15, v/v) demonstrated clearly that in gel-permeation chromatography the *trans* isomer was eluted well ahead of the *cis* isomer, most likely because of the bulkier structure of the *trans* compound. These findings are compatible with results reported for the separation of different types of geometrical isomers, namely *cis*- and *trans*-dimethylcyclohexanes on a polystyrene gel (10), which was attributed to differences in molar volumes of the isomers. As shown in Fig. 1, *cis*, *cis*-9,12-octadecadienyl ethanediol ether (*E*) was eluted from the Sephadex column together with the tetradecyl ether (*F*). This elution pattern is similar to that reported for long-chain methyl esters, when a Styragel column was eluted with diethyl ether (11). Thus, in gel chromatography the retardation observed for one

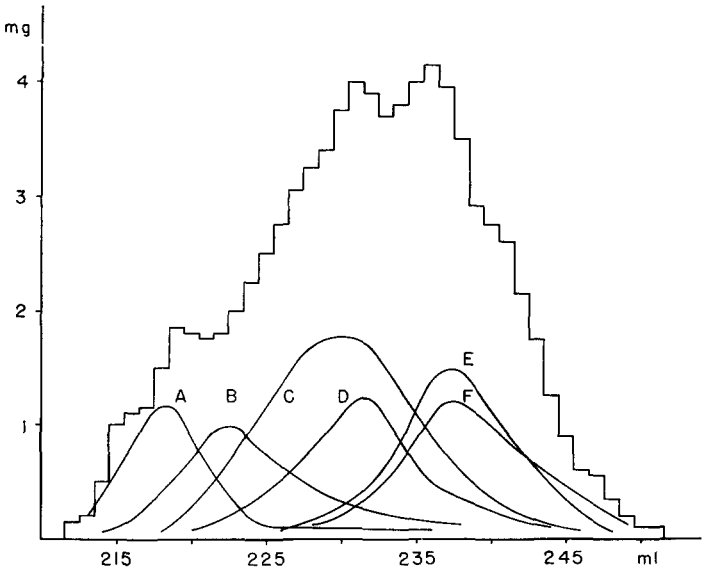

FIG. 1. Gel-permeation chromatography of a mixture of alkyl ethanediol ethers on Sephadex LH-20L. The column, 79 cm × 2.5 cm I.D., was eluted with ethanol at a flow rate of 0.5 ml/min. The composition of the fractions as determined by gas chromatography of the acetates are: *A*, eicosyl ethanediol ether; *B*, octadecyl ethanediol ether; *C*, *trans*-9′-octadecenyl followed by *cis*-9′-octadecenyl ethanediol ether; *D*, hexadecyl ethanediol ether; *E*, *cis*,*cis*-9′,12′-octadecadienyl ethanediol ether; *F*, tetradecyl ethane diol ether.

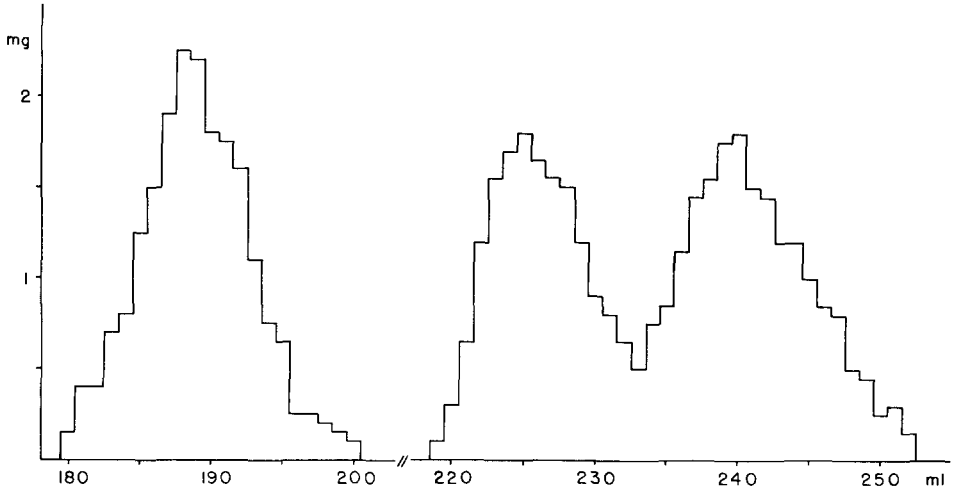

FIG. 2. Gel-permeation chromatography of a mixture of 1,2-di-*cis*-9′-octadecenyl glycerol ether (fractions 180–200), *cis*-9′-octadecenyl ethanediol ether (fractions 219–235), and cholesterol (fractions 231–252) on Sephadex LH-20. For experimental conditions, see Fig. 1.

additional *cis*-double bond is equal to that caused by a decrease in chain length by two methylene groups. The effect of one *trans*-double bond corresponds approximately to that of one methylene group.

Separations of classes of lipids according to molecular dimensions appear feasible inasmuch as subfractionation within classes, according to chain length or unsaturation, occurs only to a minor extent. Several types of lipids containing one hydroxy group show similar or identical rates of migration in adsorption chromatography. For example, cholesterol cochromatographs with ethanediol monoethers and long-chain alcohols migrate as uniform fractions together with 1,2-diglycerides.

Fig. 2 shows that in gel-permeation chromatography with ethanol as eluent, dioctadecenyl glycerol ether (12) (mol wt 593.0; fractions 180–200) is eluted ahead of and is distinctly separated from octadecenyl ethanediol ether (mol wt 312.5; fractions 219–235). A pattern of fractionation very similar to that shown in Fig. 2 was obtained when a mixture of equal amounts of 1,2-di-*cis*-9′-octadecenoyl glycerol, 1,3-di-*cis*-9′-octadecenoyl glycerol, and *cis*-9-octadecenol (The Hormel Institute

Lipids Preparation Laboratory, Austin, Minn.) was subjected to gel-permeation chromatography. Separation was not observed between the two isomeric dioleins (fractions 184–210), but both were eluted well ahead of oleyl alcohol (fractions 220–245). These hydroxy lipids are eluted quite normally in the order of decreasing molecular weights. Evidently, under the conditions employed, gel–solute interaction and differences in polarity of the lipids become negligible.

Exceptions to this normal pattern of separation were observed for compounds having vastly different shapes. Such an example is shown in Fig. 2. Although cholesterol (mol wt 386.7) has a molecular weight higher than that of octadecenyl ethanediol ether (mol wt 312.5), the ethanediol monoether (fractions 219–235) is eluted ahead of the sterol (fractions 231–252). As differences in the polarities of the two compounds are minor and of little significance with ethanol as eluent, the results can be interpreted as being due to the differences in shape or molecular volume. A similar effect was observed previously (13) and used for the resolution of paraffins from cycloparaffins.

We conclude that gel-permeation chromatography on Sephadex LH-20 with ethanol as eluent permits molecular sieving of hydroxy-substituted lipids. Chromatography on a lipophilic gel is particularly useful as a method complementary to adsorption chromatography. Mixtures of natural lipids which are not separated by adsorption chromatography can be completely resolved by gel-permeation chromatography according to differences in molecular weights or molecular shapes. For example, diglycerides of long-chain fatty acids can be separated from fatty alcohols, and long-chain monoethers of ethanediol, or the corresponding esters, can be resolved from cholesterol and other hydroxy substituted alicyclic compounds.

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