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PREPARATIVE HPLC PURIFICATION OF PROSTAGLANDIN ENDOPEROXIDES AND ISOLATION OF NOVEL CYCLOOXYGENASE-DERIVED ARACHIDONIC ACID METABOLITES

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Abstract—A preparative HPLC purification scheme for the isolation of prostaglandin endoperoxides prepared by short-time incubation of [1-^{14}C]-labelled arachidonic acid (AA) with sheep seminal vesicle microsomes was developed. Milligram quantities of prostaglandin G_2 (PGG_2) and prostaglandin H_2 (PGH_2) were obtained in ≥95% purity within shortest time. Furthermore, careful application of this HPLC technique led to the isolation of two minor [1-^{14}C]-labelled fractions which according to their spectral and chromatographic characteristics, were identical with 15(*S*)-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE) and 15(*S*)-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE). Another HETE substituted at either C_{11} or C_{12} was also present. The formation of these products was mediated by cyclooxygenase as evidenced by aspirin (100 μM) and indomethacin (10 μM) inhibition. Sulfhydryl-blocking agents such as *p*-hydroxymercuribenzoate (1 mM) and/or the 12-lipoxygenase inhibitor esculetin (100 μM) were without effect. In addition to these AA metabolites four other fractions contained arachidonate-derived endoperoxides with antiaggregatory properties, all of which released malondialdehyde upon incubation with thromboxane A_2 synthase. No thromboxane formation was observed although turnover numbers were comparable to those of PGG_2 and PGH_2. The formation of these endoperoxides did not occur via enzymatic or non-enzymatic degradation of PGG_2 or PGH_2. The exact chemical nature of these endoperoxides remains to be established.

The biosynthesis of prostaglandins and thromboxanes involves the two endoperoxides, prostaglandin G_2 and H_2 as key intermediates. Therefore, biochemical studies on prostaglandin formation require as substrates these two endoperoxides which should be available at least in milligram quantities. Due to their instability against traces of transition metals, their short half-life in aqueous solution and their tendency to undergo autoxidation, it is best to freshly prepare PGG_2 and PGH_2 or at least to rechromatograph the samples before use.

Since chemical synthesis of the two endoperoxides exceeds the capacities of most biochemical laboratories, the biosynthetic route of cyclooxygenase-mediated conversion of arachidonic acid (AA)† by sheep seminal vesicle microsomes has become the routinely used procedure (for references see [1]). Its limitations lie in the small amounts separable on TLC or in the low resolution of gravity-flow silic acid column chromatography if larger quantities are required (cf. [2]).

During our work on thromboxane synthesis from PGH_2 [3], we noticed that at least 10% of AA was converted by sheep seminal vesicle microsomes to unknown products with biological activities. We therefore developed a preparative single-step HPLC-purification procedure which allowed to separate PGG_2 and PGH_2 from these unknown metabolites, which could be partially identified and characterized.

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† Abbreviations used: AA, arachidonic acid; HETE, hydroxy eicosatetraenoic acid; HPETE, hydroperoxy-eicosatetraenoic acid; HHT, 12(*L*)-hydroxy-5,8,10-heptadecatrienoic acid; PG, prostaglandin. Enzymes: cyclooxygenase, prostaglandin H synthase (EC 1.14.99.1); 15-lipoxygenase, soybean lipoxidase type V (EC 1.13.11.12); thromboxane synthase (EC 5.3.99.5).

MATERIALS AND METHODS

Materials. [1-^{14}C]AA (2.21 GBq/mmole) was obtained from Amersham-Buchler (Braunschweig, F.R.G.) and AA from Larodan (Malmö, Sweden). Both fatty acids were found to be ≥98% pure according to TLC and GLC analyses. All solvents and substances utilized were of HPLC or analytical grade and purchased from E. Merck (Darmstadt, F.R.G.) or Sigma Chemical Co. (Deisenhofen, F.R.G.). PG and H(P)ETE reference compounds were obtained either from Sigma, Paesel (Frankfurt, F.R.G.) or Cayman Chemical Co. (Ann Arbor, MI). OKY-1581 was kindly provided by ONO Pharmaceutical Co., Ltd. (Osaka, Japan).

Endoperoxide biosynthesis. Sheep seminal vesicle microsomes were prepared according to the method of Hammarström [4]. Aliquots (1 mg) of AA (0.657 mM), labelled with tracer amounts of [1-^{14}C]AA (2000 dpm/nmole), were incubated with sheep seminal vesicle microsomes (27.6 mg of protein) for 1 min at 30° under supply of oxygen gas. Microsomes were preincubated in 5 ml of 100 mM potassium phosphate buffer, 1 mM *p*-hydroxymercuribenzoate, pH 8.0 for 2 min at 30°. PGH_2 was predominantly generated by further addition of 5 mM L-tryptophan (cf. [1]). Addition of hemin

(1 μM) had no effect on cyclooxygenase activity. The incubation mixture was acidified to pH 3.0 with 1.2 ml of 2 M citric acid and PG endoperoxides were immediately extracted from the incubation mixture with 20 ml of diethyl ether/hexane 5:1 (v/v) in two sequential steps. The combined extracts were dried over 1 mg $MgSO_4$, filtered, the solvent evaporated and the residue dissolved in 2 ml of the HPLC solvent (see below).

PGG_2 and PGH_2 were generated in a semi-preparative scale by incubating 20 mg of [1-^{14}C]-labelled AA (500 dpm/nmole) with 276 mg of microsomal protein in 20 ml buffer under the afore-mentioned conditions.

Purification by preparative HPLC. The HPLC system employed consisted of the following components. HPLC pump, model 2150 from LKB (Gräfeling, F.R.G.); injection valve, type 7125 and 1 ml-sample loop from Rheodyne (Cotati, CA) and syringes from Hamilton (Bonaduz, Switzerland). Variable wavelength u.v.-detector, model 2151 from LKB and integrator/plotter, model D-2000 from E. Merck/Hitachi (Darmstadt, F.R.G.). Solvents were continuously degassed with helium using a solvent conditioner from LKB.

The purification procedure was performed at room temperature employing a straight-phase preparative HPLC column, 10 mm (i.d.) × 250 mm, filled with LiChrosorb® Si60 7 μm-material from E. Merck. The column was additionally fitted with a 4.6 mm (i.d.) × 40 mm guard-column. The guard-column was freshly filled with silicic acid 100 mesh (Mallinckrodt, Paris, KY), activated overnight at 110°, before HPLC purification was carried out. The PG endoperoxide containing extract, dissolved in the HPLC solvent, was applied to the column (large scale preparation extracts subsequently in two 1 ml-portions) which was isocratically eluted with hexane/isopropanol/acetonitrile/acetic acid 95:5:0.05:0.05 (v/v). The flow rate was adjusted to 4 ml/min and the eluent was fractionated into 4 ml-fractions. U.v.-absorbance was continuously recorded at 200 nm and radioactivity was determined with a Raytest Ramona-D radioactivity monitor from Isomess (Straubenhard, F.R.G.). PG endoperoxide containing fractions were subsequently collected, pooled, evaporated, dissolved in isopropanol and stored at −80°. PG metabolites not eluting with the HPLC solvent system employed were rinsed from the column with hexane/isopropanol 50:50 (v/v) after endoperoxide elution.

Preparation of [1-^{14}C]12-*HETE and* [1-^{14}C]12-*HPETE.* One milligram (0.657 mM) of [1-^{14}C]AA (2000 dpm/nmole) was incubated with an aliquot of the 100,000 *g* supernatant of human platelets (36 mg of protein) in 5 ml of 100 mM Tris–HCl buffer, pH 8.5 in the presence of 100 μM indomethacin. The incubation mixture was allowed to warm up at 30° for 2 min before AA was added. After 5 min at 30° and supply of oxygen gas the reaction was terminated by adding 0.5 ml of 2 M citric acid yielding a pH of 3.0. Reaction products were immediately extracted twice with diethylether/hexane 5:1 (v/v), the organic phases were combined and dried over 1 g of $MgSO_4$.

After filtration the solvent was evaporated with a rotary evaporator and the residue was dissolved in hexane/isopropanol/acetonitrile/acetic acid 95:5:0.05:0.05 (v/v) followed by preparative HPLC.

Preparation of [1-^{14}C]15-*HETE and* [1-^{14}C]15-*HPETE.* [1-^{14}C]15-HPETE was prepared by incubating 0.5 mg (0.657 mM) of [1-^{14}C]AA (1350 dpm/nmole) with 15,000 U of soybean lipoxidase (type V, Sigma) in 2.5 ml of Tris–HCl buffer, pH 8.5 in the presence of 100 μM indomethacin. The enzyme solution was warmed up for 1 min at 30° and the reaction carried out for 2 min at 30° under supply of oxygen gas. The 15-HPETE containing extract was processed for HPLC analysis as described above. [1-^{14}C]15-HETE was obtained by treatment of HPLC-purified [1-^{14}C]15-HPETE with triphenylphosphine (1 mg/ml solvent) for 30 min at room temperature.

Straight-phase HPLC analysis of PG endoperoxides. A 4.6 mm (i.d.) × 250 mm analytical HPLC column, filled with LiChrosorb® CN 5 μm-material (E. Merck) was employed. Samples were applied to the column by using a 200 μl-sample loop and the column was isocratically eluted with hexane/isopropanol/acetonitrile/acetic acid 97.5:2.5:0.05:0.05 (v/v) at a flow rate of 1 ml/min. U.v.-absorbance was continuously recorded at 200 nm and radioactivity determined as described above. HPLC analysis was performed at room temperature.

Reversed-phase HPLC analysis of AA metabolites. Analyses were performed using a low-pressure mixing unit fitted with a model 2150 HPLC-pump and a model 2151 variable wavelength u.v.-detector from LKB. Separations were carried out on a Nucleosil® C_{18} 5 μm-material reversed-phase column (4.6 mm (i.d. × 250 mm) from Bischoff (Leonberg, F.R.G.). Samples were applied to the column using a 100 μl-sample loop and the column was eluted at room temperature with acetonitrile/methanol/water/acetic acid/phosphoric acid/ammonia 25:40:34:0.3:0.3:0.2 (v/v) (solvent A) and 40:30:29:0.5:0.3:0.2 (v/v) (solvent B), apparent pH approximately 3.5 for both solvents. The flow rate was adjusted to 1 ml/min and the gradient was started with 100% A for 3 min, increased to 100% B within 12 min and continued with 100% B for at least 20 min before the column was rinsed with methanol. Retention times of authentic and enzymatically formed HHT, 12-HETE, 15-HETE, 12-HPETE and 15-HPETE were 17.7, 29.4, 26.6, 30.4 and 27.9 min, respectively.

TLC analysis of PG endoperoxides. TLC analysis was carried out at 4° with diethylether/light petroleum (60–80°)/acetic acid 85:15:0.1 (v/v) as mobile phase (solvent system A [5]). Samples were spotted on Si60-coated aluminium sheets (E. Merck) and developed over a distance of 15 cm. Sheets were scanned for radioactivity with an IM3000 TLC linear analyser from Isomess. Authentic non-labelled reference compounds were visualized by iodine vapor. R_f values for PGG_2, PGH_2 and for non-labelled PGE_2, PGB_2 and AA were 0.50, 0.37, 0.05, 0.23 and 0.83, respectively.

Thromboxane synthase assay. Thromboxane synthase activity was determined spectrophotometrically as previously described [3]. 12-Keto-HHT

was prepared by MnO_2 oxidation according to the method of Änggård and Samuelsson [6]. Thromboxane formation was judged by TLC analysis at room temperature using the upper layer of ethyl acetate/2,2,4-trimethylpentane/acetic acid/water 110:50:20:100 (v/v) as mobile phase (solvent system B [7]), migration distance 15 cm. R_f values were compared to those of authentic, non-labelled AA (0.96), PGB_2 (0.69), PGD_2 (0.52), PGE_2 (0.39), $PGF_{2\alpha}$ (0.25), TxB_2 (0.32), 6-keto-$PGF_{1\alpha}$ (0.15), 13,14-dihydro-15-keto-PGE_2 (0.64), 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (0.53) and cyclo-PGE_2 (0.83).

Platelet aggregation studies. Blood of healthy donors (200 ml) who had not taken aspirin for at least 1 week was collected with 10% (v/v) of 3.8% (w/v) sodium citrate and centrifuged at 300 *g* for 10 min. Washed human platelets were prepared according to the method of Mustard *et al.* [8] modified by further adding 50 nM of PGE_1 (Sigma) during platelet processing. Aggregation was monitored with an Elvi 840 aggregrometer (Elvi Logos, Milan, Italy). Control measurements were performed with 4 μM AA. Endoperoxides (24–2400 ng/ml) were dissolved in isopropanol before being pipetted to the platelet suspension (250 μl, *ca* 5×10^8 platelets/ml).

RESULTS AND DISCUSSION

Isolation of PGG_2 and PGH_2

Straight-phase HPLC offers the advantage of a non-aqueous solvent in which PG endoperoxides and other AA metabolites are reasonably stable and, in addition, allows a better separation of hydroperoxide metabolites than a reversed-phase system. Therefore, in order to separate the products of incubations of AA with sheep seminal vesicle microsomes in the mg-range a straight-phase preparative HPLC system was employed and resulted in the elution profile depicted in Fig. 1.

In the absence of tryptophan about 3 mg (15% of the added radioactivity) of PGG_2 (retention time 22.5 min) and 1.2 mg (6%) of PGH_2 (r.t. 30.7 min) were recovered from 20 mg of AA. Both compounds were identified by GLC and TLC analyses after chemical conversions to known prostaglandins and comparison with reference samples as described (cf. [1]). The latter was converted to $PGF_{2\alpha}$ upon treatment with triphenylphosphine, derivatized and subjected to GLC-mass spectrometry analysis (EI-mode). Furthermore both compounds aggregated washed human platelets rapidly and irreversibly at concentrations between 0.5 and 1.0 μg/ml. This concentration is slightly higher than that reported originally by Hamberg *et al.* [9] but might be due to the addition of antiaggregatory PGE_1 in our platelet washing procedure. When PGH_2 was rechromatographed on straight-phase HPLC employing a cyano-bonded analytical column, a homogeneous band at r.t. 23.6 min was observed by radioactivity detection indicating a purity of certainly more than 95% (Fig. 2). The same was found for PGG_2 (r.t. 51.3 min). Also on TLC radiochromatography (solvent system A) and in the u.v.-spectra (350–200 nm) no contaminations were detected.

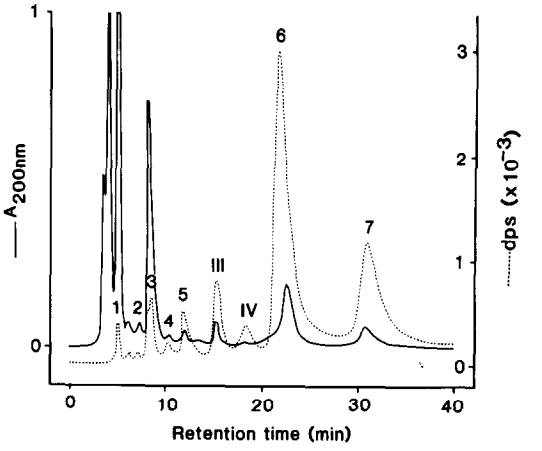

Fig. 1. Preparative HPLC of AA metabolites formed by cyclooxygenase in the absence of tryptophan. The depicted chromatogram was obtained from a 1 mg-incubation of [1-^{14}C]AA with sheep seminal vesicle microsomes in the absence of tryptophan as described in the text. The solid line represents the u.v.-absorption measured at 200 nm and the dotted line the monitored ^{14}C-radioactivity expressed as dose per sec. Indicated elution bands correspond to AA (1), 15-HETE (2), 15-HPETE (3), 12-hydroperoxy-HHT (4), HHT (5), PG endoperoxides (III, IV), PGG_2 (6) and PGH_2 (7). Relative radioactivity of the PGG_2-peak was *ca* 22.5% corresponding to 272 μg.

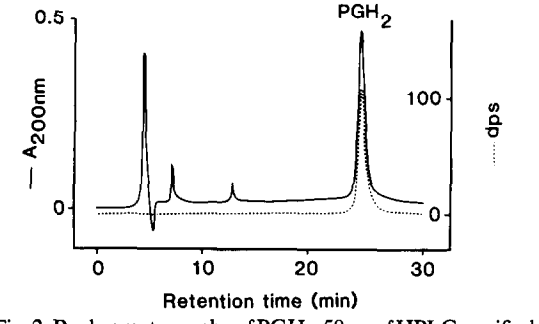

Fig. 2. Rechromatography of PGH_2. 50 μg of HPLC-purified PGH_2 (500 dpm/nmole) were applied to a LiChrosorb® CN 5 μm-material column. Radioactivity monitoring (dotted line) and u.v.-detection (solid line) were performed as described for the preparative straight-phase HPLC system.

Addition of tryptophan to the incubation is known to provide electrons for the peroxidase function of cyclooxygenase whereby PGG_2 is almost entirely converted to PGH_2 (Fig. 3). Under these conditions (5 mM tryptophan) the yield of PGH_2 increases to an average of 20–38.5% dependent on the cyclooxygenase activity of the vesicle microsomes.

Isolation of HETEs and HPETEs

It is evident from the chromatograms shown in Figs 1 and 3 that besides PGG_2 and PGH_2 several other AA metabolites are formed by seminal vesicle microsomes. 12(*L*)-hydroxy-5,8,10-heptadecatrienoic acid (HHT) could be expected as degradation product of PGH_2 and a reference sample co-chromatographed in two systems (TLC and reversed-phase HPLC) with peak no. 5 (r.t. 12.2 min). The collected material of peak no. 5 also had an u.v.-absorption at 234 nm and therefore very

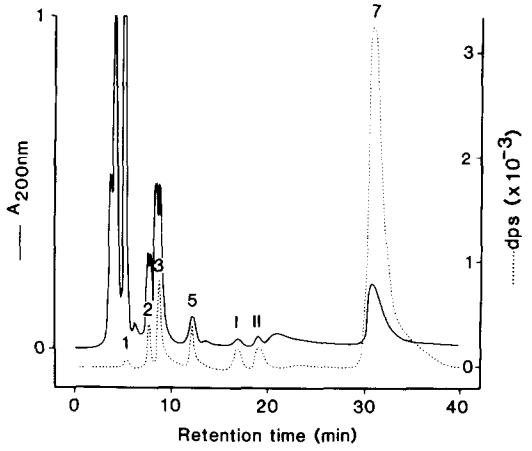

Fig. 3. Preparative HPLC of AA metabolites formed by cyclooxygenase in the presence of tryptophan. 1 mg of [1-^{14}C]AA was incubated with sheep seminal vesicle microsomes in the presence of 5 mM tryptophan as described in the experimental section. For peak designations refer to Fig. 1. The PGH_2-peak (7) contained ca. 60% of the radioactivity injected corresponding to 333 μg.

likely was identical with HHT. Under conditions where PGG_2 was present an additional peak was observed with r.t. 10.4 min. It absorbed at 235 nm, reacted with ferrous thiocyanate [10] and after reduction with triphenylphosphine migrated like HHT. We therefore assume its identity with 12-hydroperoxy-HHT.

A significant percentage (about 4%) of the radioactivity was present in peak no. 3 (r.t. 8.8 min). These fractions absorbed at 237 nm and reacted with ferrous thiocyanate. Separation on rp-HPLC and TLC resulted in a major band with cochromatographed with 15-HPETE. In agreement with the formation of 15-HPETE were the properties of peak no. 2 (r.t. 7.4 min) which corresponded in u.v.-absorption and chromatographic behavior to the presence of 15-HETE with a smaller contribution of a 11- or 12-HETE-like substance after separation on TLC and reversed-phase HPLC. Peak no. 2 was always higher when the incubation was carried out in the presence of tryptophan.

For further identification [1-^{14}C]12-HPETE and [1-^{14}C]15-HPETE were prepared as described above. Retention times on preparative HPLC were 8.8 min for both compounds which also reacted with ferrous thiocyanate. After reduction with triphenylphosphine radioactivity appeared in a single peak with a retention time of 7.4 min, identical for both compounds and ferrous thiocyanate radioactivity was lost.

According to these data peak no. 2 would represent essentially 15-HETE and peak no. 3 15-HPETE. The very minor component seen by TLC and reversed-phase HPLC analysis could be the 12-hydroxy derivative, but the corresponding 11-substituted compound could not be entirely ruled out.

Since such hydroperoxides and corresponding hydroxy fatty acids could be considered as autoxidation products of AA their occurrence in small quantities was not unexpected. However, their formation was completely inhibited by aspirin (100 μM) and indomethacin (10 μM). It was insensitive to sulfhydryl-blocking agents such as *p*-hydroxymercuribenzoate (1 mM [11]) and esculetin (100 μM), a specific inhibitor of platelet 12-lipoxygenase [12], was not effective. Heat-inactivated vesicle microsomes did not produce peak no. 2 or 3 and the fact that tryptophan (5 mM) only shifted peak no. 3 to peak no. 2, but did not diminish their formation argues against a co-oxidation process triggered by the oxidized intermediate of cyclooxygenase.

It is not unlikely and has been suggested from studies in leukocytes [13, 14] that the cyclooxygenase reaction leads to side-products for which the 15-HPETE is a probable candidate. Since the first step in the enzymatic mechanism is considered to be a hydrogen abstraction at C_{13} a peroxidation at C_{15} would be the alternative pathway if the isomerization of the double bond would occur between C_{14} and C_{15} instead of C_{11} and C_{12}. The 15-HPETE could be reduced by the peroxidase function of cyclooxygenase [15]. According to the proposed cyclooxygenase mechanism [16, 17] the minor HETE component migrating together with 15-HETE may indeed be the 11-derivative. With regard to its small amount (about 1% of 15-HETE) no further identification was attempted.

Isolation of new endoperoxide metabolites

Another pair of corresponding metabolites was found to be present in peaks I (r.t. 16.7 min, 2% of the radioactivity supplied) and II (r.t. 18.9 min, 2%) which were observed in the presence of tryptophan, and peak III (r.t. 15.8 min, 3.5%) together with peak IV (r.t. 18.5 min, 2%) in the absence of tryptophan. Aspirin (100 μM) and indomethacin (10 μM) also prevented the formation of all four peaks. None of these compounds was detected when PGH_2 (83 μg) or PGG_2 (71 μg) were incubated with sheep seminal vesicle microsomes and therefore cannot be secondary metabolites. They all were ferrous thiocyanate positive and were without significant u.v.-absorption in the range between 350 and 200 nm, even after prolonged standing in aqueous buffer. TLC analysis (solvent system A) revealed similar chromatographic behavior of peaks I and III compared with PGG_2 and peaks II and IV compared with PGH_2. In addition, all metabolites reacted with purified thromboxane synthase in a characteristic manner. With metabolites I and III a time-dependent increase in absorption at 267 nm occurred, due to the formation of malondialdehyde (Fig. 4).

Neither parallel diene conjugation nor an increase in absorbance at 279 nm indicating the formation of 12-keto-HHT derived from 15-keto-PGG_2 [18] was observed. The half-life of these compounds was 7.2 min in 50 mM potassium phosphate buffer, pH 7.4 at 20° (PGH_2: 7.4 min) and thromboxane synthase reaction was completely blocked by OKY-1581 (100 nM), a specific thromboxane synthase inhibitor [3]. Thus, they behaved like an endoperoxide lacking the Δ^{13} double bond. Such behavior is similar to that of PGH_1 which, however, is chromatographically distinct from metabolites I and III.*

* Unpublished results.

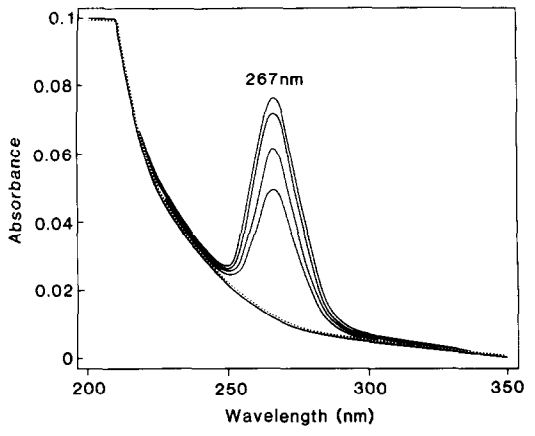

Fig. 4. U.v.-absolute spectroscopy of thromboxane synthase reaction with PG endoperoxide metabolite I. *Ca* 0.27 μg thromboxane synthase were incubated with 7.5 nmole of peak I material in 0.5 ml of 50 mM potassium phosphate buffer, pH 7.4 at 20°. The solid lines correspond to time 0 (baseline), 1, 5, 10 and 20 min. The dotted line represents the spectrum obtained after pretreatment with either 100 nM of OKY-1581 or incubation with buffer alone.

Again looking at the proposed mechanism of cyclooxygenase one could speculate that after cyclization and endoperoxide formation the remaining C_{13} radical instead of isomerization would react with molecular oxygen leading to a 13-hydroperoxy-Δ^{14}-PGH_2 derivative. This hypothesis is under further investigation.* Metabolites II and IV seem to contain a Δ^{13} double bond in contrast to I and III since their reaction with the enzyme in both cases produces absorption bands at 238 nm in addition to that of malondialdehyde at 267 nm (results not shown). Turnover numbers of metabolites II and IV were comparable to those of PGG_2 and PGH_2 whereas thromboxane synthase reaction was significantly accelerated with component III and distinctly slower with component I. However, no thromboxane-analogues compounds were produced with all four endoperoxides according to TLC analysis (solvent system B).

With regard to the biological activities metabolites I and II in a micromolar range completely blocked aggregation of washed human platelets either induced by PGG_2/PGH_2 or AA. Preincubation of platelets with metabolites III and IV only allowed a reversible aggregation. Whether these antiaggregatory properties are due to thromboxane synthase inhibition, thromboxane/endoperoxide receptor antagonism or to the formation of antiaggregatory PG metabolites has to be elucidated. All data point to a close relationship of these endoperoxides to PGG_2 and/or PGH_2, but their exact chemical nature remains to be established.

* Hecker *et al.*, in preparation.

CONCLUSIONS

The HPLC purification procedure described herein allows the rapid isolation of pure PGG_2 and PGH_2 in milligram quantities and by replacing arachidonic acid with eicosatrienoic or eicosapentaenoic acid also of the corresponding PGG_1/PGH_1 and PGG_3/PGH_3 derivatives. A high resolution HPLC system seems to be necessary in view of the formation of 15-HPETE and 15-HETEs as well as the four still unknown endoperoxide metabolites derived from a cyclooxygenase side reaction.

Note added in proof. Recent HPLC and GLC mass spectrometry (EI mode) analyses confirmed the identification data of 15-HETE, HHT and their corresponding hydroperoxy derivatives presented herein as well as the formation of 11-HPETE. Details of these studies will be published elsewhere (Hecker *et al.*, in preparation).

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REFERENCES

1. R. A. Johnson, *Adv. Prostaglandin Thromboxane Leukotriene Res.* **14**, 131 (1985).
2. I. M. Zulak, M. L. Puttemans, A. B. Schilling, E. R. Hall and D. L. Venton, *Analyt. Biochem.* **154**, 152 (1986).
3. M. Hecker, M. Haurand, V. Ullrich and S. Terao, *Eur. J. Biochem.* **157**, 217 (1986).
4. S. Hammarström, *J. biol. Chem.* **255**, 518 (1980).
5. T. Miyamoto, S. Yamamoto and O. Hayaishi, *Proc. natn. Acad. Sci. U.S.A.* **71**, 3645 (1974).
6. E. Änggård and B. Samuelsson, *J. biol. Chem.* **239**, 4097 (1964).
7. J. A. Salmon and R. J. Flower, *Meth. Enzymol.* **86**, 477 (1982).
8. J. F. Mustard, D. W. Parry, N. G. Ardlie and M. A. Packham, *Br. J. Haematol.* **22**, 193 (1972).
9. M. Hamberg, J. Svensson, T. Wakabayashi and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **71**, 345 (1974).
10. R. R. Gorman, F. F. Sun, O. V. Miller and R. A. Johnson, *Prostaglandins* **13**, 1043 (1977).
11. S. Narumiya, J. A. Salmon, F. H. Cottee, B. C. Weatherley and R. J. Flower, *J. biol. Chem.* **256**, 9583 (1986).
12. K. Sekiya, H. Okuda and S. Arichi, *Biochim. biophys. Acta* **713**, 68 (1982).
13. M. I. Siegel, R. T. McConell, N. A. Porter, J. L. Selph, J. F. Truax, R. Vinegar and P. Cuatrecasas, *Biochem. biophys. Res. Commun.* **92**, 688 (1980).
14. R. W. Randall, K. E. Eakins, G. A. Higgs, J. A. Salmon and J. E. Tateson, *Agents Actions* **10**, 553 (1980).
15. L. J. Marnett, P. H. Siedlik and L. W. M. Fung, *J. biol. Chem.* **257**, 6957 (1982).
16. B. Samuelsson, *J. Am. chem. Soc.* **87**, 3011 (1965).
17. R. A. Appleton and K. Brown, *Prostaglandins* **18**, 29 (1979).
18. G. Graff, E. W. Dunham, T. P. Krick and N. D. Goldberg, *Lipids* **14**, 334 (1979).