Purification, cDNA cloning and expression of 15-oxoprostaglandin 13-reductase from pig lung

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15-Oxoprostaglandin 13-reductase (PGR) has been purified to apparent homogeneity from pig lung. The enzyme was estimated to have a molecular mass of 36 kDa by both SDS/PAGE and non-denaturing PAGE, indicating that the enzyme is a monomer. 15-Oxo-PGE_1, 15-oxo-PGE_2 and 15-oxo-$PGF_{2\alpha}$ were found to be substrates for the enzyme, whereas the corresponding 15-hydroxyprostaglandins were not. The reverse reaction, the oxidation of 13,14-dihydro-15-oxo-PGE_1 to 15-oxo-PGE_1, was not observed. Either NADH or NADPH could serve as a coenzyme. However, the V_{max} with NADH was approx. 3-fold that with NADPH, while the K_m for NADPH was approx. one-tenth that for NADH. Cloning of the cDNA was achieved by PCR and library screening. A 600 bp PCR product containing the sequences of three different tryptic peptides derived from purified PGR was used for cDNA library screening by plaque hybridization. A cDNA clone that contained the entire PGR coding sequence of 987 bp was obtained. The sequence codes for a protein of 329 amino acid residues with a calculated molecular mass of 35791 Da. Homology analysis indicated that the sequence is virtually identical with that of leukotriene B_4 (LTB_4) 12-hydroxydehydrogenase [Yokomizo, Ogawa, Uozumi, Kume, Izumi and Shimizu (1996) J. Biol. Chem. **271**, 2844–2850]. Expression of this cDNA in *Escherichia coli* resulted in a protein exhibiting both PGR and LTB_4 12-hydroxydehydrogenase activities. However, the specific activity of PGR with 15-oxo-PGE_1 as a substrate was approx. 300-fold that of LTB_4 12-hydroxydehydrogenase. These results indicate that the cloned cDNA codes for a protein with two different enzyme activities, with 15-oxoprostaglandins as the preferred substrates.

INTRODUCTION

The lung is a major organ for the biosynthesis and catabolism of prostaglandins [1]. The lungs are responsible largely for the degradation of prostaglandins, especially of the E and F series, which enter the circulation after synthesis in the peripheral tissues. This uptake by the lung ensures that large amounts of prostaglandins do not circulate and exert systemic effects. Prostaglandin E_1 (PGE_1), PGE_2 and $PGF_{2\alpha}$ are inactivated rapidly in a single passage through the pulmonary circulation, with the 13,14-dihydro-15-oxo prostaglandins being the major metabolites [2]. The two enzymes responsible for the formation of these metabolites are the NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and 15-oxoprostaglandin 13-reductase (PGR). 15-PGDH oxidizes the 15-hydroxy group of prostaglandins to produce 15-oxo metabolites that generally exhibit greatly decreased biological activities compared with their respective 15-hydroxyprostaglandins. The 13,14 double bond of the 15-oxo metabolite is then reduced by PGR. This reduction can result in a further decrease in biological activity of the prostaglandin. It has been shown that the lung is particularly rich in 15-PGDH and PGR activities [3]. Of these two enzymes, 15-PGDH has been the most extensively studied, whereas relatively little is known about PGR. PGR has been previously purified from chicken heart [4], bovine lung [5], human placenta [6,7] and bovine iris ciliary body [8]. Characterization of the PGR enzymes suggests that there are species and tissue differences and/or more than one form of PGR.

To elucidate the interrelationship of various forms of PGR and to understand the structure and function of the enzyme better, it is essential to determine the enzyme's primary structure. Furthermore the cDNA for the enzyme will be valuable for the study of the enzyme's pathophysiological roles. Therefore we performed the cloning, sequencing and expression of the cDNA coding for pig lung PGR. Surprisingly, the primary structure of PGR is virtually identical with that of LTB_4 12-hydroxydehydrogenase, indicating that the enzyme is capable of carrying out the reduction of a double bond as well as the oxidation of a hydroxy group.

EXPERIMENTAL

Materials

DEAE-Sephacel, adenosine 2′,5′-diphosphate agarose, Cibacron Blue-Sepharose, NAD^+, $NADP^+$, NADH, NADPH and Freund's adjuvant were purchased from Sigma. All the prostaglandins and LTB_4 were obtained from Cayman. *Taq* DNA polymerase was supplied by Gibco-BRL. Hydroxyapatite (Biogel HTP) was obtained from Bio-Rad. Oligonucleotides were synthesized by Integrated DNA Technologies. λgt11 pig lung cDNA library was obtained from Clontech. TA cloning kit was pur-

Abbreviations used: LTB_4, leukotriene B_4; PG, prostaglandin; 15-PGDH, 15-hydroxyprostaglandin dehydrogenase; PGR, 15-oxoprostaglandin 13-reductase.

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The nucleotide sequence results reported here will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession number BankIt 93753 U87622.

chased from Invitrogen. Plasmid purification kit was supplied by Qiagen. Immobilon PVDF-P membrane was obtained from Millipore. Silica gel G thin-layer plates were purchased from EM Science.

Purification of PGR from pig lung

The procedure used to purify the pig lung PGR was a combination of the methods described by Hansen [5] and Westbrook [7], with some additional modifications. Pig lung was obtained from the University of Kentucky College of Agriculture. Lung tissue (300 g) was homogenized in 700 ml of 5 mM potassium phosphate (pH 7.0)/20 % (w/v) glycerol/1 mM EDTA (buffer A) in a Waring blender. The homogenate was centrifuged at 10000 ***g*** for 30 min; the supernatant was then recentrifuged at 100000 ***g*** for 1 h. $(NH_4)_2SO_4$ (0.298 g per ml of high-speed supernatant) was added slowly and the solution was stirred at 4 °C for 1 h. The solution was then centrifuged at 10000 ***g*** for 30 min. Additional $(NH_4)_2SO_4$ (0.197 g/ml) was added to the supernatant; the solution was stirred at 4 °C for 1 h and then centrifuged at 10000 ***g*** for 30 min. The pellet was resuspended in a small volume of buffer A (50–60 ml final volume). The solution was dialysed against four changes of 2 litres of buffer A for a total of 36 h. The dialysed solution was then applied to a DEAE-Sephacel column (2.5 cm × 11 cm) equilibrated with buffer A. This step separates 15-PGDH activity, which binds to DEAE, from PGR activity, which does not bind. The flow-through fraction was applied directly to a Cibacron Blue-Sepharose column (2.5 cm × 10 cm) equilibrated with 10 mM potassium phosphate (pH 7.0)/1 mM EDTA (buffer B). The bound protein was then eluted with a 400 ml gradient of 0–1.0 M KCl in buffer B; 6 ml fractions were collected. The most active fractions (fractions 6–22) were pooled and desalted by repeated concentration by ultrafiltration and dilution with 10 mM potassium phosphate, pH 7.0 (buffer C). The desalted enzyme was then applied to a hydroxyapatite column (2.5 cm × 6.5 cm) equilibrated with buffer C and the enzyme was eluted with a gradient formed from 200 ml buffer C and 200 ml 200 mM potassium phosphate, pH 7.0; 4 ml fractions were collected. Active fractions (52–65) were pooled, concentrated and diluted with buffer B and then concentrated to less than 20 ml. The concentrated solution was applied to an adenosine 2′,5′-diphosphate agarose column (5 ml of gel) equilibrated with buffer B. The protein was eluted with a 50 ml gradient of 0–0.6 M KCl in buffer B; 2 ml fractions were collected. The active fractions (25–34) were pooled, concentrated to 5 ml and dialysed overnight against 2 litres of 20 mM Tris/HCl (pH 8.0)/1 mM EDTA. The dialysed enzyme was further purified by FPLC on a Mono-Q column (Pharmacia) with a 30 ml gradient of 0–0.5 M NaCl in 20 mM Tris/HCl (pH 8.0)/1 mM EDTA. The purified enzyme was stored at −80 °C. The degree of purification was examined by SDS/PAGE and staining with Coomassie Blue.

Assay of PGR activity

Because 15-PGDH can catalyse the reduction of the 15- oxo group of prostaglandins, PGR activity cannot be accurately assayed in crude extracts containing 15-PGDH activity. DEAE effectively separates 15-PGDH and PGR activities, so PGR activity can be assayed after this step in the purification procedure. 15-PGDH activity was assayed by the ^3H release assay as described [9]. For assaying PGR activity during purification the chromaphore method described by Hansen [5] was used. 15-oxo-PGE_1 and 15-oxo-PGE_2 will form labile red chromophores in alkaline solution. For determining activity during the purification of PGR, the reaction mixture contained 0.1 M sodium phosphate, pH 7.4, 1 mM 2-mercaptoethanol, 20 μg 15-oxo-PGE_1 or 15-oxo-PGE_2, and 1 mM NADH. The reaction was started by adding enzyme (5–40 μg depending on the purification step) to give a final volume of 1 ml. The reaction was incubated at 37 °C for 10 min, after which 0.25 ml of 2 M NaOH was added. The amount of 15-oxo-prostaglandin remaining was measured by reading the maximal absorption at 500 nm, which was reached approx. 1–2 min after the addition of NaOH. One unit of enzyme is defined as the amount of enzyme catalysing the oxidation of 1 μmol of substrate/min under the conditions of the assay. Under the conditions used in the PGR assay, the absorption coefficient of the labile chromophore of 15-oxo-PGE_1 was determined as 15160 $M^{-1}\cdot cm^{-1}$, while that of 15-oxo-PGE_2 was 30300 $M^{-1}\cdot cm^{-1}$. For determining kinetic values the assay conditions were the same except that variable amounts of 15-oxo-PGE_1, 15-oxo-PGE_2, NADH and NADPH were used. For the 15-oxo-prostaglandins, a concentration range between 3.5 and 57 μM was used with 1 mM NADH. For NADH, concentrations between 50 and 400 μM were used, and for NADPH, a range of 5–40 μM was used. For determining kinetic values for NADH and NADPH, 57 μM 15-oxo-PGE_1 was used. The reactions were started by adding enzyme (8.5 μg). Aliquots (1 ml) were removed at 1 min intervals and treated with 0.25 ml of 2 M NaOH to determine the rate of each reaction. Under these conditions the reaction rate was found to be linear for at least 10 min. The apparent K_m and V_{max} values were based on Michaelis–Menten kinetics by using the direct linear-plot technique [10].

Assay of LTB_4 12-hydroxydehydrogenase activity

The enzyme activity was assayed by the procedure described by Yokomizo et al. [11] with the use of HPLC to separate LTB_4 and its metabolites.

TLC

TLC was used to determine what type of prostaglandins could be used as substrates by PGR. The following prostaglandins were tested: PGE_1, PGE_2, $PGF_{2\alpha}$, 15-oxo-PGE_1, 15-oxo-PGE_2, 15-oxo-$PGF_{2\alpha}$ and 13,14-dihydro-15-oxo-PGE_1. Each reaction was performed as described above with 20 μg of prostaglandin at 37 °C for 20 min. The reaction was stopped by adding 100 μl of 1 M citric acid and then extracted twice with diethyl ether. The ether extract was evaporated under nitrogen and the sample redissolved in 50 μl methanol and spotted on a silica gel TLC plate. Two separate solvent systems were used to examine each set of reactions. The first system was the top organic layer from a mixture of ethyl acetate/acetic acid/iso-octane/water (11:2:5:10, by vol.). The second system consisted of chloroform/methanol/acetic acid (95:1:5, by vol.). Products were revealed by exposure of plates to iodine vapour. Migration of products was compared with known standards run at the same time.

Production of antibody

A rabbit was immunized with purified native PGR by subcutaneous injection along the back of the animal [12]. The first injection was with Freund's complete adjuvant. Incomplete adjuvant was used for booster injections, which were given at intervals of 4 weeks. Approx. 100 μg of purified enzyme was used for each injection. Blood was collected via the marginal ear vein and serum was tested for antibody production by the antigen-coated plate method [12] and by Western blot analysis. Significant antibody production was detected after the second booster injection.

Western blot analysis

Proteins were subjected to SDS/PAGE and the proteins were electrophoretically transferred to an Immobilon PVDF-P membrane. The membranes were blocked in TBST [50 mM Tris/HCl (pH 7.5)/150 mM NaCl/0.05 % (v/v) Tween-20] containing 5 % (w/v) dried non-fat milk for 30 min and then incubated with antiserum diluted 1:250 in TBST containing 5 % (w/v) dried non-fat milk for 1 h at room temperature or overnight at 4 °C. The membranes were then washed with TBST four times for 15 min each wash and incubated with goat anti-(rabbit IgG) conjugated with alkaline phosphatase, diluted 1:1000 in TBST containing 5 % (w/v) dried non-fat milk. The membranes were washed and then developed with Nitro Blue Tetrazolium (0.2 mg/ml) and 5-bromo-4-chloro-3-indoyl phosphate (0.1 mg/ml) in substrate buffer [100 mM Tris/HCl (pH 9.5)/100 mM NaCl/5 mM $MgCl_2$].

N-Terminal and internal amino acid residue sequencing of PGR

Purified protein was subjected to SDS/PAGE and then transblotted to PVDF membranes, which were then stained with Amido Black. The band at 36 kDa was excised and sequenced by the method of Matsudaira [13]. The N-terminal amino sequence was determined by the Macromolecular Structure and Analysis Facility of the University of Kentucky. The internal tryptic peptide sequences were determined by the Wistar Protein Microchemistry Core Facility (Philadelphia, PA, U.S.A.).

Cloning of the PGR cDNA

On the basis of the N-terminal and internal peptide sequences of PGR, degenerate 17-base sense and 17-base anti-sense oligonucleotides were designed from regions of low codon degeneracy (sense, 5′-AAA(G)AAA(G)GGT(CAG)TTC(T)GT-T(CAG)GG-3′; anti-sense, 5′-TTA(G)TCA(G)AAA(G)TAA-(G)CAA(G)TCA(G)TA-3′). The PCR was performed with these two degenerate oligonucleotides as primers and with the use of a λgt11 pig lung cDNA library as a template. Conditions of the PCR were: denaturation at 94 °C for 1 min, annealing at 42 °C for 1 min and elongation at 72 °C for 2 min. After 30 cycles of reaction the PCR products were separated on a 0.75 % agarose gel: three different bands were recovered from the gel with a Qiagen gel purification kit. Each band was cloned into PCRII with a TA cloning kit and the plasmids were transformed into INVoF′. Plasmids were purified with a Qiagen plasmid purification kit and sequenced by the chain-termination method. The 600 bp PCR product was found to contain sequences coding for the three peptides previously sequenced. This 600 bp DNA fragment was radiolabelled by random primer labelling and used as a probe to screen the pig lung λgt11 cDNA library by plaque hybridization. One positive clone was plaque-purified from 3×10^5 phages and the cDNA insert was amplified by PCR with *Taq* DNA polymerase with λgt11 primers that flank the insertion site of the cDNA. The PCR product was subcloned into PCR II and sequenced by Sequenase version 2.0.

Expression and purification of recombinant PGR

The cDNA insert was digested from PCRII by *Eco*RI and ligated into the *Eco*RI site of pGBT-T19, an expression vector that contains an isopropyl β-D-thiogalactoside-inducible *tac* promoter. The ligation reaction mixture was used to transform *Escherichia coli* JM109; transformants expressing PGR activity were isolated. Large-scale purification of recombinant PGR was performed as follows. The PGR-expressing JM109 cells were grown in 700 ml of Luria–Bertani medium containing 100 μg/ml ampicillin and 1 mM isopropyl β-D-thiogalactoside overnight at 37 °C. Cells were pelleted by centrifugation at 4000 ***g*** for 10 min. The cells were resuspended in 20 ml of cold 40 mM potassium phosphate buffer, pH 7.0, containing 20 % (v/v) glycerol and 1 mM EDTA, then lysed by sonication. The lysate was centrifuged at 100000 ***g*** for 20 min and the supernatant was applied to a Cibacron Blue-Sepharose column (2.5 cm × 10 cm) equilibrated with buffer B as described above. The bound protein was then eluted with a 400 ml gradient of 0–1.0 M KCl in buffer B; 6 ml fractions were collected. The most active fractions (fractions 13–23) were pooled and desalted by repeated concentration by ultrafiltration and dilution with buffer B, then concentrated to 5 ml. The desalted enzyme was further purified by FPLC on a Mono Q column (Pharmacia) with a 30 ml gradient of 0–0.5 M NaCl in 20 mM Tris/HCl, pH 8.0, containing 1 mM EDTA. The purified enzyme was stored at −20 °C. The degree of purification was examined by SDS/PAGE followed by staining with Coomassie Blue.

RESULTS

Purification of pig lung PGR

We have purified 15-oxoprostaglandin 13- reductase (PGR) from pig lung to apparent homogeneity. The procedure used was a combination of those used for the purification of the human placental and bovine lung PGRs [5,7], with some additional modifications. Purification steps included: (1) homogenization of tissue and isolation of high-speed supernatant; (2) $(NH_4)_2SO_4$ fractionation; (3) DEAE-Sephacel chromatography; (4) Cibacron Blue chromatography; (5) hydroxyapatite chromatography; (6) chromatography on adenosine 2′,5′-diphosphate-agarose; (7) FPLC on a Mono Q column. The amount of PGR activity present in the crude extract or in the $(NH_4)_2SO_4$ fraction could not be accurately determined because of the presence of 15-PGDH activity, which can catalyse the reverse reaction from 15-oxoprostaglandin to 15-hydroxprostaglandin and thereby interfere with the chromophore assay used to detect PGR activity. The DEAE chromatography effectively separates PGDH activity, which binds to DEAE, from PGR, which does not bind to DEAE. Starting with 300 g of tissue, approx. 400 μg of purified enzyme was isolated (Table 1). Figure 1 shows the results of SDS/PAGE of protein from each of the purification steps.

Properties of PGR

We have tested 15-oxo-PGE_1, 15-oxo-PGE_2 and 15-oxo-$PGF_{2\alpha}$ and found that all three are substrates for the purified enzyme. On TLC, the products produced from each of the above 15-oxo-prostaglandins co-migrated with their respective 13,14-dihydro-15-oxo-prostaglandin standards in two separate solvent systems. PGE_1, PGE_2 and $PGF_{2\alpha}$ are not substrates for the enzyme, indicating a specificity for the 15-oxo-prostaglandins.

The apparent K_m for 15-oxo-PGE_1 was 7.7 μM, whereas that for 15-oxo-PGE_2 was 19 μM (Table 2). The reaction was dependent on the presence of cofactor. Both NADH (apparent K_m 153 μM) and NADPH (apparent K_m 15 μM) can be used by the pig lung enzyme as cofactors; however, the V_{max} with NADH is approx. 3-fold that with NADPH. The turnover number (k_{cat}) of the enzyme with 15-oxo-PGE_1 and NADH is 91 min^{-1}, compared with 31 min^{-1} with 15-oxo-PGE_2 and NADH. These low turnover numbers are similar to the turnover numbers (60 and 42 min^{-1} in two separate experiments with 15-oxo-PGE_1) determined for the bovine lung PGR [5]. It has been suggested that these low turnover numbers are compensated for by a high

Table 1 Purification of PGR from pig lung

Activities were determined with 15-oxo-PGE_1 and NADH as substrates. Because of the presence of 15-PGDH activity, PGR activity could not be determined accurately in the steps before the DEAE column.

Step	Total A_{280} (units)	Specific activity (m-units/mg)	Total activity (m-units)	Purification (fold)	Recovery (%)
Cytosol	17500	–[2]	–	–	–
$(NH_4)_2SO_4$	1906	–	–	–	–
DEAE	544	9.5	5168	1	100
Cibacron Blue	55	75	4131	8	80
HPT	4.5	556	2502	59	48
ADP-agarose	0.9	1715	1530	180	30
Mono Q	0.4	2562	1025	270	20

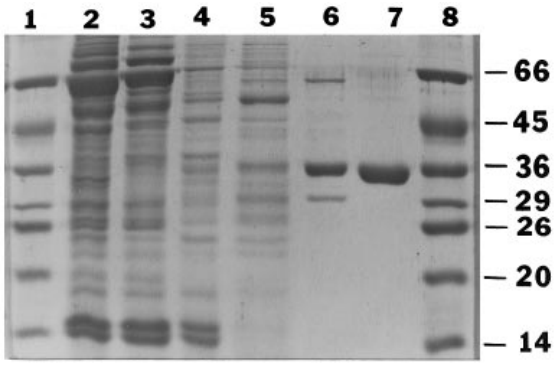

Figure 1 SDS/PAGE of samples from steps in purification of PGR from pig lung

Lanes 1 and 8, molecular mass standards (values in kDa are shown at the right); lane 2, $(NH_4)_2SO_4$ fraction, 50 μg; lane 3, DEAE flow-through, 30 μg; lane 4, Cibacron Blue column, 15 μg; lane 5, hydroxyapatite column, 10 μg; lane 6, ADP-agarose column, 6 μg; lane 7, Mono Q column, 4 μg. Protein was stained with Coomassie Brilliant Blue R.

concentration of the enzyme in the lung, which results in a high metabolic capacity to catabolize 15-oxo-prostaglandins [5].

The reverse reaction, the oxidation of 13,14-dihydro-15-oxo-PGE_1 to 15-oxo-PGE_1, was not observed. The molecular mass of the enzyme is estimated to be 36 kDa by both SDS/PAGE and by non-denaturing PAGE, indicating that the enzyme is a monomer.

N-terminal and internal amino acid sequences and cDNA cloning

The purified enzyme (P1) was subjected to N-terminal sequencing and the sequence was found to be XVRAKS(L)TLKK(G)FVGY. The purified enzyme was also subjected to tryptic digestion followed by HPLC analysis of tryptic peptides. Two internal peptides (P2 and P3) were isolated and sequenced. The sequences of these two peptides were: P2, ASPEGYDCYFDNNGGE-FSNAVTSQMK; P3, XAAFPTGTIVVALLGXTX(G/H)(S)-(I)(S/G)(D/L). The parenthesis indicate those amino acids that were not identified unambiguously in the sequence analysis. A sense primer corresponding to part of the P1 sequence (KKGFVG) and anti-sense primer corresponding to part of the P2 sequence (YDCYFDN) were synthesized and used in a PCR with a pig lung cDNA library as template. A 600 bp product was obtained and sequenced. The deduced amino acid sequence of the PCR product contained the sequence of P3 located near the middle of the product, indicating the PCR product is part of the PGR cDNA sequence. With this 600 bp DNA fragment as a probe, we screened the pig lung cDNA library by plaque hybridization. A positive clone having the complete PGR coding sequence was found. The nucleotide sequence of this cDNA and the deduced amino acid sequence are shown in Figure 2. The peptide sequences corresponding to P1, P2 and P3 are underlined. The open reading frame has 987 bp and codes for a protein of 329 amino acid residues with a calculated molecular mass of 35791 Da. A computer search for homologous sequences revealed that the sequence of PGR is virtually identical with that of LTB_4 12-hydroxydehydrogenase [14] except for two nucleotide differences at nt 387, where C replaces G, and nt 394, where G replaces A in the LTB_4 12-hydroxydehydrogenase sequence (underlined in Figure 2). The change at nt 394 results in an amino

Table 2 Apparent K_m and V_{max} values for pig lung PGR

The amount of enzyme used for kinetic determinations was 8.5 μg for either native or recombinant enzyme. Values for 15-oxo-PGE_1 and 15-oxo-PGE_2 were determined with NADH; values for NADH and NADPH were determined with 15-oxo-PGE_1; values for LTB_4 were determined with $NADP^+$.

	K_m (μM)		V_{max} (m-units/mg)		k_{cat} (min^{-1})	
Substrate	Native	Recombinant	Native	Recombinant	Native	Recombinant
15-oxo-PGE_1	7.7	8.3	2470	2270	91.0	84.1
15-oxo-PGE_2	19.0	18.2	847	835	31.0	30.8
NADH	153.0	160.0	2352	2247	87.0	83.1
NADPH	15.0	16.0	729	823	27.0	30.4
LTB_4	–	10.0	–	7	–	0.3

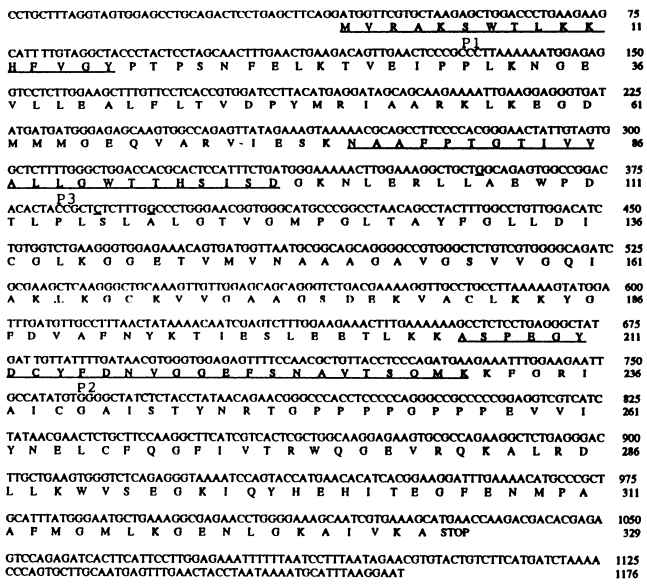

Figure 2 cDNA and deduced amino acid sequences of pig lung PGR

The underlined letters indicate peptide sequences from the purified pig lung PGR.

acid difference (Thr-118 in PGR, compared with Ala-118 in LTB_4 12-hydroxydehydrogenase) between the two enzymes.

Expression of cDNA and characterization of recombinant enzyme

The PGR cDNA was subcloned into pGBT-T19 and expressed in *E. coli* as described in the Experimental section. The crude extract was found to contain PGR activity. The enzyme was purified from the crude extract by a two-step procedure as indicated in Table 3. The enzyme was purified to apparent homogeneity as shown in Figure 3. The specific activity of the purified recombinant enzyme was found to be comparable to that of the native enzyme, as shown in Tables 1 and 2. The kinetic parameters of the recombinant enzyme are also comparable to those determined for the native enzyme, as shown in Table 2. Because the sequence of PGR is virtually identical with that of LTB_4 12-hydroxydehydrogenase, the ability of the recombinant enzyme to use LTB_4 as a substrate was investigated. The recombinant enzyme was able to oxidize LTB_4 in the presence of $NADP^+$: the K_m and V_{max} values determined with LTB_4 were found to be 10 μM and 7.0 nmol/min per mg respectively (Table 2), which are comparable to the values reported for the LTB_4 12-hydroxydehydrogenase [11]. It is interesting to note that the the specific activities of PGR with 15-oxo-PGE_1 and 15-oxo-PGE_2 as substrates are respectively 300-fold and 100-fold that of LTB_4 12-hydroxydehydrogenase.

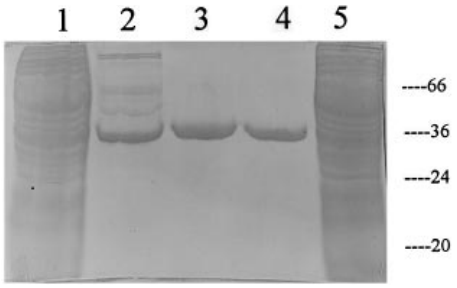

Figure 3 SDS/PAGE of samples from steps in purification of recombinant PGR from *E. coli*

Lane 1, pGBT-PGR JM109 lysate, 740 μg; lane 2, Cibacron Blue column, 30 μg; lane 3, Mono Q column, 10 μg; lane 4, native PGR from pig lung, 10 μg; lane 5, JM109 lysate, 100 μg. Protein was stained with Coomassie Brilliant Blue R. The positions of molecular mass markers (in kDa) are shown at the right.

Antibody production and Western blot analysis

Polyclonal antibody specific for PGR was produced by immunization of a rabbit with the purified native enzyme. Western blot analysis of crude extracts and of Cibacron Blue and Mono Q preparations from *E. coli* expressing PGR as well as the purified native enzyme detected a protein of the correct size (Figure 4). The availability of a specific antibody against the pig lung PGR should be extremely useful for immunocytochemical and other studies of this enzyme.

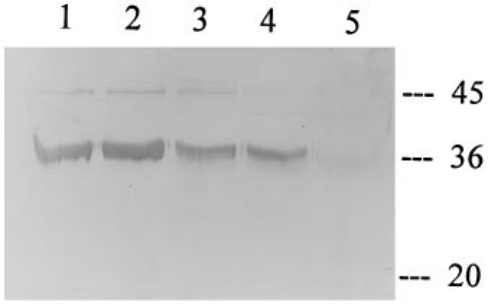

Figure 4 Western blot analysis with PGR antibody

Lane 1, pGBT-PGR JM109 lysate, 37 μg; lane 2, Cibacron Blue column from pGBT-PGR JM109 lysate, 2 μg; lane 3, Mono Q column from pGBT-PGR JM109 lysate, 1 μg; lane 4, native PGR from pig lung, 1 μg; lane 5, JM109 lysate, 100 μg. The positions of molecular mass markers (in kDa) are shown at the right.

Table 3 Purification of recombinant PGR from *E. coli*

Step	Total protein (mg)	Specific activity (m-units/mg)	Total activity (m-units)	Purification (fold)	Recovery (%)
Lysate	360.0	21	7560	1	100
Cibacron Blue	12.0	511	6132	24	81
FPLC	1.4	2600	3681	124	49

DISCUSSION

It is clear that the removal and catabolism of prostaglandins by the lung have important implications for both pulmonary and systemic functions. The lung contains large amounts of both 15-PGDH and PGR, which is consistent with the finding that 13,14-dihydro-15-oxo-PG metabolites are the most abundant products released from perfused lungs [2]. Of the two major prostaglandin catabolic enzymes, 15-PGDH and PGR, 15-PGDH has been the most extensively studied. Prostaglandins can have a wide range of physiological effects on the lung: they can influence bronchoconstriction, lung compliance, transpulmonary pressure and total lung resistance. They can also relax or contract airway smooth muscle and stimulate the secretion of mucus and Cl^- ions [15,16]. The 15-oxoprostaglandin metabolites, such as 15-oxo-PGE_2 and 15-oxo-$PGF_{2\alpha}$, produce biological responses in airways similar to their respective 15-hydroxyprostaglandin compounds [17–19]. However, 13,14-dihydro-15-oxoprostaglandins have less than 10% of the activity of the primary prostaglandins. This demonstrates that the reduction of the 13,14 double bond by PGR can be important in regulating prostaglandin activity.

PGR has been previously purified from chicken heart [4], bovine lung [5], human placenta [6,7] and bovine iris ciliary body [8]. A comparison of these tissues shows that bovine lung is the richest source of the enzyme. The enzymes from all of these sources are specific for 15-oxo-prostaglandins but there are clearly some differences in their characteristics. First, there are differences in their cofactor requirements: the chicken heart PGR and bovine ciliary body PGRs use NADPH as a cofactor much more efficiently than NADH, whereas the bovine lung enzyme uses NADH more efficiently than NADPH; the placental enzyme is specific for NADH. There are also differences in the reported sizes of these enzymes. When estimated by SDS/PAGE the placental and bovine lung enzymes have a molecular mass of 39.5 kDa. When using gel filitration, the size of the placental enzyme was estimated to be 68.5 kDa, suggesting that it is a dimer [6,7]. However, estimation of the size of the bovine lung enzyme by gel filtration gave a molecular mass of 56 kDa [5]. It was suggested that the enzyme was not a dimer; rather, the overestimation of size of the bovine lung and placental enzymes by gel filtration was due to the molecule's being non-spherical. The molecular mass of the chicken heart enzyme, as estimated by gel filtration, was 70–80 kDa. The size of this enzyme was not determined by SDS/PAGE. The bovine ciliary body PGR was found to have a molecular mass of 55–57 kDa by both gel filtration and SDS/PAGE.

The size of the pig lung PGR was determined as 36 kDa by both SDS/PAGE and non-denaturing PAGE, suggesting that it is a monomer. The properties of the pig lung enzyme most closely resemble those of the bovine lung PGR [5]. These two enzymes have the same cofactor dependence, with lower K_m values for NADPH (15 μM for the pig PGR and 10 μM for bovine lung PGR) than for NADH (153 μM for pig PGR and 88–94 μM for bovine PGR). However, the velocity of the reaction with NADH is approx. 3-fold that with NADPH for both enzymes. Also, the k_{cat} is very low for both enzymes. The two enzymes are similar in size.

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The fact that the bovine lung and bovine ciliary body enzymes have different cofactor requirements and seem to be of different sizes, yet are from the same species, suggests that there is more than one form of the enzyme. This suggestion was recently supported by the isolation of four different forms of PGR from rat liver [20]: molecular sizes of 40 (I), 25 (II), 64 (III) and 70 (IV) kDa were recognized. Form I seemed to be related to the PGR reported here, on the basis of comparable molecular size, specific activity and substrate specificity. An interrelationship of different forms of PGR could be revealed by a determination of the primary structure of each form. The availability of the cDNA reported here should help with cloning the cDNA of other forms if they are structurally related.

It is particularly interesting to note that the structure of PGR is virtually identical with that of LTB_4 12-hydroxydehydrogenase. The expressed recombinant enzyme was also shown to have LTB_4 12-hydroxydehydrogenase activity, with kinetic constants comparable to the reported values [11], indicating that the enzyme has dual enzyme activities. However, comparison of the k_{cat} values for 15-oxo-prostaglandins and LTB_4 indicates that the enzyme has a much higher catalytic efficiency for the reduction of the 13,14 double bond of 15-oxo-prostaglandins than for the oxidation of the 12-hydroxy group of LTB_4.

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