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Note

Separation and quantitation of [^{14}C]prostaglandin E_1 from lung effluent metabolites by high-performance liquid chromatography

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Prostaglandins (PG) are derivatives of poly-unsaturated fatty acids containing twenty carbon atoms. Classically, prostaglandin metabolites have been separated by thin-layer (TLC) or column chromatography (CC). Recently, Banschach and Love[1] have reported their minicolumn techniques for the separation of prostaglandins using acid-washed Florisil as an adsorbent. Separation of prostaglandins has also been reported on triethylaminoethyl cellulose and on a strong anion-exchange pellicular support[2].

The use of high-performance liquid chromatography (HPLC) for prostaglandin separation has only been applied in the past few years. Mikeš *et al.*[3] compared TLC and HPLC separations of epimeric prostaglandins and reported better resolution with the latter method. Andersen and Leovey[4] separated a number of closely related prostaglandins using a pellicular silica support column (Corosil II). Alam *et al.*[5] combined HPLC–radioimmuno assay procedures for the determination of several prostaglandin metabolites in cell culture fluid. Many methods require the synthesis of derivatives before separation; some examples include: (a) formation of *p*-nitrophenacyl esters on a microparticulate silica gel column[6]; (b) formation of pentafluorobenzyl oxime derivatives[7]; (c) formation of 4-bromomethyl-7-methoxycoumarin fluorescent esters[8].

In work dealing with the ability of developing lungs to metabolize circulating PGE_1, we need a reliable and fast method for separation and quantitation of PGE_1 and its metabolites without derivatization. This report describes the first HPLC method developed by us for the resolution and quantitation of ^{14}C-labelled PGE_1 and metabolites in isolated, vascularly perfused rabbit lung effluent samples.

MATERIALS AND METHODS

Apparatus

A composite, high-performance liquid chromatographic system incorporating dual pumps (Altex, Model 110A) was employed. Samples were injected into a Rheo-

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dyne syringe loading sample injector (Model 7125, equipped with a 20-μl loop), with a Hamilton (Model S705 LT) 50-μl syringe. The chromatography column employed was a prepacked, reversed-phase column (30 cm $\times$ 3.9 mm I.D.) (μBondapak C_{18}; Waters Assoc., Milford, Mass., U.S.A.) which was operated at ambient temperature. The void volume of this chromatographic system was 4.7 ml. Column effluent fractions were collected with a Brinkman instrument (Model linear II) fraction collector. Radioactivity was counted in a liquid scintillation counter (Nuclear-Chicago, Isocap/300).

Reagents

[1-^{14}C]PGE_1 (specific activity 40–45 mCi/mmole; 0.22–0.25 μmoles/ml) was purchased from New England Nuclear (Boston, Mass., U.S.A.) and Amersham Company (Chicago, Ill., U.S.A.). Unlabeled prostaglandin E_1 was supplied by Dr. John E. Pike of the Upjohn Company (Kalamazoo, Mich., U.S.A.). PPO (2,5-diphenyloxazole), POPOP [1,4-bis(5-phenyloxazolyl-2)benzene] and Triton X-100 were obtained from Research Products International Corporation (Elkgrove, Ill., U.S.A.). Acetic acid was purchased from Drake Brothers (Menomonee Falls, Wisc., U.S.A.). Toluene was obtained from Mallinckrodt (Paris, Ky., U.S.A.), methanol from Fisher Scientific (Fair Lawn, N.J., U.S.A.), Pic A (tetrabutylammonium phosphate) from Waters Assoc. Thin-layer plates (Baker-flex silica gel IB2) were purchassed from J. T. Baker (Phillipsburg, N.J., U.S.A.) and 3.5% phosphomolybdic acid spray reagent was obtained from E.M. Laboratories (Elmsford, N.J., U.S.A.).

Chromatographic procedures

For HPLC the reservoir flask for pump A contained aqueous methanol–acetic acid–Pic A (96.85:3:0.15). In the chromatography described the amount of methanol used in pump A ranged from 56 to 70 parts; water was added so that the total amount of the methanol–water mixture used was 96.85 parts. For example, the most commonly used solvent is referred to as "62% methanol", and contained methanol–water–acetic acid–Pic A (62:34.85:3:0.15.) The flask for pump B (used only when a gradient was desired) contained methanol–acetic acid–Pic A (96.85:3:0.15). The column was washed at 0.7 ml/min with more than 15 ml of methanol and then equilibrated with solvent A for at least 45 min. Prostaglandin samples in a volume of 10–20 μl were chromatographed at a solvent flow-rate of 0.7 ml/min (approximately 500–800 p.s.i.). The column was routinely washed with methanol after each sample. 1-min fractions were collected directly into scintillation vials. The scintillation cocktail used contained 1 l toluene, 1 l Triton X-100, 12.0 g PPO and 0.5 g POPOP.

A stock solution of unlabeled PGE_1 in chloroform (10 mg/ml) was used for TLC. One microliter samples were spotted and developed for 45 min in a solvent containing chloroform–methanol–water (90:5:5), air dried and visualized with phosphomolybdic acid spray reagent.

RESULTS AND DISCUSSION

Fig. 1 is the elution profile of stock [^{14}C]PGE_1 using a 56% methanol solvent. A 4% increase in methanol reduced the PGE_1 elution time by 17 min (from 44 to

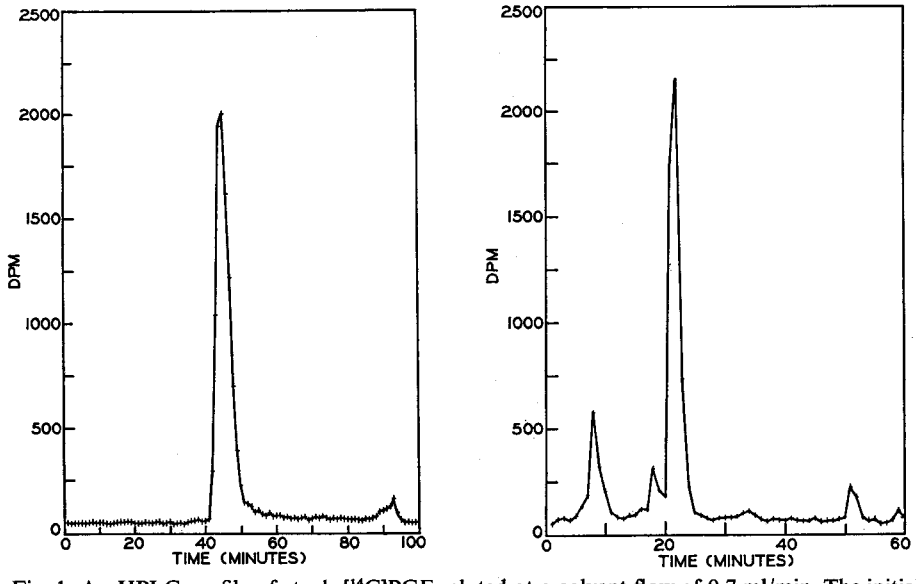

Fig. 1. An HPLC profile of stock [^{14}C]PGE$_1$ eluted at a solvent flow of 0.7 ml/min. The initial 56% methanol solvent contained methanol–water–acetic acid–Pic A (56:40.85:3:0.15). After 60 min the column was eluted for 25 min with a non-linear gradient which asymptotically approached a 96.85% methanol solvent (methanol–acetic acid–Pic A (96.85:3:0.15). The final fifteen fractions were then eluted with an 100% methanol wash.

Fig. 2. Separation of [^{14}C]PGE$_1$ and metabolites in an ethanol concentrate from perfusate which had passed through the vasculature of an isolated, adult rabbit lung. A 62% methanol solvent containing methanol–water–acetic acid–Pic A (62:34.85:3:0.15) was pumped at 0.7 ml/min for 45 min followed by 15 min of 100% methanol wash.

27 min at a flow of 0.7 ml/min). When methanol was increased further to 62, 65 or 70% (maintaining flow constant at 0.7 ml/min) PGE$_1$ elution time was reduced to 22, 17 and 12 min respectively.

After each HPLC elution a small, reproducible fraction of ^{14}C remained on the column, and was removed in the methanol wash. The methanol washes of fourteen experimental chromatographs contained $3.2 \pm 2.0\%$ of the column dpm (mean $\pm$ standard deviation). After rechromatography of five separate [^{14}C]PGE$_1$ peak tubes, $2.9 \pm 2.2\%$ of total dpm (mean $\pm$ standard deviation) were recovered in the methanol wash confirming the hypothesis that the counts in the methanol wash represent non-specific binding. The use of a methanol wash after each column has continued to substantiate this, and we do not include these dpm in determining the recovery of unchanged PGE$_1$ or PGE$_1$ metabolites.

Chromatographic resolution was demonstrated by separating PGE$_1$ stock from two chemically altered forms of PGE$_1$. First, a methyl derivative of [^{14}C]PGE$_1$ stock was made with diazomethane according to the method of Vogel[9]. Methylated [^{14}C]PGE$_1$ was injected onto the column and eluted at a flow of 0.7 ml/min with the 65% methanol solvent. In this situation 100% of the ^{14}C eluted at 47 min as a single symmetrical peak instead of at 17 min as observed for untreated [^{14}C]PGE$_1$. Second, to oxidize [^{14}C]PGE$_1$, 3 μl of [^{14}C]PGE$_1$ stock and 3 μl of unlabeled PGE$_1$ stock diluted with 30 μl of chloroform were treated with 200 μl of potassium dichromate solution

and heated over a 38° water-bath for 10 min[10]. TLC of this oxidized sample revealed that the PGE_1 spot ($R_F = 0.29$) was no longer present, but chromatographed as a new spot with an R_F of 0.63. HPLC done on this sample showed that all of the radioactivity disappeared from the [^{14}C]PGE_1 peak area, and 100% of the counts were recovered after one void volume (7 min collection at 0.7 ml/min flow).

Fig. 2 shows the [^{14}C]PGE_1 profile following ethanol deproteination and concentration of perfused adult rabbit lung effluent. Similar profiles for all the experimental samples were obtained by using identical concentration and HPLC conditions. Using these profiles, the percent dpm in the [^{14}C]PGE_1 peak was calculated relative to the % dpm eluted prior to PGE_1 and the % dpm eluted after the PGE_1 peak. The percentage of [^{14}C]PGE_1 in the ethanol concentrates from the infusate when compared to the percent [^{14}C]PGE_1 in the concentrates from isolated lung effluents are used to calculate the amount of [^{14}C]PGE_1 metabolized by the isolated lung preparation.

A cross section of the methods currently available for the measurement of prostaglandins are reviewed comprehensively by Hubbard *et al.*[11]; some are non-specific while others are insensitive. The HPLC method we have developed is relatively simple, quite sensitive and specific for applications where unchanged PGE_1 recovery is of interest. In the present report no attempt was made to establish the identity of each PGE_1 metabolite, although it is possible to identify and quantitate the different PGE_1 metabolites by co-chromatography with known standards.

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REFERENCES

1 W. M. Banschach and P. K. Love, *Prostaglandins*, 17 (1969) 193.
2 W. Morozowich, *J. Pharm. Sci.*, 63 (1974) 800.
3 F. Mikeš, V. Scharig and E. Gil-Av, *J. Chromatogr.*, 83 (1973) 91.
4 N. H. Andersen and E. M. K. Leovey, *Prostaglandins*, 6 (1974) 361.
5 I. Alam, K. Ohuchi and L. Levine, *Anal. Biochem.*, 93 (1979) 339.
6 W. Morozowich and S. L. Douglas, *Prostaglandins*, 10 (1975) 19.
7 F. A. Fitzpatrick, M. A. Wynalda and D. G. Kaiser, *Anal. Chem.*, 49 (1977) 1032.
8 J. Turk, S. J. Weiss, J. E. Davis and P. Needleman, *Prostaglandins*, 16 (1978) 291.
9 A. I. Vogel, *A Textbook of Practical Organic Chemistry*, Wiley, New York, 1966, p. 971.
10 A. I. Vogel, *A Textbook of Practical Organic Chemistry*, Wiley, New York, 1966, p. 53.
11 W. C. Hubbard, J. T. Watson and B. J. Sweetman, in G. L. Hawk (Editor), *Biological/Biomedical Applications of Liquid Chromatography*, Marcel Dekker, New York, 1979, p. 31.