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Structural similarity of bovine lung prostaglandin F synthase to lens ε-crystallin of the European common frog

(recombinant DNA/DNA sequence/liver aldehyde reductase/lactate dehydrogenase)

KIKUKO WATANABE*, YUTAKA FUJII*, KAZUHISA NAKAYAMA†, HIROAKI OHKUBO†, SEIKI KURAMITSU‡, HIROYUKI KAGAMIYAMA‡, SHIGETADA NAKANISHI†, AND OSAMU HAYAISHI*§

*Hayaishi Bioinformation Transfer Project, Kyoto Laboratory, Research Development Corporation of Japan, Nishioji-Hachijo, Minami-ku, Kyoto 601, Japan; †Institute for Immunology, Kyoto University Faculty of Medicine, Kyoto 606, Japan; and ‡Department of Medical Chemistry, Osaka Medical College, Takatsuki 569, Japan

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ABSTRACT **Cloned cDNA sequences specific for prostaglandin F (PGF) synthase have been isolated from a cDNA library of bovine lung mRNA sequences. Nucleotide-sequence analyses of cloned cDNA inserts have revealed that PGF synthase consists of a 969-base pair open reading frame coding for a 323-amino acid polypeptide with a M_r of 36,666. The sequence analysis indicates that bovine lung PGF synthase shows 62% identical plus conservative substitutions compared with human liver aldehyde reductase [Wermuth, B., Omar, A., Forster, A., Francesco, C., Wolf, M., Wartburg, J. P., Bullock, B. & Gabbay, K. H. (1987) in *Enzymology and Molecular Biology of Carbonyl Metabolism: Aldehyde Dehydrogenase, Aldo-Keto Reductase, and Alcohol Dehydrogenase*, eds. Weiner, H. & Flynn, T. G. (Liss, New York), pp. 297–307], which is similar to PGF synthase in molecular weight and substrate specificity. However, comparison of the amino acid sequence of PGF synthase with the National Biomedical Research Foundation protein data base reveals that the sequences of 225 amino acids from C termini of ε-crystallin of the European common frog (*Rana temporaria*) [Tomarev, S. I., Zinovieva, R. D., Dolgilevich, S. M., Luchin, S. V., Krayev, A. S., Skryabin, K. G. & Gause, G. G. (1984) *FEBS Lett.* 171, 297–302] and of PGF synthase show 77% identical and conservative substitutions without deletions/additions. The result suggests that European common frog lens ε-crystallin is identical to bovine lung PGF synthase.**

In 1981 we found an enzyme that catalyzed the reduction of prostaglandin D_2 (PGD_2) to prostaglandin F_2 (PGF_2) in rat lung (1), and we purified the enzyme prostaglandin F (PGF) synthase from bovine lung to apparent homogeneity (2). The purified enzyme was a monomeric protein with a M_r of 30,500; the enzyme showed a broad substrate specificity and reduced not only PGD_2, but also PGH_2 and other carbonyl compounds. The enzyme catalyzed the reduction of PGH_2 to $PGF_{2\alpha}$ and that of PGD_2 to (5*Z*,13*E*)-(15*S*)-9α,11β,15-trihydroxyprosta-5,13-dien-1-oic acid (9α,11β-PGF_2), which is a stereoisomer of $PGF_{2\alpha}$ (3), at different active sites on the same molecule (2, 4, 5). As an initial step to study the primary structure and molecular mechanism of the enzyme, we isolated a cDNA sequence encoding PGF synthase.

We now report the cDNA sequence encoding the entire bovine lung PGF synthase¶ and the deduced 323-amino acid sequence. The amino acid sequences of PGF synthase and human liver aldehyde reductase (alcohol: $NADP^+$ oxidoreductase, EC 1.1.1.2) (6) show 39% identical and 23% conservative substitutions. However, by comparison of the amino acid sequence of PGF synthase with the National Biomedical Research Foundation protein data base, sequences of PGF synthase and European common frog (*Rana temporaria*) lens ε-crystallin show 58% identical and 19% conservative substitutions.

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MATERIALS AND METHODS

Peptide Purification and Sequencing. S-carboxymethylation of PGF synthase was done as described (7). A portion of the carboxymethylated preparation was further citraconylated by the method of Habeeb and Atassi (8). Tryptic digestion of the chemically modified preparations of PGF synthase was done in 50 mM Tris·HCl, pH 8.5, with 5% (wt/wt) trypsin at 37°C for 24 hr. Peptides were purified by HPLC on a Cosmosil 5-μm C_{18} column (0.46 × 15 cm, Nakarai Chemical, Kyoto, Japan). Chromatography was done using a linear gradient of 0–60% acetonitrile in 0.1% trifluoroacetic acid at 40°C with a flow rate of 1 ml/min for 8 hr. Absorbance was recorded at 210 nm. The obtained peptides were further characterized by amino acid analysis (9) and by automated Edman degradation with an Applied Biosystems (Foster City, CA) gas-phase sequencer.

Cloning Procedures. Total RNA was extracted from a bovine lung as described (10), and poly(A)-containing RNA was isolated by subjecting the total RNA extracted to oligo(dT)-cellulose chromatography (11). A cDNA library was constructed by the method of Watson and Jackson (12). *Escherichia coli* MC 1061 or HB 101 was used for transformation as described (13), and ampicillin-resistant transformants were screened at 42°C with a mixture of 16 synthetic oligonucleotides I (Fig. 1) and at 38°C with a mixture of 16 synthetic oligonucleotides II (Fig. 1), described in *Results*. Further details of the cloning procedures have been described (14). All cloning procedures were conducted in accordance with the guidelines for research involving recombinant DNA molecules issued by the Ministry of Education, Science and Culture of Japan.

Analytical Procedures. Restriction endonucleases were obtained from Takara Shuzo (Kyoto, Japan), New England Biolabs, and Bethesda Research Laboratories and were used under the conditions described by the suppliers. 5′-End-labeling of a mixture of oligonucleotides and restriction fragments was done as described (15). DNA fragment was labeled by the method of Feinberg and Vogelstein with the use of [α-^{32}P]dCTP (16). DNA sequence analysis was done by

Abbreviations: PG, prostaglandin; 9α,11β-PGF_2 (11-epi-$PGF_{2\alpha}$), (5*Z*,13*E*)-(15*S*)-9α,11β,15-trihydroxyprosta-5,13-dien-1-oic acid; PGF synthase, prostaglandin F synthase; nt, nucleotide.

§To whom reprint requests should be addressed at: Osaka Medical College, 2-7 Daigakumachi, Takatsuki 569, Japan.

¶The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03570).

Amino acid sequence I: Lys Glu Asn Met Gln Val

mRNA: 5'-AA(A/G) GA(A/G) AA(U/C) AUG CA(A/G) GU(U/C/A/G)-3'

Oligonucleotide I: 3'-TT(T/C) CT(T/C) TT(A/G) TAC GT(T/C) CA -5'

Amino acid sequence II: Pro Glu Asp Met Lys

mRNA: 5'-CC(U/C/A/G) GA(A/G) GA(U/C) AUG AA(A/G)-3'

Oligonucleotide II: 3'-GG(A/G/T/C) CT(T/C) CT(A/G) TAC TT -5'

FIG. 1. Two synthetic oligonucleotides. As hybridization probes, two mixtures of oligonucleotides were synthesized according to the cDNA sequences predicted from hexapeptide I and pentapeptide II sequences (excluding the third nucleotide residue of the sixth valine codon and that of the fifth lysine codon, respectively) present in PGF synthase.

the procedure of Maxam and Gilbert (17) and the method of Messing (18), according to the strategy indicated in Fig. 2. RNA blot hybridization analysis was done according to the procedure of Alwine *et al.* (19); poly(A)-containing RNA isolated from bovine lung was denatured with 1 M glyoxal/50% dimethyl sulfoxide (20), electrophoresed on a 1.5% agarose gel, and transferred to a nylon membrane. The hybridization probe was labeled by the method described above.

Sequence Comparison. The amino acid sequence deduced from the cDNA sequence was subjected to the National Biomedical Research Foundation protein data base.[‖] For sequence comparison, conservative substitutions were defined as pairs of residues belonging to one of the following groups: serine, threonine, proline, alanine, and glycine; asparagine, aspartic acid, glutamic acid, and glutamine; histidine, arginine, and lysine; methionine, isoleucine, leucine, and valine; phenylalanine, tyrosine, and tryptophan (21). The degree of sequence identity described in the text was determined by counting a continuous stretch of gaps as one substitution regardless of its length.

[‖]Protein Identification Resource (1987) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 12.

RESULTS

Partial Amino Acid Sequence. To design an oligonucleotide suitable for screening a PGF synthase-specific cDNA library, tryptic peptides were prepared from carboxymethylated PGF synthase. Purified fractions isolated by HPLC were subjected to sequence analysis. From the tryptic octadecapeptide Ile-Lys-Glu-Asn-Met-Gln-Val-Phe-Asp-Phe-Glu-Leu-Thr-Pro-Glu-Asp-Met-Lys, 17-mer oligonucleotides (Fig. 1 I) coding for amino acid residues 2–7 and 14-mer oligonucleotides (Fig. 1 II) coding for amino acid residues 14–18 in the peptide were deduced.

We also analyzed the amino acid sequence of the N-terminal portion of the PGF synthase. However, several cycles of automated Edman degradation released no amino acid at any step, indicating that the N terminus was blocked. Among the purified tryptic peptides, a tripeptide with an amino acid composition of aspartic acid, proline, and lysine was resistant to Edman degradation. Therefore, this peptide must be from the N-terminal portion of the protein. Lysine was likely to be the C-terminal residue of the peptide as judged by trypsin specificity. To determine the sequence of aspartic acid and proline, we treated the peptide with 70% formic acid at room temperature for 2 days because the Asp-Pro bond is known to be cleaved by the acid treatment, and the Pro-Asp bond is not cleaved (22). Two steps of Edman degradation of acid-treated peptide yielded proline in step 1 followed by lysine in step 2. These results indicate the N-terminal sequence of Asp-Pro-Lys, in which the N-terminal aspartic acid is masked.

Isolation of cDNA Clones. Our approach for isolating the cloned cDNA sequences specific for PGF synthase was to screen a library of cDNA clones by hybridization with two mixtures of oligonucleotides complementary to all possible coding sequences for two portions of the amino acid sequence of the protein, as shown in Fig. 1. One hybridization-positive clone, which hybridized with both oligonucleotide probes, was isolated from about 40,000 transformants. This clone, pPF13, was subjected to nucleotide-sequence analysis and found to contain cDNA sequence corresponding to four tryptic peptides (amino acid residues 217–236, 259–263, 277–294, and 311–323) (Fig. 3). Because clone pPF13 did not encode the N-terminal amino acid sequence of Asp-Pro-Lys of the enzyme determined above, we constructed the cDNA library using the mixture of oligonucleotides I (Fig. 1 I, and Fig. 2) described above as a primer. As hybridization probe, we used the complete cDNA insert of pPF13. Five hybridization-positive clones were isolated from about 80,000

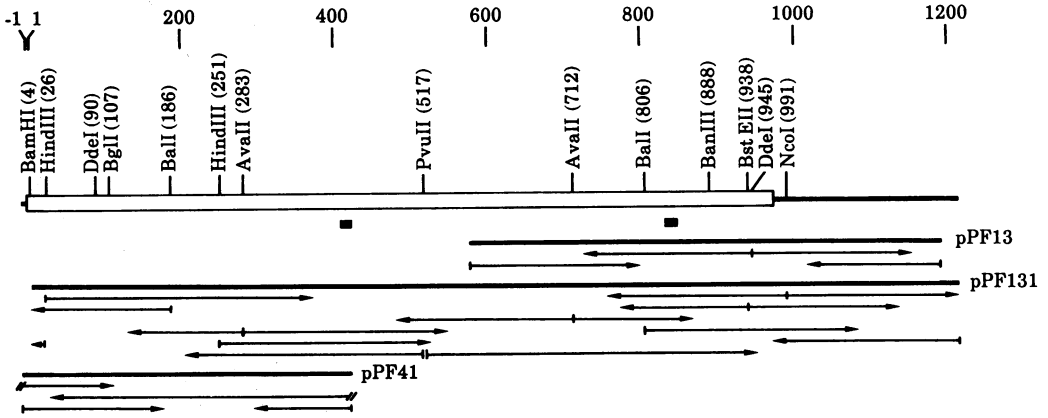

FIG. 2. Sequence strategy for cDNA. The map displays only the relevant restriction endonuclease sites, which are identified by numbers indicating the 5'-terminal nucleotide generated by cleavage (for nucleotide numbers, see Fig. 3). Sequence corresponding to the coding region is indicated by the open box. Sequences of synthetic oligonucleotides used for specific primer of reverse transcription are indicated by small solid boxes directly beneath the restriction map. Direction and extent of the sequence determinations are shown by horizontal arrows.

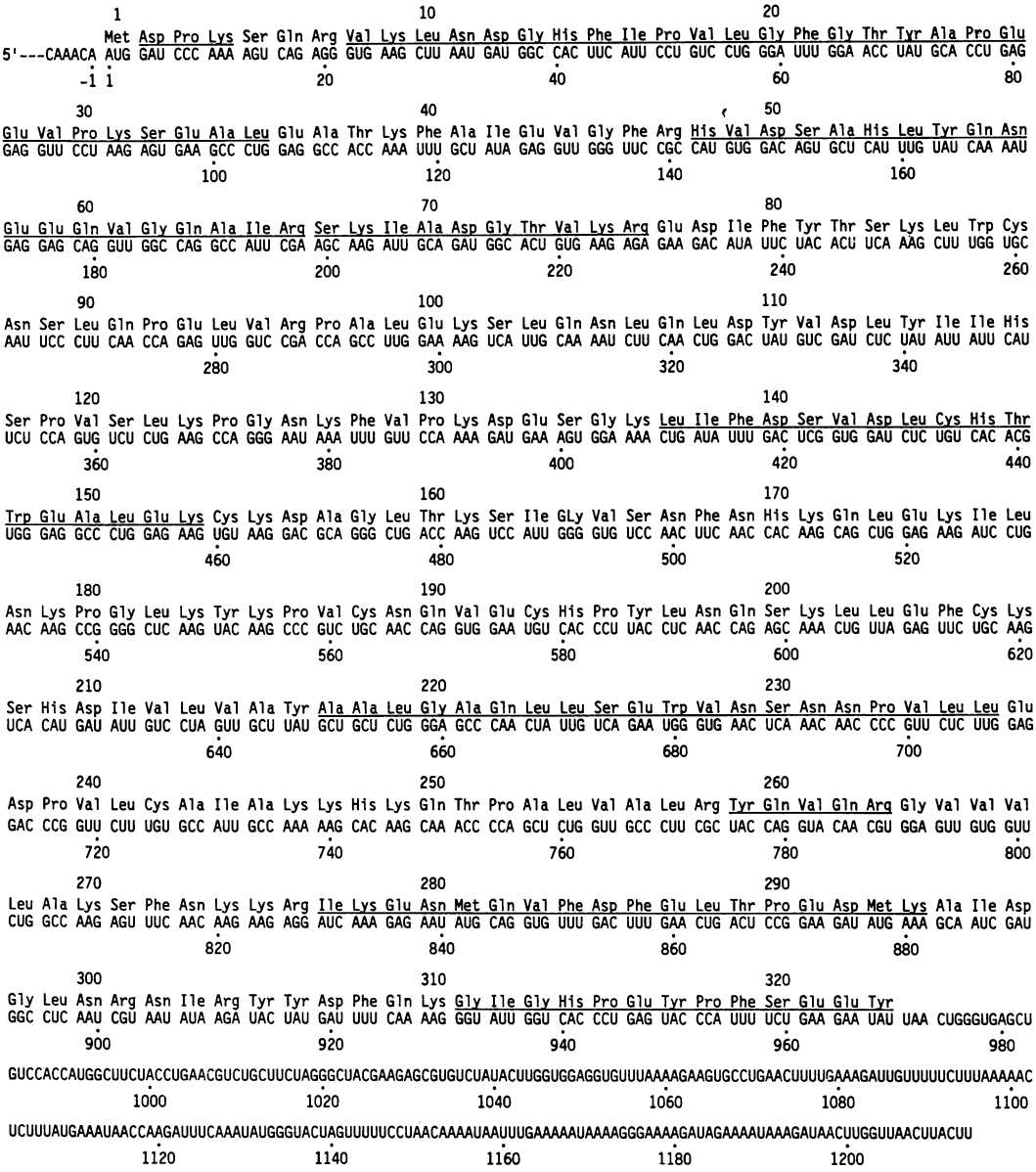

FIG. 3. Primary structure of bovine lung PGF synthase mRNA. The nucleotide sequence of mRNA was deduced from that of the cDNA inserts in clones pPF13, pPF131, and pPF41. Nucleotide residues are numbered in the 5′ to 3′ direction beginning with the first residue of the AUG triplet that codes for the initiation methionine. Nucleotides on the 5′ side of residue 1 are indicated by negative numbers. The predicted amino acid sequence of PGF synthase is displayed above the nucleotide sequence. Underlined amino acids completely matched the amino acids of PGF synthase identified by peptide analysis of the chemically modified fractions of the enzyme as described.

transformants. Clone pPF131, which carried the largest cDNA insert of the five, was chosen for further analysis. Sequence analysis showed that pPF131 contained a poly(A) tail at the 3′-end of its insert. This result can be explained by assuming that the thymine-rich sequence of oligonucleotides I served as a primer for reverse transcriptase reactions from adenine-rich sequences in the mRNA, such as poly(A) tail. Because this clone did not contain the initiation codon ATG, we constructed the cDNA library using a synthetic oligonucleotide complementary to nucleotide residues 410–424 as a primer (Fig. 2). By using the *Bgl* I (residue 107)–*Pvu* II (residue 517) fragment from pPF131 as a hybridization probe, 84 positive clones were isolated from 50,000 transformants. Twelve of them were analyzed with several restriction endonucleases, either individually or in pairs. Clone pPF41, which carried the largest cDNA insert among them, was subjected to nucleotide-sequence analysis and was shown to contain the initiation codon ATG. The initiation methionine codon was assigned from the amino acid sequence (Asp-Pro-Lys) of the tryptic peptide, of which the amino acid terminus was blocked.

Nucleotide Sequence Analysis. Sequences of the above three representative cDNA clones were determined to verify absolute fidelity of the cDNA sequence as a copy of the mRNA. Fig. 2 shows the restriction map and sequence-analysis strategy. The complete nucleotide sequence is shown in Fig. 3; the entire sequence was obtained from both the message and complementary strand, and agreement between nucleotide sequences obtained from overlapping areas was complete. The insert contained 1220 nucleotides (nt)—the length of the poly(A) tail was not included in this determination. The cDNA inserts contained 6 nt in the 5′ noncoding region, 969 nt in the coding region that were followed by the termination codon UAA, and 245 nt in the 3′ untranslated region preceding the poly(A) tail. The hexanucleotide 5′ AAUAAA 3′, which could function as a signal for poly(A) addition or RNA processing (23), was found in

position 22 and 43 nt upstream from the poly(A) tail in the 3′ untranslated region.

Predicted Amino Acid Sequence of PGF Synthase. The amino acid sequence deduced from nucleotide-sequence analyses is shown in Fig. 3. PGF synthase contains 323 amino acids, and the calculated M_r is 36,666. The M_r of the mature protein is 36,517 excluding the initiation methionine. As shown in Fig. 3, nine tryptic peptides (amino acid residues, 2–4, 8–35, 48–66, 67–76, 137–153, 217–236, 259–263, 277–294, and 311–323) completely matched those of PGF synthase identified by peptide analysis of the purified enzyme. Furthermore, the amino acid composition of the purified enzyme nearly matched with that deduced from cDNA sequence (data not shown). *E. coli* harboring the complete sequence constructed from pPF41 and pPF131 showed PGF synthase enzyme activity (data not shown). All these results support the authenticity of the amino acid sequence deduced from the cloned cDNA sequence.

Similarity of PGF Synthase with Human Liver Aldehyde Reductase and European Common Frog Lens ε-Crystallin. PGF synthase shows a broad substrate specificity and catalyzes the reduction of carbonyl compounds as well as of prostaglandins (2). On the other hand, human liver aldehyde reductase (24) catalyzes the reduction of aldehyde compounds—e.g., 4-nitrobenzaldehyde and 4-carboxybenzaldehyde, which were substrates for PGF synthase. Furthermore, the M_r of the aldehyde reductase was ≈36,000 and similar to that of PGF synthase. Wermuth *et al.* (6) recently reported the amino acid sequence of human liver aldehyde reductase. The amino acid sequence of PGF synthase showed 39% identical and 23% conservative substitutions with deletions/additions to that of human liver aldehyde reductase (Fig. 4). The amino acid sequence of European common frog lens ε-crystallin has been determined concerning 225 amino acids from the C terminus (25). Its sequence shows 58% identity with that from the C terminus of PGF synthase without deletions/additions, and the similarity between the two sequences increases to 77% when amino acids are compared on the basis of conservative substitutions (Fig. 4). Fig. 4 shows that part of the amino acid sequence (residues 176–199) of PGF synthase was identical to that of ε-crystallin except for one amino acid.

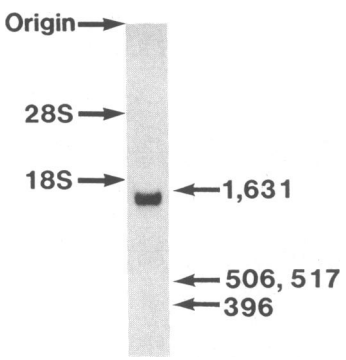

FIG. 5. Blot hybridization analysis of PGF synthase. A band was obtained with 10 μg of poly(A)$^+$ RNA from bovine lung; the probe used was *Dde* I (residue 90)–*Ban* III (residue 888) fragment. Size markers at left and right are bovine ribosomal RNA (indicated by S value), and the *Hin*fI cleavage products of pBR322 DNA (indicated in nt), respectively.

Identification and Size Determination of PGF Synthase mRNA by Blot Hybridization Analysis. Fig. 5 shows the result of blot hybridization analysis of bovine lung mRNA using the *Dde* I (residue 90)–*Ban* III (residue 888) fragment as a probe. From its migration in a denaturing gel system, the sequence of PGF synthase mRNA from bovine lung is estimated to be 1400 nt. Therefore, assuming a length for the poly(A) tail of ≈100–150 nt, the insert cDNA sequence of 1220 nt extends nearly the full length of the mRNA.

DISCUSSION

We isolated a cDNA sequence encoding PGF synthase of bovine lung and determined its primary structure. The calculated M_r of PGF synthase deduced from the cloned cDNA sequence was 36,517, excluding the initiation methionine. However, this value is 16% larger than the M_r of 30,500 for the purified enzyme reported earlier (2). *E. coli* containing

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                10        20        30        40        50        60        70        80        90       100
AR      AASCVLLHTGQKMPLIGLGTWKSEPGQVKAAV---KYALSVGYRHIDCAAIYGNEPEIGEALKEDVGPGKAVPREELFVTSKLWNTKHHPEDVEPALR
          :  | |  |  :|::| ||: :|      |:   |:|: ||:||:| | :| || ::|:|::  :: | :| ||::| |||||     || | |||
PS      MDPKSQRVKLNDGHFIPVLGFGTYAPEEVPKSEALEATKFAIEVGFRHVDSAHLYQNEEQVGQAIRSKIADG-TVKREDIFYTSKLWCNSLQPELVRPALE
                                                                                                          ||
EC                                                                                                        LE


                110       120       130       140       150       160       170       180       190       200
AR      KTLADLQLEYLDLYLMHWPYAFERGDNPFPKNADGTICYDSTHYKETWKALEALVAKGLVQALGLSNFNSRQIDDILSVASV--RPAVLQVECHPYLAQN
        |:| :|||:|:|||::| | :   |:   ||:  | : :||     || |||     ||  ::|:|||| :|:: ||  :::  :|   |||||||| |
PS      KSLQNLQLDYVDLYIIHSPVSLKPGNKFVPKDESGKLIFDSVDLCHTWEALEKCKDAGLTKSIGVSNFNHKQLEKILNKPGLKYKPVCNQVECHPYLNQS
        :|| :: :||:||:::| ||||||:    | |     |:| |||| ||||||  ||||| :|:|||||||::|||:||||||||||||||||||| ||||
EC      RSLRDVGMDYLDLFLMHWPVSLKPSGASDPSDKDKPFIYDNVDLCATWEALEARKDAGLVRSLGVSNFNRRQLERILNKPGLKYKPVCNQVECHVYLNQN


                210       220       230       240       250       260       270       280       290       300
AR      ELIAHCQARGLEVTAY-PLGSS-DRAWRDPDEPVLLEEPVVLALAEKYGRSPAQILLRWQVQRKVICIPKSITPSRILQNIKVFDFTFSPEEMKQLNALN
          |:  | :: : : || :||:     | ::::|||||:||: |:| |   :|| : ||:|||| |: ::||    || :|: ||||  :||:|| :::||
PS      KLLEFCKSHDIVLVAYAALGAQLLSEWVNSNNPVLLEDPVLCAIAKKHKQTPALVALRYQVQRGVVVLAKSFNKKRIKENMQVFDFELTPEDMKAIDGLN
        ||  :|||:|||||:|: ||:   :||:   ||||:||:| :| |   |:| :|:|: :|:|:|||||||   |||:|: ||:||| |||||:::|:
EC      KLHSYCKSKDIVLVTYSVLGSHRDRNWVDLSLPVLLDDPILNKVAAKYNRTSAEIAMRFILQKGIVVLAKSFTPARIKQNLGVFEFELKPEDMKSLESLD


                310       320
AR      KNWRYIVPMLTVDGKRVPRDAGHPLYPFNDPY
        :| ||    ::     |
PS      RNIRYYDFQKGIGHPEYPFSEEY
        ||::|  |    |||||| :||
EC      RNLHYGPFREVKQHPEYPFHDEY
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FIG. 4. Comparison of PGF synthase (PS) with human liver aldehyde reductase (AR) and European common frog lens ε-crystallin (EC). Amino acid sequences are described with the standard single-letter notation for amino acid residues. Amino acid residues are numbered according to PGF synthase. Bars and colons between the sequences indicate exact matches and conservative substitutions, respectively.

the complete sequence of PGF synthase expressed PGF synthase enzyme activity, and the expressed protein in *E. coli* comigrated with the purified enzyme on $NaDodSO_4$/polyacrylamide gel (data not shown). Furthermore, the purified enzyme contained both N- and C-terminal regions deduced from cDNA sequence (Fig. 3). These results suggest that the correct M_r of bovine lung PGF synthase is 36,517.

PGF synthase is similar to aldehyde reductase in molecular weight and substrate specificity. The similarity in the amino acid sequence between PGF synthase and human liver aldehyde reductase (6) was 62% (Fig. 4). However, deletions/additions were seen between the two sequences, suggesting that PGF synthase is similar, but not exactly identical, to human liver aldehyde reductase. PGF synthase and aldehyde reductase belong to a group of aldo-keto reductases in terms of substrate specificity. Possibly the amino acid sequences for the active sites of these enzymes show similarity, and a group of aldo-keto reductases may form a gene family.

Crystallins, the principal component of the lens, have been regarded simply as soluble, structural proteins. Recently, Wistow and Piatigorsky reported that the major taxon-specific crystallins of vertebrates and invertebrates are either enzymes, or closely related to enzymes (26). ε-Crystallin of avian and crocodilian lenses is identical to lactate dehydrogenase (27), and δ-crystallin is closely related to arginosuccinate lyase, τ-crystallin is related to enolase, and S_{III}-crystallin is related to glutathione *S*-transferase (26). Comparison of the amino acid sequence of PGF synthase with the National Biomedical Research Foundation protein data base[||] revealed no significant similarity with sequences of chicken lactate dehydrogenase A (28), chicken and pig lactate dehydrogenase B (29), yeast arginosuccinate lyase (30), yeast enolase (31), or rat glutathione *S*-transferase (32) but revealed 77% similarity with that of European common frog lens ε-crystallin (Fig. 4) (25). These results suggest that PGF synthase is not related to these former enzymes and that ε-crystallin of European common frog lens is different from ε-crystallin of avian and crocodilian lenses, which is identical to lactate dehydrogenase (27).

The ε-crystallin of European common frog is one structural protein of lens (25). The sequence of ε-crystallin is 889 nt without its poly(A) tail and contains an open reading frame of 675 nt; this length accounts for about three-fourths of the total mRNA length from the 3′-nucleotide terminus (25). The amino acid sequences of PGF synthase and European common frog ε-crystallin are highly similar in 225 amino acids from the C terminus without deletions/additions. Considering that the M_r of European common frog ε-crystallin is ≈35,000, the N-terminal region of the amino acid sequence of ε-crystallin may also be similar to PGF synthase, and ε-crystallin of European common frog lens may be identical to PGF synthase. These results raise the interesting possibilities that ε-crystallin of European common frog has PGF synthase activity, besides being the structural protein of lens, and that prostaglandins play some biological role(s) in the lens.

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