Bioorganic & Medicinal Chemistry Letters 21 (2011) 801–804

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure of rat aldose reductase-like protein AKR1B14 holoenzyme: Probing the role of His269 in coenzyme binding by site-directed mutagenesis

Krithika Sundaram [a,†], Urmi Dhagat [a,†], Satoshi Endo [b,†], Roland Chung [a], Toshiyuki Matsunaga [b], Akira Hara [b], Ossama El-Kabbani [a,*]

[a] Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia
[b] Laboratory of Biochemistry, Gifu Pharmaceutical University, Daigaku-Nishi, Gifu 501-1196, Japan

ARTICLE INFO

Article history:
Received 7 October 2010
Revised 17 November 2010
Accepted 19 November 2010
Available online 24 November 2010

Keywords:
Aldose reductase-like protein
Aldo-keto reductase
Coenzyme binding
AKR1B14

ABSTRACT

Rat aldose reductase-like protein (AKR1B14) is the ortholog of mouse vas deferens protein (AKR1B7) playing roles in detoxification of reactive aldehydes and synthesis of prostaglandin $F_{2\alpha}$. The crystal structure of the binary complex (AKR1B14-NADPH) was determined at 1.86 Å resolution, and showed that the adenine ring and the 2′-phosphate group of the coenzyme formed π-stacking and electrostatic interactions with the imidazole ring and ND1 atom, respectively, of His269, which is not conserved in other aldose reductase-like proteins. The interactions were supported by site-directed mutagenesis of His269 to Arg, Phe and Met, which increased the K_m for NADPH by 4, 7 and 127-fold, respectively. This is the first report of the tertiary structure of a rodent AKR1B7 ortholog, which describes the role of a novel dual interaction for the non-conserved His269 in coenzyme binding.

Aldose reductase (AR) is a NADPH-dependent enzyme that converts a broad range of aldehydes including glucose into their corresponding alcohols, and structurally belongs to the aldo-keto reductase (AKR) superfamily.[1] Human AR, named AKR1B1 in this superfamily, catalyzes the first step in the polyol pathway and has been implicated in the development of secondary diabetic complications.[2–4] AKR1B1 is also involved in the metabolism of retinoids, steroids and xenobiotics, and defensive mechanisms against oxidative stress.[5–8]

Recent studies have identified human and rodent AR-like proteins, which exhibit high overall amino acid sequence identity (67–71%) to ARs, but clearly differ from ARs in their inability to reduce glucose. In humans, an AR-like protein (AKR1B10) has been characterized as a NADPH-dependent reductase, that is similar to AKR1B1 in its broad substrate specificity and sensitivity to AR inhibitors.[9,10] Major differences of AKR1B10 from AKR1B1 are its efficient reduction of retinals, isoprenyl aldehydes and isatin,[7,10] inability to convert prostaglandin H_2 to prostaglandin $F_{2\alpha}$,[11] and high up-regulation in lung carcinomas.[12] In mice, two AR-like proteins, AKR1B7 and AKR1B8, are differently distributed in the tissues and also called androgen-regulated vas deferens protein[13,14] and fibroblast growth factor-inducible protein,[15,16] respectively. The two AR-like proteins differ in their substrate specificity and susceptibility to inhibition by AR inhibitors such as sorbinil and tolrestat. AKR1B7 reduces isocaproaldehyde, a product of the side-chain cleavage of cholesterol during steroidogenesis[14] and cytotoxic 4-hydroxynonenal, a major product of lipid peroxidation,[17] and exhibits prostaglandin $F_{2\alpha}$ synthase activity.[11] AKR1B8 shows high catalytic efficiency towards long-chain aliphatic aldehydes including 4-hydroxynonenal[16] and does not exhibit prostaglandin $F_{2\alpha}$ synthase activity.[11] While AKR1B7 is insensitive to AR inhibitors,[14,17] AKR1B8 is moderately inhibited by AR inhibitors.[16] In rats, three AR-like proteins, AKR1B13,[18] AKR1B14[19] and rat AKR1B10,[20] have been characterized. AKR1B13 and AKR1B14 are orthologues of mouse AKR1B8 and AKR1B7, respectively, sharing high sequence identity (>87%) between the corresponding AR-like proteins. AKR1B13 exhibits almost identical substrate specificity and inhibitor sensitivity to those of mouse AKR1B8.[18] AKR1B14 shows prostaglandin $F_{2\alpha}$ synthase activity[21] and reduces the above substrates of mouse AKR1B7.[19] However, AKR1B14 displays broad substrate specificity for various aldehydes, α-dicarbonyl compounds and several aromatic ketones, and is distinct from the mouse ortholog, AKR1B7, in its sensitivity towards AR inhibitors.[19] In addition, compared to other AR-like proteins, AKR1B14 exhibits low K_m values for the coenzymes, particularly, $NADP^+$, which is equal to 0.14 μM.

The crystal structures of AKR1B8[22] and AKR1B10[23] in complex with the coenzyme and an AR inhibitor have been solved, but those of AKR1B7 and its rat ortholog AKR1B14 have yet to be reported. Among the members of the AKR1B subfamily, the outstanding feature of AKR1B7 and AKR1B14 is their high prostaglandin $F_{2\alpha}$ synthase activity that is comparable to AKR1B1.[11,21] In contrast,

* Corresponding author. Tel.: +61 3 9903 9691; fax: +61 3 9903 9143.
E-mail address: ossama.el-kabbani@monash.edu (O. El-Kabbani).
† Contributed equally to the work.

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.11.086

Table 1
Data collection and refinement statistics

Unit cell parameters	
Space group	$P2_1$
Cell dimensions	a = 50.66 Å
	b = 69.14 Å
	c = 87.83 Å
	β = 96.4°
Diffraction data[a]	
Radiatio source	Rotating anode
Wavelength (Å)	1.54178
Number of unique reflections	49,358 (3640)
Redundancy	3.4 (3.0)
Completeness (%)	97.6 (90.6)
I/σ (I)	13.5 (2.3)
R_{merge} (%)	4.7 (27.9)
Refinement statistics	
Resolution (Å)	30–1.86
Protein residues	630
Solvent molecules	639
Cofactor molecules	2
R_{free} (%)	24.7
R_{cryst} (%)	18.3
RMSDs	
Bonds (Å)	0.023
Angles (°)	1.93
Ramachandran plot	
Residues in most favored regions (%)	92
Residues in allowed regions (%)	8
Estimated coordinated error	
Luzzati mean coordinate error (Å)	0.208
Mean B factors (Å²)	
Protein	25.9
NADPH	27.0

[a] Statistics for the highest resolution shell (1.93–1.86 Å) are shown in parentheses.

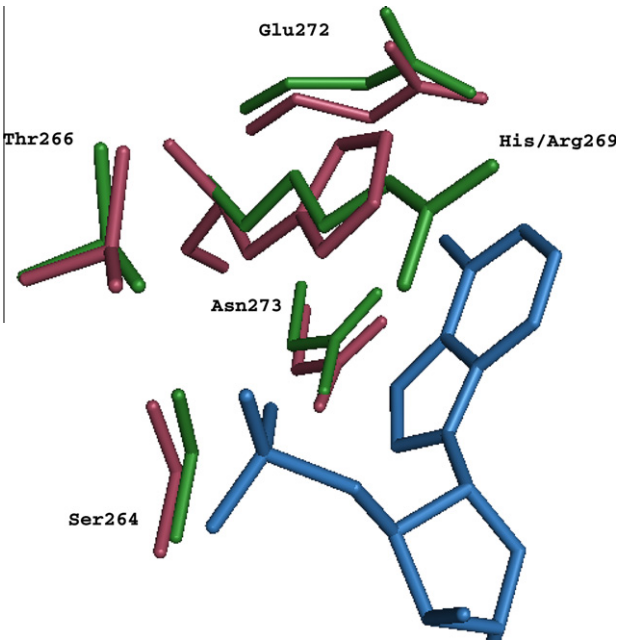
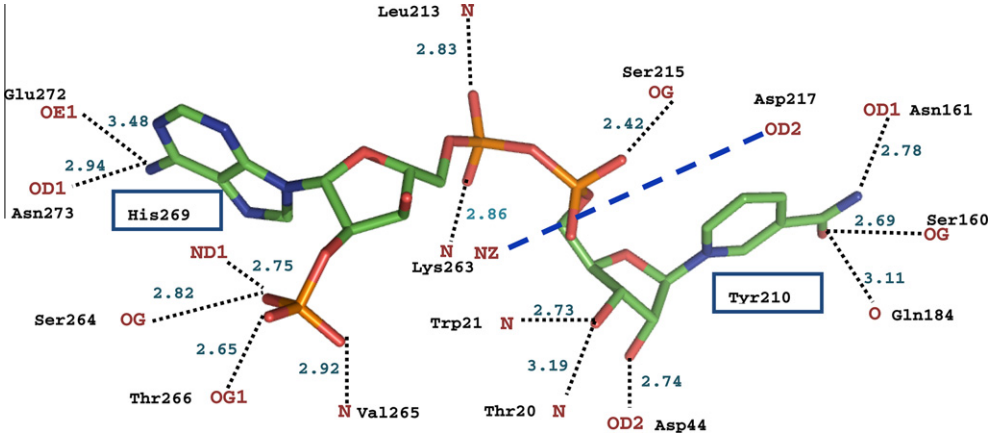


Figure 1. Superimposition of the structures of AKR1B14 (magenta) and AKR1B1 (green) in the vicinity of the 2′-phosphate adenosine moiety of NADPH (blue), showing the corresponding side-chains of His and Arg at position 269, respectively.

AKR1B7 and AKR1B14 are different from each other in their sensitivity to AR inhibitors despite of their high sequence identity. In order to understand the structural reasons for exhibiting prostaglandin $F_{2\alpha}$ synthase activity and their difference in selectivity towards AR inhibitors, we have determined the first three-dimensional structure of AKR1B14 in complex with the coenzyme NADPH.

Structure of AKR1B14 holoenzyme: The crystal structure of AKR1B14 in complex with NADPH was refined at 1.86 Å resolution with a final R_{cryst} of 18.3% and R_{free} of 24.7%. The backbone dihedral angles of 92% of the residues were in the favored regions and the remaining 8% were in the allowed region of the Ramachandran plot. A summary of the data collection and refinement statistics is presented in Table 1. Like other AR-like proteins (AKR1B8[22] and AKR1B10[23]), AKR1B14 consists of a conserved α_8/β_8 TIM-barrel core with the least conserved residues occurring in three loop regions lining the active site pocket. The NADPH binding site residues are conserved with the exception of His269, and are located mainly at the C-terminal end of the TIM-barrel. Residues that participate in hydrogen bonding interactions with the NADPH molecule include Glu272, Asn273 (with amino group on the adenine ring), Ser264, Val265, Thr266 and His269 (with the 2′- phosphate group), Asn161 (with the amide nitrogen of the nicotinamide ring) and Ser160, Gln184 (with the amide oxygen of the nicotinamide ring), Asp44, Thr20 and Trp21 (with the ribose sugar). The pyrophosphate bridge of NADPH interacts with Ser215, Lys22, Leu213, Asp36, and Lys263. Tyr210 forms π stacking interactions with the nicotinamide ring. These interactions with the corresponding



Scheme 1. Interactions in the coenzyme binding site between AKR1B14 and NADPH with distances shown in angstroms. The salt link between the side-chain of Asp217 and Lys263 acting as a safety belt in coenzyme binding is illustrated as a blue-dashed line. The side-chains of Tyr210 and His269 that form π-stacking interactions with the nicotinamide and adenine rings, respectively, of NADPH are designated as blue boxes.

Table 2
Kinetic alterations in the reduction and oxidation reactions by site-directed mutagenesis of His269 in AKR1B14

Parameters[a]	Wild-type	His269Arg		His269Phe		His269Met	
Reduction							
K_m NADPH (μM)	1.5 ± 0.1	6.2 ± 0.5	(4)	10 ± 1	(7)	185 ± 13	(127)
K_m 4NBA (μM)	1.5 ± 0.3	4.8 ± 0.8	(3)	4.1 ± 0.1	(3)	5.3 ± 0.1	(4)
k_{cat} (min^{-1})	1.6 ± 0.2	4.0 ± 0.1	(3)	4.2 ± 0.5	(3)	17 ± 1	(12)
k_{cat}/K_m NADPH (min^{-1} μM^{-1})	1.1	0.65	(0.6)	0.42	(0.4)	0.09	(0.08)
Oxidation							
K_m $NADP^+$ (μM)	0.14 ± 0.01	1.2 ± 0.2	(9)	3.3 ± 0.3	(24)	269 ± 21	(1920)
K_m geraniol (μM)	37 ± 9	85 ± 10	(2)	117 ± 14	(3)	192 ± 13	(5)
k_{cat} (min^{-1})	0.21 ± 0.02	0.28 ± 0.01	(2)	0.65 ± 0.01	(3)	0.70 ± 0.03	(4)
k_{cat}/K_m $NADP^+$ (min^{-1} μM^{-1})	1.5	0.23	(0.2)	0.20	(0.1)	0.0026	(0.002)

[a] The K_m and k_{cat} values for NADP(H) were determined with 0.1 mM 4-nitrobenzaldehyde (4NBA) and 0.5 mM geraniol as the fixed substrates in the reduction and oxidation reactions, respectively. In the case of the His269Met mutant enzyme the concentration of geraniol was kept at 1.0 mM. The K_m values for 4NBA and geraniol were determined with 0.1 mM NADPH and 0.25 mM $NADP^+$, respectively. In the case of the His269Met mutant enzyme the respective coenzyme concentrations were 0.15 and 1.0 mM, respectively. Values in parentheses are the ratios of mutant enzyme to wild-type enzyme.

hydrogen bond distances given in angstroms are illustrated in Scheme 1.

Sequence comparison of the AKRs (1B1, 1B7, 1B8, 1B10, 1B13 and 1B14) and rat AKR1B10 revealed that 16 of the 17 NADPH-binding residues shown in Scheme 1 are conserved in all the enzymes and only His269 of AKR1B14 is replaced by Arg in the other enzymes. A comparison of the crystal structures of AKR1B14 and AKR1B1 suggested that the side-chains of His269 in AKR1B14 and Arg269 in AKR1B1 lie in the same plane where they can form both stacking interactions with the adenine ring and electrostatic interactions with the 2′-phosphate group of NADPH (Fig. 1).

Role of His269 of AKR1B14 in coenzyme binding: The dual role of His269 in coenzyme binding was verified by site-directed mutagenesis of His269 in AKR1B14 with Arg (the corresponding residue of AKR1B1 and other AR-like proteins), Phe (without a positively charged side-chain) and Met (without both aromatic and positively charged side-chain). Since AKR1B14 catalyzes the $NADP^+$-dependent oxidation of several alcohols such as geraniol,[19] the effects of the three mutations on the kinetic constants in the NADPH-linked 4-nitrobenzaldehyde reduction and $NADP^+$-linked geraniol oxidation were examined (Table 2). All the mutations resulted in increases in the K_m values for both coenzymes and substrates, of which the K_m values for the coenzymes were more greatly affected than those for the substrates. The increase in K_m value for $NADP^+$ by the mutations was larger than that for NADPH. $NADP^+$ inhibited the reductase activity competitively with respect to NADPH, showing K_i values of 0.20 ± 0.04, 2.3 ± 0.4, 3.1 ± 0.5 and 287 ± 70 μM for the wild-type, His269Arg, His269Phe and His269Met enzymes, respectively. Because the K_i value implies the dissociation constant (K_d) for the coenzyme in the kinetic ordered mechanism of AKR1B14,[19] the affinity for $NADP^+$ is suggested to be increased more than 10-fold by the mutations. Although AKR1B14 utilizes NADH as a less efficient coenzyme,[19] no significant changes in the K_m values for NADH were observed by the mutagenesis. The values of the His269Arg and His269Met enzymes were 313 ± 20 and 373 ± 11 μM, respectively, which are less than 1.5-fold changes versus 227 ± 30 μM for the wild-type enzyme. The alteration of the K_m and K_d values for NADP(H) by the His269Arg mutation was low but significant, suggesting that the aromatic imidazole ring of His269 contributes to the stacking interactions with the adenine ring of the coenzyme more strongly than the side-chain of the replaced Arg in the other AKRs. This may also be related to the low K_m values of AKR1B14 for NADP(H)[19] compared to those of other AR-like proteins which possess Arg269. The moderate kinetic changes induced by the His269Phe mutation support the importance of the electrostatic interaction between His269 and the coenzyme. The kinetic changes by the His269Met mutation were large and seem to be a synergistic effect due to the loss of the two above interactions. Thus, His269 of the wild-type AKR1B14 is involved in two interactions which are complementary in their effect on the binding of coenzyme. Considering the low K_m and K_d values for $NADP^+$ and the increases in k_{cat} values by the mutagenesis, it is also suggested that the rate-limiting step of enzyme catalysis is the dissociation of $NADP^+$, which may be facilitated by the decrease in the binding caused by the mutation.

In conclusion, structural and kinetic studies on the binding of coenzyme to the AR-like protein AKR1B14 suggested that the π-stacking interaction between the imidazole ring of the non-conserved His269 and the adenine ring of the coenzyme, and the electrostatic interaction between the ND1 of His269 and the 2′-phosphate group of the coenzyme contributed equally to coenzyme binding. In addition to the role of the AR-like protein AKR1B10 in regulating cellular response to carbonyl stress, together with human AR they are upregulated in diverse types of cancers and thus are being considered as potential targets for anti-cancer agents.[24] Indeed AR inhibitors developed for the treatment of diabetic complications have been shown to inhibit AKR1B10 and therefore may act as lead compounds in the search of new cancer treatments.[25]

The atomic coordinates were deposited in the PDB (entry ID code 3O3R) and will be released immediately upon publication.

Acknowledgements

U.D. is the recipient of a Monash Graduate School postgraduate scholarship. This work was partly supported by a Grant-in-Aid for Young Scientists (to S.E.).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.086.

References and notes

1. Bohren, K. M.; Bullock, B.; Wermuth, B.; Gabbay, K. H. *J. Biol. Chem.* **1989**, *264*, 9547.
2. Oates, P. *J. Curr. Drug Targets* **2008**, *9*, 14.
3. Dunlop, M. *Kidney Int. Suppl.* **2000**, *77*, S3.
4. Yabe-Nishimura, C. *Pharmacol. Rev.* **1998**, *50*, 21.
5. Conklin, D.; Prough, R.; Bhatanagar, A. *Mol. BioSyst.* **2007**, *3*, 136.
6. Petrash, J. M. *Cell. Mol. Life Sci.* **2004**, *61*, 737.
7. Crosas, B.; Hyndman, D. J.; Gallego, O.; Martras, S.; Parés, X.; Flynn, T. G.; Farrés, J. *Biochem. J.* **2003**, *373*, 973.
8. Wermuth, B.; Monder, C. *Eur. J. Biochem.* **1983**, *131*, 423.
9. Cao, D.; Fan, S. T.; Chung, S. S. *J. Biol. Chem.* **1998**, *273*, 11429.
10. Endo, S.; Matsunaga, T.; Mamiya, H.; Ohta, C.; Soda, M.; Kitade, Y.; Tajima, K.; Zhao, H. T.; El-Kabbani, O.; Hara, A. *Arch. Biochem. Biophys.* **2009**, *487*, 1.
11. Kabututu, Z.; Manin, M.; Pointud, J. C.; Maruyama, T.; Nagata, N.; Lambert, S.; Lefrançois-Martinez, A. M.; Martinez, A.; Urade, Y. *J. Biochem.* **2009**, *145*, 161.

12. Fukumoto, S.; Yamauchi, N.; Moriguchi, H.; Hippo, Y.; Watanabe, A.; Shibahara, J.; Taniguchi, H.; Ishikawa, S.; Ito, H.; Yamamoto, S.; Iwanari, H.; Hironaka, M.; Ishikawa, Y.; Niki, T.; Sohara, Y.; Kodama, T.; Nishimura, M.; Fukayama, M.; Dosaka-Akita, H.; Aburatani, H. *Clin. Cancer Res.* **2005**, *11*, 1776.
13. Pailhoux, E. A.; Martinez, A.; Veyssiere, G. M.; Jean, C. G. *J. Biol. Chem.* **1990**, *265*, 19932.
14. Lefrançois-Martinez, A. M.; Tournaire, C.; Martinez, A.; Berger, M.; Daoudal, S.; Tritsch, D.; Veyssière, G.; Jean, C. *J. Biol. Chem.* **1999**, *274*, 32875.
15. Donohue, P. J.; Alberts, G. F.; Hampton, B. S.; Winkles, J. A. *J. Biol. Chem.* **1994**, *269*, 8604.
16. Srivastava, S.; Harter, T. M.; Chandra, A.; Bhatnagar, A.; Srivastava, S. K.; Petrash, J. M. *Biochemistry* **1998**, *37*, 12909.
17. Martinez, A.; Aigueperse, C.; Val, P.; Dussault, M.; Tournaire, C.; Berger, M.; Veyssière, G.; Jean, C.; Lefrançois Martinez, A. *Chem. Biol. Interact.* **2001**, *130–132*, 903.
18. Endo, S.; Matsunaga, T.; Mamiya, H.; Hara, A.; Kitade, Y.; Tajima, K.; El-Kabbani, O. *Chem. Biol. Interact.* **2009**, *178*, 151.
19. Endo, S.; Matsunaga, T.; Fujita, A.; Tajima, K.; El-Kabbani, O.; Hara, A. *Biol. Pharm. Bull.* **2010**, *33*, 1886.
20. Endo, S.; Matsunaga, T.; Kuragano, T.; Ohno, S.; Kitade, Y.; Tajima, K.; El-Kabbani, O.; Hara, A. *Arch. Biochem. Biophys.* **2010**, *503*, 230.
21. Lambert-Langlais, S.; Pointud, J. C.; Lefrançois-Martinez, A. M.; Volat, F.; Manin, M.; Coudoré, F.; Val, P.; Sahut-Barnola, I.; Ragazzon, B.; Louiset, E.; Delarue, C.; Lefebvre, H.; Urade, Y.; Martinez, A. *PLoS ONE* **2009**, *4*, e7309.
22. Wilson, D. K.; Nakano, T.; Petrash, J. M.; Quiocho, F. A. *Biochemistry* **1995**, *34*, 14323.
23. Gallego, O.; Ruiz, F. X.; Ardèvol, A.; Domínguez, M.; Alvarez, R.; de Lera, A. R.; Rovira, C.; Farrés, J.; Fita, I.; Parés, X. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 20764.
24. Yan, R.; Zu, X.; Ma, J.; Liu, Z.; Adeyanju, M.; Cao, D. *Int. J. Cancer* **2007**, *121*, 2301.
25. Liu, J.; Wen, G.; Cao, D. *Recent Pat. Anticancer Drug Discov.* **2009**, *4*, 246.