CHROM. 4620

Group separation of prostaglandins on Sephadex LH-20

The prostaglandins constitute a family of closely related lipids with potent biological properties[1]. Recent reviews on their separation have appeared[2,3]. Group separation of these compounds can be achieved by silicic acid chromatography[4,5] or thin-layer chromatography[6]. The individual prostaglandins in each group may then be separated by either partition chromatography[7-10], ion exchange or thin-layer argentation chromatography[6,11,12] or paper chromatography[8,9]. Most of these methods suffer from the drawback of low recoveries in the submicrogram range. Lately, SJÖVALL, *et al.*[13] have demonstrated that different lipophilic dextran derivatives can be used advantageously for the isolation and analysis of microgram amounts of steroids and related substances. The present report shows that a lipophilic dextran derivative, Sephadex LH-20®, may be used for group separation of the methyl esters of prostaglandins E, F and A or B.

Experimental

Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala) was refluxed three times with methanol–chloroform (1:1), filtered and dried at 40°. This material was equilibrated with an excess of heptane–chloroform–ethanol (10:10:1) for a few hours. The swollen gel slurry was briefly evacuated by suction and then poured into the column (10 × 750 mm) and allowed to settle under free flow. The top gel surface was protected by a small disc of porous Teflon (Filter 4290-04, LKB-produkter AB, Stockholm). The top of the column was connected to a reservoir containing the eluting solvent mixture. The prostaglandin esters were dissolved and applied in 0.1–0.2 ml of the solvent. The flow rate was about 0.2 ml/cm² per min. Fractions of about 0.7 ml were collected. The elution volumes are given relative to β-carotene which appears at about 1.3 × void volume of the column. The prostaglandin E and A compounds were detected by their absorption at 280 nm after conversion into the respective prostaglandin B by dilute NaOH. The prostaglandins of the F group were analyzed by quantitative gas–liquid chromatography (GLC) after their conversion into the trimethylsilyl ether derivatives. Some of the prostaglandins were tritium-labeled and were measured by conventional liquid scintillation counting. In the recovery experiments, the appropriate quenching corrections were performed.

Results and discussion

The separation of the methyl esters of prostaglandins E_2, $F_{2\alpha}$, A_2 and B_2 is shown in Fig. 1. Baseline separations are obtained for prostaglandins E_2, $F_{2\alpha}$ and A_2. Prostaglandins A_2 and B_2 are not separated. The peaks were symmetric. The prostaglandin esters within each group (*e.g.* dihydroprostaglandin E_1, prostaglandins E_1, E_2 and E_3) showed only minor variations in the retention volume. The retention factors relative to β-carotene are shown in Table I. Since the prostaglandins appear roughly in order of increasing polarity and since no evidence for adsorption was noticeable, the mechanism of separation seems to be a liquid–gel partition chromatography of "straight-phase" type.

The recovery of 50 ng of the methyl ester of prostaglandin E_2 (PGE_2-Me) was

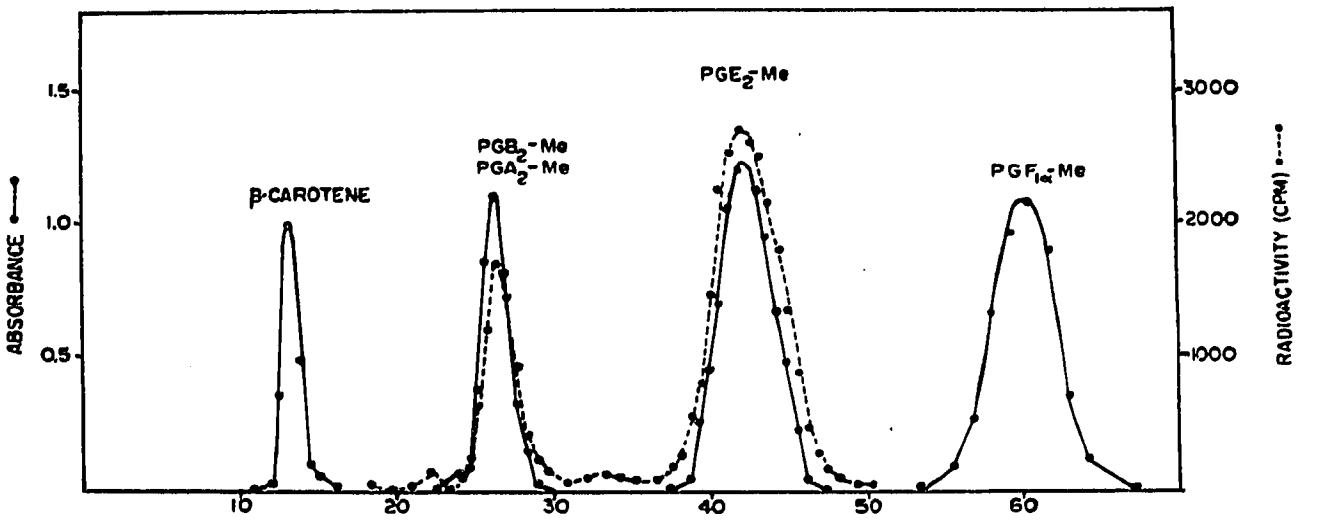

Fig. 1. Chromatography on Sephadex LH-20 of the methyl esters of prostaglandin A_2 (PGA_2), prostaglandin B_2 (PGB_2), prostaglandin E_2 (PGE_2) and prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$). ^{3}H-labeled PGE_2 and PGA_2 (specific activity, 50 mCi/mmole) were combined with about 240 μg of PGE_2, PGB_2 and $PGF_{1\alpha}$ and esterified with diazomethane. Solvent system: heptane–chloroform–ethanol (10:10:1).

TABLE I

RELATIVE RETENTION VOLUMES OF PROSTAGLANDIN METHYL ESTERS

Chemical name of free acid	*Trivial name*	*Elution volume of methyl ester relative to β-carotene*
11α,15-Dihydroxy-9-ketoprost-13-enoic	PGE_1	3.13
11α,15-Dihydroxy-9-ketoprostanoic	Dihydro-PGE_1	3.25
11α,15-Dihydroxy-9-ketoprosta-5,13-dienoic	PGE_2	3.26
11α,15-Dihydroxy-9-ketoprosta-5,13,17-trienoic	PGE_3	3.32
15-Hydroxy-9-ketoprosta-10,13-dienoic	PGA_1	1.95
15-Hydroxy-9-ketoprosta-5,10,13-trienoic	PGA_2	1.95
15-Hydroxy-9-ketoprosta-8(12),13-dienoic	PGB_1	1.95
15-Hydroxy-9-ketoprosta-5,8(12),13-trienoic	PGB_2	1.95
9α,11α,15-Trihydroxyprost-13-enoic	$PGF_{1\alpha}$	4.48
9α,11α,15-Trihydroxyprosta-5,13-dienoic	$PGF_{2\alpha}$	4.49

studied using the tritium-labeled compound added to extracts of acidic lipids from human plasma followed by chromatography and collection of 12 ml of the eluate at the expected position of PGE_2-Me. The mean recovery from 6 experiments was 89 % ± 4 % (SEM). The recovery of 12 ng of the methyl ester of ^{3}H-$PGF_{2\alpha}$ was 91.2 % ± 1 % (SEM).

The advantage of liquid–gel chromatography over available methods seems to be the satisfactory and reproducible recoveries of submicrogram amounts. The compounds appear in a small volume of organic solvent which is easily removed. Other attractive features are speed (2–4 h) and relatively high efficiency of separation. The columns can also be used repeatedly without repacking. If subsequent biological

assay is desired the methyl esters of the PGF compounds may be quantitatively hydrolyzed in weak alkali. The other biologically active prostaglandins, however, are labile in alkali. A useful application of the described separations seems to be in the isolation of prostaglandins prior to analysis by GLC and/or mass spectrometry.

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Gelchromatographie von strukturisomeren Phenylalanin-Peptiden an Sephadex G-15

Über die unterschiedlich starke Retardation von aromatischen und heterocyclischen Verbindungen an Dextrangelen berichteten schon PORATH[1] und GELOTTE[2]. Der Mechanismus dieses in der Gelchromatographie als "reversible Adsorption" bezeichneten Effekts scheint ziemlich komplizierte Wechselwirkungen zwischen Netzwerk[3,4], Substanz[5,6] und Elutionsmittel[3,5] einzubeziehen. Dieser Adsorptionseffekt kann sich bei der Siebanalyse von Proteinhydrolysaten wie den Peptonen nachteilig auswirken, da hochmolekulare Peptide, die aromatische Aminosäuren enthalten, gemeinsam mit anderen niedermolekularen Peptiden von der Säule eluiert werden. Aus der Literatur sind aber auch Beispiele bekannt, wo dieser Adsorptionseffekt zur Lösung spezieller Probleme ausgenutzt wurde wie z.B. zur Trennung von homologen