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Note

Chromatography of B prostaglandins on β-cyclodextrin silica: application to analysis of major E prostaglandins in human seminal fluid

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Reversed-phase high-performance liquid chromatography (HPLC) of prostaglandins (PG) and other eicosanoids on octadecasilane silica (C_{18}) has been developed into a standard chromatographic procedure, because of its high reproducibility, efficiency and resolving power [1,2]. The separation is mainly based on the polarity of the compounds. Recently, cyclodextrins chemically bonded to silica were introduced as stationary phases for reversed-phase HPLC, and they are now commercially available (see refs. 3–5 for review). These α-, β- and γ-cyclodextrins consist of six, seven or eight glucose molecules, which are joined by α(1,4)-linkages. The interior of the cyclodextrin cavities is relatively hydrophobic, and the primary and secondary hydroxyl groups are located on each edge of the cone. The cyclodextrin silica stationary phases are also claimed to separate by mechanisms other than the polarity of the analytes. Cyclodextrin bonded to silica has been found to retain certain molecules via formation of inclusion complexes, when eluted with aqueous–organic mobile phases [3–7]. Since cyclodextrins are chiral molecules, resolution of enantiomers has also been reported [3–5,8].

Cyclodextrins, as mobile phase modifiers or chemically bonded to silica, have also been used for chromatography of prostaglandins [5,9,10]. Uekama et al. [9] demonstrated that A, B and E prostaglandins could form useful complexes with α- and β-cyclodextrins in buffer. Recently, Snider [10] reported that *cis–trans* isomers of the 5,6 double bond of some prostaglandins and prostaglandin analogues could be resolved on β-cyclodextrin silica. So far, β-cyclodextrin silica has been little used for analysis of prostaglandins or other compounds in the complexity of biological samples.

The objective of the present study was to assess whether analysis of the relative

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amounts of the four major E prostaglandins in human seminal fluid by reversed-phase HPLC on β-cyclodextrin silica would offer any advantages over reversed-phase HPLC on C_{18}. Since E prostaglandins can be dehydrated to B prostaglandins by alkalis, and the latter can be quantified by their UV absorption, the β-cyclodextrin silica phase was first evaluated by chromatography of a series of B prostaglandins, derived from naturally occurring seminal E prostaglandins.

EXPERIMENTAL

Instrumentation

The HPLC apparatus consisted of a pump (LDC ConstaMetric III), an injector (Rheodyne 7125), a variable-wavelength UV detector (Kratos Spectroflow 757) set at 278 nm for detection of B prostaglandins and an integrator (Hitachi D-2000 Chromato-Integrator). The column (250×4.6 mm I.D.) with β-cyclodextrin bonded to silica (5 μm; Cyclobond I, Astec, Whippany, NJ, U.S.A.) was eluted (1 ml/min; 140 bar) with methanol–water–acetic acid (600:400:0.1, v/v/v; the apparent pH of the mobile phase was 4.5) at ambient temperature. Methanol and water for HPLC were from Merck (Darmstadt, F.R.G.). The samples were dissolved in the mobile phase and 20-μl samples were injected. For comparison, some biological samples were also chromatographed on C_{18} (5μm, Nucleosil; 150×3.9 or 4.6 mm I.D.), eluted stepwise first with methanol–water–acetic acid (520:480:0.1) for 15 min after injection of the sample, and then with a more polar system (methanol–water–acetic acid, 650:350:0.1; flow-rate 0.7 ml/min).

Prostaglandins and seminal fluid

The following gifts are gratefully acknowledged: PGE_1, PGE_2, PGE_3 and methyl 19(*R*)-hydroxy-PGE_1 (cf. ref. 11 for hydrolysis of the methyl ester) from the Upjohn Company (Kalamazoo, MI, U.S.A.; courtesy of Drs. J.E. Pike and J.C. Sih), 20-hydroxy-PGE_1 from the ONO Pharmaceutical Company (Osaka, Japan; courtesy of Dr. M. Tsuboshima). *cis*-17,18-Dehydro-PGE_1 and 19,20-dehydro-PGE_2 were obtained by biosynthesis from their precursor fatty acids [12,13], while 19(*R*)-hydroxy-PGE_2 was purified from human seminal fluid [11]. PGE compounds were converted into PGB compounds as described below for the biological samples. Chromatographic resolution of the standards was assessed by coinjection of equal amounts.

Fresh human seminal fluid was combined with 5–10 ml of ethanol and stored at −20°C until analysis. The precipitated proteins were removed by centrifugation and the supernatant was evaporated to dryness. The residue was dissolved in 0.5 ml of water, and 0.5 ml of 0.5 *M* potassium hydroxide in methanol was added. After 10 min at room temperature, 5 ml of water were added and prostaglandins were extracted three times with diethyl ether at pH 3 (dilute hydrochloric acid). After evaporation under reduced pressure, the residue was dissolved in 1–3 ml of the mobile phase for HPLC and centrifuged before analysis.

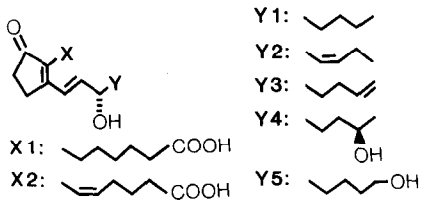

Fig. 1. Summary of the studied B prostaglandins.

TABLE I

RETENTION TIMES OF B PROSTAGLANDINS

Prostaglandin	Formula*		Retention time on β-cyclodextrin silica** (min)
	X	Y	
PGB_1	1	1	16.2
PGB_2	2	1	19.2
PGB_3	2	2	16.2
cis-17,18-Dehydro-PGB_1	1	2	14.2
19,20-Dehydro-PGB_2	2	3	16.2
19-Hydroxy-PGB_1	1	4	11.3
19-Hydroxy-PGB_2	2	4	12.6
20-Hydroxy-PGB_1	1	5	11.3

*See Fig. 1.
**The retention times changed with the condition of the column and are therefore approximate.

RESULTS AND DISCUSSION

The elution times of eight B prostaglandins on β-cyclodextrin silica are shown in Table I, and their structures are summarized in Fig. 1. Chromatography on β-cyclodextrin silica changed the order of elution of some of the PGB compounds relative to the elution order on C_{18}. On β-cyclodextrin silica, the following five primary PGB compounds eluted as follows: (i) *cis*-17,18-dehydro-PGB_1, (ii) PGB_1, 19,20-dehydro-PGB_2 and PGB_3 (without separation) and (iii) PGB_2. The separation of *cis*-17,18-dehydro-PGB_1, PGB_1 and PGB_2 is shown in Fig. 2 (left). On C_{18} eluted with methanol–water, the elution order of these PGB compounds has been found [12,14] to be: (i) 19,20-dehydro-PGB_2 and PGB_3 (almost without separation), (ii) *cis*-17,18-dehydro-PGB_1, (iii) PGB_2 and (iv) PGB_1. As regards the more polar compounds, e.g. 19-hydroxy-PGB_2 and 19-hydroxy-PGB_1, they eluted before the primary B prostaglandins on both C_{18} and on β-cyclodextrin silica, but in the reverse order: 19-hydroxy-PGB_1 eluted before 19-hydroxy-PGB_2 on β-cyclodextrin silica (Fig. 2, right, or Fig. 3, top). 19-Hydroxy-PGB_1 and 20-hydroxy-PGB_1 were not separated under these conditions (Table I).

These results show that introduction of a *cis* double bond in the 5,6 position of B prostaglandins increases the retention time on β-cyclodextrin silica relative to the compounds without this double bond (e.g. 19-hydroxy-PGB_2/19-hydroxy-PGB_1, PGB_2/PGB_1 or PGB_3/*cis*-17,18-dehydro-PGB_1). This is in agreement

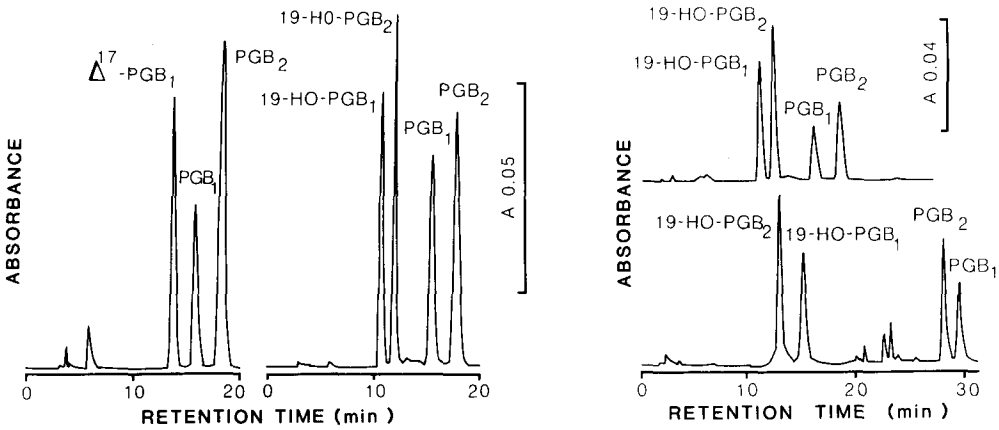

Fig. 2. Reversed-phase HPLC on β-cyclodextrin silica. (Left) Separation of *cis*-17,18-dehydro-PGB_1 (designated Δ-17-PGB_1 in the figure), PGB_1 and PGB_2. (Right) Separation of the four major B prostaglandins derived from alkali treatment of human seminal fluid.

Fig. 3. Reversed-phase HPLC on β-cyclodextrin silica and on C_{18} of B prostaglandins derived from the same sample of human seminal fluid. (Top) β-Cyclodextrin silica, isocratic elution with 60% methanol in water with acetic acid (0.01%). (Bottom) C_{18}, eluted stepwise with 52 to 65% methanol in water with acetic acid (0.01%). The amount of 19-hydroxy-PGB_2 injected on the columns was ca. 0.7 μg.

with the separation of 5,6 *cis–trans* isomers of prostaglandins, since the geometry of a 5,6-*trans* double bond and the saturated counterpart could be expected to be fairly similar [5,10]. On the other hand, introduction of a *cis* double bond in position 17,18 was found to shorten the retention time relative to the compound without this double bond. Thus, PGB_3 eluted before PGB_2, and *cis*-17,18-dehydro-PGB_1 eluted before PGB_1. In this context, it was somewhat disappointing to find that the β-cyclodextrin silica column could not separate the compounds with a 17,18 *cis* double bond and a terminal double bond. Thus, PGB_3 and 19,20-dehydro-PGB_2 were not separated under present conditions.

Separations on cyclodextrin silica are believed to proceed via formation of inclusion complexes rather than merely by differences in polarity. Many publications support this notion [3–10]. The present report is no exception. The separation obtained for the B prostaglandins indicates that the form of the molecules may strongly affect the retention time. In this respect, β-cyclodextrin silica appears to be a useful complement to C_{18}. Unlike chromatography on C_{18}, however, the retention times were slightly shortened after each injection, be it injection of a standard or a biological sample. Using the very same solvent, the retention time of PGB_2, for example, decreased from 19.7 to 16 min during analysis of about fifteen seminal samples; the rate of this decrease with each injection then slowed down (to a few seconds per injection) but did not appear to halt. The retention times could only be partly restored by washing of the β-cyclodextrin silica column with ethanol and water (as suggested by the manufacturer). β-Cyclodextrin silica thus appears to be less reproducible than C_{18}.

Biosynthesis of primary E prostaglandins and 19-hydroxy E prostaglandins

TABLE II

RELATIVE AMOUNTS OF MAJOR E PROSTAGLANDINS IN HUMAN SEMINAL FLUID

CD = β-cyclodextrin silica; C_{18} = octadecasilane silica. Semen sample 2 was obtained 14.5 h after sample 1 and sample 3 3.25 h after sample 2. Samples 4–8 were delivered at 24-h intervals. Treatment with cimetidine (0.8 g daily for two days) was started directly after sample 6. Chromatography of sample 1 is shown in Fig. 3. R_1 = 19-hydroxy-PGE_1/PGE_1, R_2 = 19-hydroxy-PGE_2/PGE_2.

Semen sample	Column	Amounts of E prostaglandins relative to PGE_2 (100%)			19-OH-PGE/PGE	
		19-OH-PGE_1	19-OH-PGE_2	PGE_1	R_1	R_2
1	CD	101	136	63	1.6	1.4
	C_{18}	111	154	72	1.5	1.5
2	CD	63	89	85	0.74	0.89
	C_{18}	60	90	73	0.82	0.90
3	CD	40	63	66	0.61	0.63
	C_{18}	40	64	70	0.57	0.64
4	CD	117	155	73	1.6	1.6
5	CD	77	110	69	1.1	1.1
6	CD	66	93	70	0.94	0.93
7	CD	66	91	74	0.89	0.91
8	CD	64	86	71	0.90	0.86

occurs in human seminal vesicles [15,16]. Analysis of the relative amounts of the four major E prostaglandins, viz. PGE_1, PGE_2, 19(*R*)-hydroxy-PGE_1 and 19(*R*)-hydroxy-PGE_2, in human seminal fluid was done after dehydration to the corresponding B prostaglandins, which were separated by reversed-phase HPLC on β-cyclodextrin silica (Figs. 2 and 3). Since human seminal fluid contains very small amounts of PGE_3 and 19,20-dehydro-PGB_2 relative to PGB_1 [17], the incomplete separation of these compounds on β-cyclodextrin silica is of little quantitative importance. It is also important to note that human seminal fluid lacks 20-hydroxy-PGE compounds [15], so that the incomplete separation of 19- and 20-hydroxy-PGB compounds on β-cyclodextrin silica does not hamper the analysis.

Reversed-phase HPLC of the same seminal sample on β-cyclodextrin silica and on C_{18} is shown in Fig. 3. On C_{18}, 19-hydroxy-PGB_1 and 19-hydroxy-PGB_2 are much more polar than PGB_1 and PGB_2, so that stepwise or gradient elution is needed to obtain resolution of these four prostaglandins within reasonable retention times. A comparison of the relative amounts of the four major E prostaglandins in three seminal samples as judged from HPLC on C_{18} and on β-cyclodextrin silica is given in Table II. These three seminal samples were obtained consecutively at short intervals from one individual, who appears to be a slow hydroxylator of seminal prostaglandins, since PGE and 19-hydroxy-PGE were in the same order of magnitude. Two days treatment with cimetidine, an inhibitor of some human liver cytochrome P-450 isozymes [18], does not appear to effect the $\omega - 1$ hydroxylation of seminal E prostaglandins to any appreciable extent in this par-

ticular individual, as judged from the small variation in PGE and 19-hydroxy-PGE from day to day (Table II).

In summary, β-cyclodextrin silica is an interesting alternative to C_{18} for the reversed-phase HPLC of B prostaglandins, and this technique appears to be useful and convenient for analysis of major E prostaglandins in human semen. The separation is presumably based on formation of inclusion complexes between the B prostaglandin and the cyclodextrin molecules and may thus be different in principle from the reversed-phase separation on C_{18}. One major limitation of cyclodextrin silica is that the retention times are less reproducible than on C_{18}.

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