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JB Commentary

# Recent reports about enzymes related to the synthesis of prostaglandin (PG) $F_2$ ($PGF_{2\alpha}$ and 9α, 11β-$PGF_2$)

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**Kikuko Watanabe***

Department of Nutrition, Koshien University, 10-1 Momijigaoka, Takarazuka, Hyogo 665-0006, Japan

*Kikuko Watanabe, Department of Nutrition, Koshien University, 10-1 Momijigaoka, Takarazuka, Hyogo 665-0006, Japan.
Tel: +81-797-87-8370, Fax: +81-797-87-8370,
E-mail: kwatana@koshien.ac.jp

**Prostaglandin (PG) $F_{2\alpha}$ is widely distributed in various organs and exhibits various biological functions, such as luteolysis, parturition, aqueous humor homeostasis, vasoconstriction, rennin secretion, pulmonary fibrosis and so on. The first enzyme reported to synthesize $PGF_2$ was referred to as PGF synthase belonging to the aldo-keto reductase (AKR) 1C family, and later $PGF_{2\alpha}$ synthases were isolated from protozoans and designated as members of the AKR5A family. In 2003, AKR1B5, which is highly expressed in bovine endometrium, was reported to have $PGF_{2\alpha}$ synthase activity, and recently, the paper entitled 'Prostaglandin $F_{2\alpha}$ synthase activities of AKR 1B1, 1B3 and 1B7' was reported by Kabututu *et al.* (*J. Biochem.*145, 161–168, 2009). Clones that had already been registered in a database as aldose reductases (AKR1B1, 1B3, and 1B7) were expressed in *Escherichia coli*, and these enzymes were found to have $PGF_{2\alpha}$ synthase activity. Moreover, in the above-cited article, the effects of inhibitors specific for aldose reductase on the $PGF_{2\alpha}$ synthase activity of AKR1B were discussed. Here, I present an overview of various PGF/$PGF_{2\alpha}$ synthases including those of AKR1B subfamily that have been reported until now.**

*Keywords*: aldo-keto reductase (AKR) family/AKR1B/AKR1C/PGF synthase/prostaglandin (PG) $F_2$ ($PGF_{2\alpha}$, 9α, 11β-$PGF_2$).

*Abbreviations*: AKR, aldo-keto reductase; PG, prostaglandin; Trx, thioredoxin.

Prostaglandin (PG)$F_{2\alpha}$ is one of the primary PGs, together with $PGE_2$. These PGs were found as the compounds in human semen having an effect on the motility of uterine strips isolated from non-pregnant patients (*1*), and their structures were identified by mass spectrometric analysis performed by Berström *et al.* (*2*, *3*) in Sweden. For the synthesis of $PGF_2$, the following three pathways have been proposed (Fig. 1): (i) the 9-keto group of $PGE_2$ is reduced to $PGF_2$, (ii) the 11-keto group of $PGD_2$ is reduced to $PGF_2$ and (iii) the 9, 11-endoperoxide group of $PGH_2$ is reduced to $PGF_2$. In 1981, in the process of studying the metabolism of $PGD_2$, we found $PGD_2$ 11-ketoreductase activity that catalyses the reduction of $PGD_2$ to $PGF_2$, which was later known as 9α, 11β-$PGF_2$, in rat lung (*4*). 9α, 11β-$PGF_2$ is a stereoisomer of $PGF_{2\alpha}$ and has biological function like $PGF_{2\alpha}$ (*5*). This enzyme is a dual-function enzyme and catalyses the reduction of $PGH_2$ to $PGF_{2\alpha}$ as well as that of $PGD_2$ to 9α, 11β-$PGF_2$ on the same molecule in the presence of NADPH (*6*). Therefore, we named it PGF synthase. This enzyme has broad substrate specificity, reducing the keto group of xenobiotic carbonyl compounds such as phenanthrenequinone. In 1988, we assigned PGF synthase to the aldo-keto reductase (AKR) family, based on its amino acid sequence, molecular weight, NADPH as cofactor and substrate specificities (*7*). This was the first report of an enzyme synthesizing $PGF_2$ and belonging to the AKR family. In 1975, Levine *et al.* (*8*) first found a PGE 9-ketoreductase that synthesized $PGF_{2\alpha}$ from $PGE_2$, and later, in 1995, Wintergalen *et al.* (*9*) reported that a PGE 9-ketoreductase belongs to the AKR family and that the enzyme has 20α-hydroxysteroid dehydrogenase activity. This enzyme is identical with carbonyl reductase (*10*).

Most of AKRs are monomeric enzymes that fold into a typical $(\alpha\beta)_8$-barrel structure (*11*). Based on their amino acid sequence, AKRs have been grouped into 15 different families, AKR1–AKR15, having <40% amino acid identity with any other family, while subfamilies may be defined by >60% identity in amino acid sequence among subfamily members (*11*, *12*). According to the website (http://www.med.upenn.edu/akr/) created by Hyndman and Penning, human, bovine lung and bovine liver types of PGF synthase are designated as AKR1C3, AKR1C7 and AKR1C11 respectively, and PGE 9-ketoreductase, as AKR1C5. In 2000, Kubata *et al.* (*13*) purified $PGF_{2\alpha}$ synthase from *Trypanosoma brucei* and referred to it as AKR5A2, and another protozoan $PGF_{2\alpha}$ synthase, known as AKR5A1, was found in *Leishmania* (*14*). These enzymes were purified from native tissues and protozoans by detecting the activity that synthesized $PGF_2$, and their amino acid sequences were determined. Therefore, these enzymes contribute to the synthesis of $PGF_2$ in each organ and protozoans.

In 2003, an enzyme (AKR1B5) that had already been registered in the database of the AKR family was expressed in *Escherichia coli* by Madore *et al.* (*15*), and found to have $PGF_{2\alpha}$ synthase activity. They found it to be highly expressed in bovine endometrium. This was the first report about an enzyme

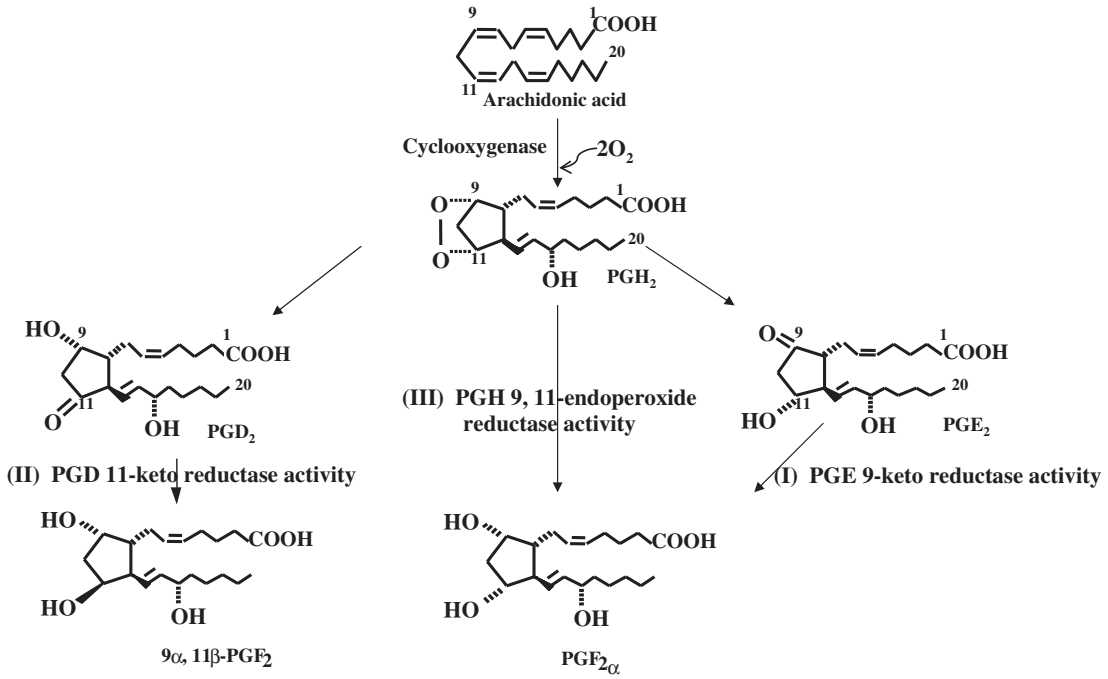


**Fig. 1 Arachidonic acid cascade.** The numbers next to arachidonic acid, $PGH_2$, $PGD_2$ and $PGE_2$ are the positions of carbon atoms.

belonging to the AKR1B subfamily and having $PGF_{2\alpha}$ synthase activity. Moreover, Kabututu *et al.* reported that AKR1B1, 1B3 and 1B7 also have $PGF_{2\alpha}$ synthase activities (*16*). The $K_m$ values for $PGH_2$ of AKR1B1, 1B3 and 1B7 are about 2, 9 and 4 μM, respectively, and $V_{max}$ values are about 26, 53 and 4 nmol/min/mg, respectively, indicating $k_{cat}$ values of ~0.9, 1.9 and 1.6 $min^{-1}$, respectively. Recently, they reported $K_m$ values for $PGH_2$ of AKR1B1 and AKR1B3 to be 29 and 33 μM, respectively, and $V_{max}$ values of 169 and 240 nmol/min/mg, respectively, giving $k_{cat}$ values of ~6.1 and 8.6 $min^{-1}$, respectively (*17*). Kabututu *et al.* (*16*) reported that the $K_m$ value for $PGH_2$ of AKR1C3 is 17.7 μM, and the $V_{max}$ value, 4 nmol/min/mg. However, we obtained different $K_m$ and $V_{max}$ values, i.e. 10 μM and 260 nmol/min/mg, respectively, indicating a $k_{cat}$ value of about 9.6 $min^{-1}$ (*18*). These data are summarized on the left side of Table I.

The reports of Madore *et al.* (*15*) and Kabututu *et al.* (*16*) opened up the field of AKR1B subfamily members involved in the synthesis of $PGF_{2\alpha}$, and recently reports about the $PGF_{2\alpha}$ synthase of this subfamily have been increased (*19*). However, many of these enzymes of the AKR1B subfamily were found to have aldose reductase activity (*12*). Although it is interesting that these enzymes show $PGF_{2\alpha}$ synthase activity, we must never forget the activity toward other substrates. The substrate specificities of the enzymes belonging to the AKR1B subfamily are shown on the right side of Table 1. Ruiz *et al.* (*19*) and Spite *et al.* (*20*) examined the substrate specificities of various enzymes belonging to the AKR family. The $k_{cat}/K_m$ values of AKR1B1 and 1B3 for glyceraldehyde are ~0.5 − 0.6 $min^{-1}.\mu M^{-1}$ and 0.42 − 0.78 $min^{-1}.\mu M^{-1}$, respectively, whereas Gui *et al.* (*21*) reported that mouse aldose reductase (AKR1B3) shows a $k_{cat}/K_m$ value of 1.8 $min^{-1}.\mu M^{-1}$ for this substrate. Moreover, $k_{cat}/K_m$ values for phospholipid aldehyde of AKR1B1 and 1B3 are about 3 $min^{-1}.\mu M^{-1}$ and 1 $min^{-1}.\mu M^{-1}$, respectively (*20*). On the other hand, the $k_{cat}/K_m$ values of AKR1B1 and 1B3 for $PGH_2$ were calculated to 0.49 and 0.20 $min^{-1}.\mu M^{-1}$, respectively, (*16*), and 0.21 and 0.26 $min^{-1}.\mu M^{-1}$, respectively (*17*). The $PGF_{2\alpha}$ synthase activity belonging to the AKR1B subfamily is detected for enzymes overexpressed in *E. coli*, using the clones that have already been registered in a database as other enzymes, but is not isolated from native tissues unlike $PGF/PGF_{2\alpha}$ synthases belonging to the AKR1C subfamily and AKR5A subfamily. We must never forget that the AKR1B subfamily has a broad substrate specificity for naturally occurring compounds.

Ever since we found that bovine lung-type PGF synthases belong to the AKR1C subfamily (AKR1C7), many other enzymes synthesizing $PGF_{2\alpha}$ have been reported. AKR was originally reported to catalyse the reduction of a keto group. $PGH_2$ has an endoperoxide group, not a keto one. Therefore, when we found that PGF synthase belonging to the AKR1C subfamily acts as a dual-function enzyme, based on the reduction of $PGH_2$ to $PGF_{2\alpha}$ as well as that of $PGD_2$ to $9_\alpha$, 11β-$PGF_2$, some people claimed this dualism to be strange. Now, in addition to the AKR1C subfamily, AKR5A and AKR1B subfamilies are also known to catalyze the reduction of the endoperoxide-group of $PGH_2$. Kabututu *et al.* (*16*) reported that tolrestat or sorbinil inhibits the reduction of $PGH_2$ in a noncompetitively or mixed-type manner. Tolrestat and sorbinil are competitive inhibitors for the reduction of glyceraldehyde. These results suggest that the binding site of

**Table I. Substrate specificities of various enzymes belonging to AKR family.**

| Family | Species and tissue | Mr | $K_m$ $PGH_2$ (μM) | $k_{cat}$ $PGH_2$ ($min^{-1}$) | $k_{cat}/K_m$ $PGH_2$ ($min^{-1}.\mu M^{-1}$) | $K_m$ $PGD_2$ (μM) | $kcat$ $PGD_2$ ($min^{-1}$) | $kcat/Km$ $PGD_2$ ($min^{-1}.\mu M^{-1}$) | $K_m$ $PGE_2$ (μM) | $kcat$ $PGE_2$ ($min^{-1}$) | $kcat/Km$ $PGE_2$ ($min^{-1}.\mu M^{-1}$) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AKR family | | | | | | | | | | | | |
| AKR5A2 | Trypanosoma brucei $PGF_{2\alpha}$ synthase | 30,991 | 1.3 | 61.98 | 47.68 | | | | | | | (*13*) |
| AKR1B1 | Human placental aldose reductase | 35,852 | 1.9 | 0.93 | 0.491 | | | | | | | (*16,17*) |
| AKR1B3 | Mouse kidney aldose reductase | 35,730 | 9.3 | 1.89 | 0.204 | | | | | | | (*16,17*) |
| AKR1B5 | Bovine edometrium $PGF_{2\alpha}$ synthase | 35,917 | 7.1 | 0.86 | 0.121 | | | | | | | (*15*) |
| AKR1B7 | Mouse vas deferens protein $PGF_{2\alpha}$ synthase | 35,987 | 3.8 | 1.58 | 0.417 | | | | | | | (*16*) |
| AKR1C3 | Human PGF synthase | 36,842 | 10 | 9.58 | 0.958 | 3.4 | 73.68 | 21.67 | n.d. | | | (*18*) |
| AKR1C5 | Corpus luteum of pseudopregnant rabbit | 36,668 | | | | | | | 122 | 4.8 | 0.039 | (*9*) |
| AKR1C7 | Bovine lung PGF synthase | 36,666 | 10 | 2.09 | 0.209 | 120 | 4.77 | 0.040 | n.d. | | | (*7,18*) |
| AKR1C11 (native) | Bovine liver PGF synthase | 36,742 | 25 | 0.11 | 0.004 | 10 | 3.49 | 0.349 | n.d. | | | (*18*) |
| AKR1C11 (expressed) | Bovine liver PGF synthase | 36,742 | 25 | 0.33 | 0.013 | 15 | 0.85 | 0.056 | n.d. | | | (*18*) |
| Trx superfamily | Swine brain Prostamide/PGF synthase | 21,669 | 6.9 | 14.95 | 2.167 | | | | | | | (*23*) |

| AKR1B family | Substrate | $K_m$ (μM) | $k_{cat}$ ($min^{-1}$) | $k_{cat}/K_m$ ($min^{-1}.\mu M^{-1}$) | Ref. |
|---|---|---|---|---|---|
| AKR1B1 | glyceraldehyde | 64 | 32.00 | 0.50 | (*20*) |
| | phopholipid aldehyde | 8.8 | 25.90 | 2.94 | (*20*) |
| | $PGH_2$ | 1.9 | 0.93 | 0.49 | (*16*) |
| | $PGH_2$ | 29 | 6.06 | 0.21 | (*17*) |
| AKR1B3 | glyceraldehyde | 48 | 20.30 | 0.42 | (*20*) |
| | glyceraldehyde | 40 | 31.00 | 0.78 | (*19*) |
| | glyceraldehyde | 44 | 78.00 | 1.77 | (*21*) |
| | phopholipid aldehyde | 18.7 | 17.80 | 0.95 | (*20*) |
| | $PGH_2$ | 9.3 | 1.89 | 0.20 | (*16*) |
| | $PGH_2$ | 33 | 8.58 | 0.26 | (*17*) |
| AKR1B7 | glyceraldehyde | 140 | 1.60 | 0.01 | (*20*) |
| | glyceraldehyde | 800 | 4.50 | 0.01 | (*19*) |
| | phopholipid aldehyde | 165 | 9.5 | 0.06 | (*20*) |
| | $PGH_2$ | 3.8 | 1.58 | 0.42 | (*16*) |

n.d.: not detected

$PGH_2$ is different from that of the carbonyl compounds such as glyceraldehyde, as suggested in our reports about the PGF synthase belonging to the AKR1C subfamily (*6*).

$PGF_{2\alpha}$ exhibits physiological and pathological roles *in vivo* (*22*). Many types of PGF synthase may contribute to synthesis of $PGF_{2\alpha}$/9α, 11β-$PGF_2$. In 2008, we found a new type of PGF synthase in mouse brain and purified this enzyme from swine brain (*23*). This enzyme belongs to the thioredoxin (Trx) superfamily, having the Cys-x-x-Cys motif at its active site, and catalyses the reduction of $PGH_2$ to $PGF_{2\alpha}$ in the presence of the reduced form of Trx as a reducing equivalent donor. In addition to the contribution of the enzymes belonging to the AKR family to the synthesis of $PGF_2$, some enzymes belonging to the Trx superfamily may also contribute to the synthesis of $PGF_2$. When PGF synthase is isolated by detection of the enzyme activity from native tissues, other novel types of PGF synthase with more specific and higher catalytic activity may be found in the future.

## References


1. Kurzrok, R. and Lieb, C.C. (1930) Biochemical studies of semen II. The action of semen on the human uterus. *Proc. Soc. Exp. Biol. Med.* **28**, 268
2. Bergström, J. and Sjövall, J. (1957) The isolation of prostaglandin. *Acta Chem. Scand. II.* **11**, 1086
3. Barden, T.P. (1974) Induction of labor with prostaglandins in *The Prostaglandins* (Ramwell, P. W., ed.) Vol. 3, pp. 109–133, Plenum Press, New York
4. Watanabe, K., Shimizu, T., and Hayaishi, O. (1981) Enzymatic conversion of prostaglandin $D_2$ to $F_{2\alpha}$ in the rat lung. *Biochem. Int.* **2**, 603–610
5. Liston, T.E. and Roberts, L.J. II (1985) Transformation of prostaglandin D2 to 9 alpha, 11 beta-(15S)-trihydroxyprosta-(5Z,13E)- dien-1-oic acid (9 alpha, 11 beta-prostaglandin F2): a unique biologically active prostaglandin produced enzymatically *in vivo* in humans. *Proc. Natl Acad. Sci. USA* **82**, 6030–6034
6. Watanabe, K., Yoshida, R., Shimizu, T., and Hayaishi, O. (1985) Enzymatic formation of prostaglandin F2 alpha from prostaglandin H2 and D2. Purification and properties of prostaglandin F synthetase from bovine lung. *J. Biol. Chem.* **260**, 7035–7041
7. Watanabe, K., Fujii, Y., Nakayama, K., Ohkubo, H., Kuramitsu, S., Kagamiyama, H., Nakanishi, S., and Hayaishi, O. (1988) Structural similarity of bovine lung prostaglandin F synthase with ρ-crystallin of European common frog. *Proc. Natl Acad. Sci. USA* **85**, 11–15
8. Levine, L., Wu, K. Y., and Pong, S. S. (1975) Stereospecificity of enzymatic reduction of prostaglandin E2 to F2alpha. *Prostaglandins* **9**, 531–544
9. Wintergalen, N., Thole, H.H., Galla, H.J., and Schlegel, W. (1995) Prostaglandin- E2 9-reductase from corpus luteum of pseudopregnant rabbit is a member of the aldo-keto reductase superfamily featuring 20 alpha-hydroxysteroid dehydrogenase activity. *Eur. J. Biochem.* **234**, 264–270
10. Schieber, A., Frank, R. W., and Ghisla, S. (1992) Purification and properties of prostaglandin 9-ketoreductase from pig and human kidney. Identity with human carbonyl reductase. *Eur. J. Biochem.* **206**, 491–502
11. Barski, O. A., Tipparaju, S. M., and Bhatnagar, A. (2008) The aldo-keto reductase superfamily and its role in drug metabolism and detoxification. *Drug Metab. Rev.* **40**, 553–624
12. Hyndman, D. and Penning, T. M. AKR family, Members of the Aldo-Keto Reductase Superfamily. http://www.med.upenn.edu/akr/ (4 September 2011, date last accessed)
13. Kubata, B.K., Duszenko, M., Kabututu, Z., Rawer, M., Szallies, A., Fujimori, K., Inui, T., Nozaki, T., Yamashita, K., Horii, T., Urade, Y., and Hayaishi, O. (2000) Identification of a novel prostaglandin f(2alpha) synthase in *Trypanosoma brucei*. *J. Exp. Med.* **192**, 1327–1338
14. Kabututu, Z., Martin, S.K., Nozaki, T., Kawazu, S., Okada, T., Munday, C.J., Duszenko, M., Lazarus, M., Thuita, L.W., Urade, Y., and Kubata, B.K. (2003) Prostaglandin production from arachidonic acid and evidence for a 9,11-endoperoxide prostaglandin H2 reductase in *Leishmania*. *Int J. Parasitol.* **33**, 221–228
15. Madore, E., Harvey, N., Parent, J., Chapdelaine, P., Arosh, J.A., and Fortier, M.A. (2003) An aldose reductase with 20 alpha-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin f2 alpha in the bovine endometrium. *J. Biol. Chem.* **278**, 11205–11212
16. Kabututu, Z., Manin, M., Pointud, J.C., Maruyama, T., Nagata, N., Lambert, S., Lefrançois-Martinez, A.M., Martinez, A., and Urade, Y. (2009) Prostaglandin F2alpha synthase activities of aldo-keto reductase 1B1, 1B3 and 1B7. *J. Biochem.* **145**, 161–168
17. Nagata, N., Kusakari, Y., Fukunishi, Y., Inoue, T., and Urade, Y. (2011) Catalytic mechanism of the primary human prostaglandin F2α synthase, aldo-keto reductase 1B1–prostaglandin D2 synthase activity in the absence of NADP(H). *FEBS J.* **278**, 1288–1298
18. Watanabe, K. (2010) Prostaglandin synthases with thioredoxin domain. *Seikagaku* **82**, 1041–1046
19. Ruiz, F.X., Moro, A., Gallego, O., Ardèvol, A., Rovira, C., Petrash, J. M., Parés, X., and Farrés, J. (2011) Human and rodent aldo-keto reductases from the AKR1B subfamily and their specificity with retinaldehyde. *Chem. Biol. Interact.* **191**, 199–205
20. Spite, M., Baba, S.P., Ahmed, Y., Barski, O.A., Nijhawan, K., Petrash, J.M., Bhatnagar, A., and Srivastava, S. (2007) Substrate specificity and catalytic efficiency of aldo-keto reductases with phospholipid aldehydes. *Biochem. J.* **405**, 95–105
21. Gui, T., Tanimoto, T., Kokai, Y., and Nishimura, C. (1995) Presence of a closely related subgroup in the aldo-ketoreductase family of the mouse. *Eur. J. Biochem.* **277**, 448–453
22. Woodward, D.F., Jones, R.L., and Narumiya, S. (2011) International union of basic and clinical pharmacology. LXXXIII: classification of Prostanoid Receptors, Updating 15 Years of Progress. *Pharmacol. Rev.* **63**, 471–538
23. Moriuchi, H., Koda, N., Okuda-Ashitaka, E., Daiyasu, H., Ogasawara, K., Toh, H., Ito, S., Woodward, D.F., and Watanabe, K. (2008) Molecular characterization of a novel type of prostamide/prostaglandin F synthase, belonging to the thioredoxin-like superfamily. *J. Biol. Chem.* **283**, 792–801