

Aldo-keto reductase-catalyzed detoxication of endogenous aldehydes associated with diabetic complications

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Numerous reactive aldehydes elevated in diabetic patients are key intermediates in the formation of Advanced Glycation Endproducts and likely contribute to development of long-term diabetic complications. These aldehydes include formaldehyde, glyoxal, methylglyoxal, glucosone, 3-deoxyglucosone, xylosone, 3-deoxyxylosone, and 4-hydroxynonenal. All of these aldehydes are substrates of human aldehyde and aldose reductases (AKR1A1, AKR1B1), two members of the aldo-keto reductase superfamily. The broad specificity of aldose and aldehyde reductases for these endogenous aldehydes suggests that detoxication of reactive aldehydes is one of their main functions. The structural features that contribute to substrate recognition include the presence of an oxidized carbon at the 2-position. Remaining structural features can vary widely. Thus, aldose and aldehyde reductases mainly appear to recognize a reactive aldehyde functional group. This imparts to these reductases an exceptionally broad protective function against the toxicity of reactive aldehydes.

A number of Advanced Glycation Endproducts (AGE) produced by modification of proteins by glucose have been identified either from in vitro or in vivo studies. These are thought to contribute to the cross-linking that is associated with the development of long-term diabetic complications. Although it initially was assumed that AGE are formed primarily from glycation of proteins by glucose, it is now clear that AGE can be formed from a variety of compounds besides glucose, including fructose, trioses, ribose, and ascorbate, and even from lipoxidation pathways (1-14). Therefore, it may be preferable to define AGE as Advanced Glycation/Lipoxidation Endproducts. In addition, reactive 2-oxoaldehydes appear to be key intermediates in the formation of many of the AGE identified thus far (figure 1). The recent success in developing potential therapeutic agents that degrade AGE points to the importance of AGE in the etiology of diabetic complications (15-18). The known AGE primarily are the products of reactions involving the 2-oxoaldehydes glyoxal, methylglyoxal and 3-deoxyglucosone. The importance of methylglyoxal (MeG) deserves special attention: 1) Antibodies against MeG-derived AGE cross-react with AGE produced by modification of proteins with glucose, fructose, ribose, glyceraldehyde, glyoxal, ascorbate and ascorbate oxidation products, suggesting that MeG may be a common intermediate in AGE formation from a wide variety of glycating agents (8); 2) MeG-derived AGE and glyoxal-derived AGE as well as 3-deoxyglucosone-derived AGE are elevated in diabetes (6-8); 3) AGE derived from MeG and other 2-oxoaldehydes catalyze the production of free radicals (19); and 4) Enzymes involved with metabolism of MeG are elevated in diabetes (20). These recent observations support the suggestion that MeG and other 2-oxoaldehydes play an essential role in the chemistry of AGE production.

Numerous studies support a role for aldose reductase (aldo-keto reductase, AKR1B1) in the development of diabetic complications in experimental models (21-23). A role for AKR1B1 in the development of diabetic complications in man is less certain. All of the endogenous aldehydes that have been implicated in AGE formation are substrates of AKR1B1; this includes MeG, glucose, glucosone, 3-deoxyglucosone, glyoxal, xylosone and 3-deoxyxylosone. The lipid-derived aldehyde 4-hydroxynonenal is also an excellent substrate of aldose reductase (24-31). This raises the question of the physiological roles of AKR1B1 and other aldo-keto reductases. In the present study, we compare the catalytic properties of AKR1B1 and the related aldo-keto reductase AKR1A1 (aldehyde reductase) with a range of reactive aldehydes and demonstrate that the broad specificities of AKR1A1 and AKR1B1 are consistent with the suggestion that a major function of these aldo-keto reductases is the detoxication of reactive aldehydes. We also demonstrate that glutathione plays a role in the detoxication of certain reactive aldehydes catalyzed by AKR1A1 and AKR1B1 and that there appears to be a glutathione binding site at the active sites of both AKR1A1 and AKR1B1.

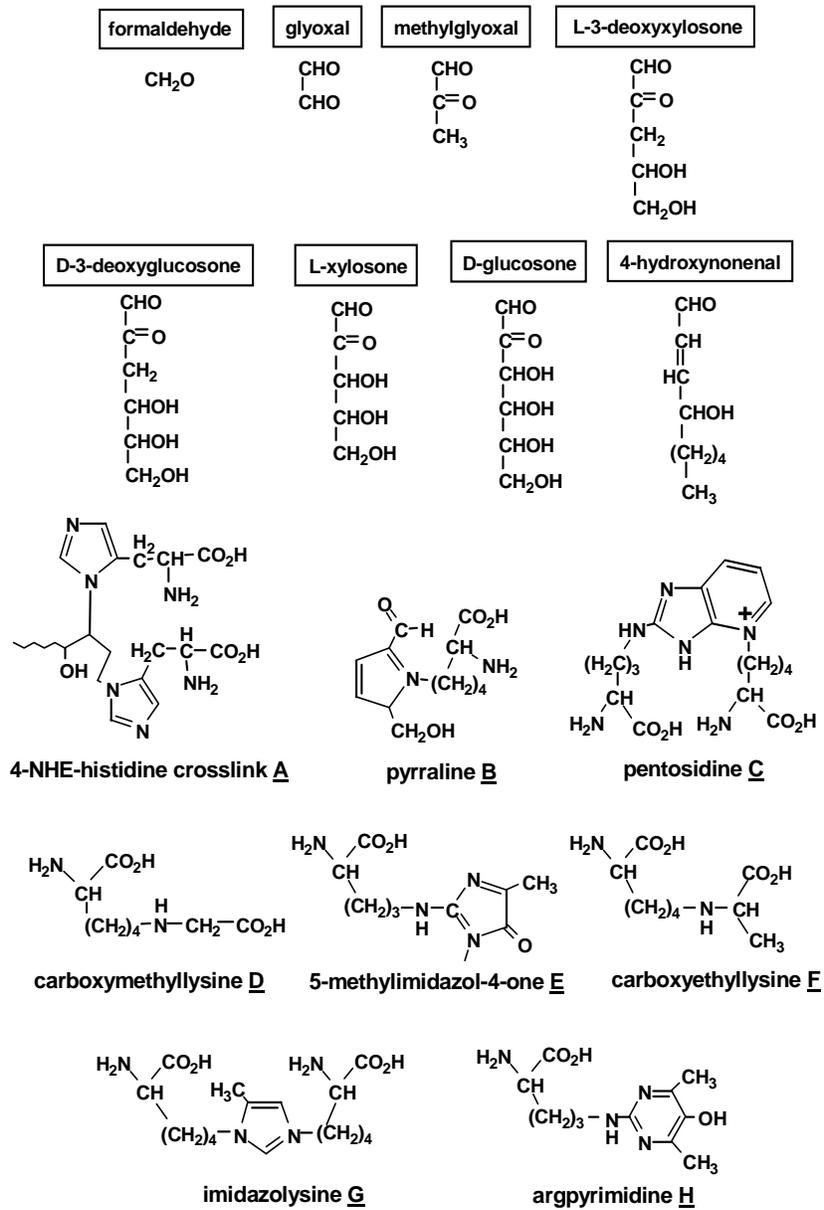


Figure 1: Structures of reactive aldehydes and AGEs implicated in the development of diabetic complications. AGEs E - H are derived from methylglyoxal

Materials and Methods

Chemicals

MeG was prepared by hydrolysis of pyruvic aldehyde dimethylacetal with sulfuric acid followed by azeotropic distillation with water. 3-Deoxyglucosone, glucosone, 3-deoxyxylosone, xylosone, and 4-hydroxynonenal were synthesized by literature procedures. All other aldehydes were from Sigma.

Purification of AKR1A1 and AKR1B1

AKR1A1 and AKR1B1 were purified from human liver and skeletal muscle, respectively, as described previously (24-26).

Enzyme Assay

AKR1A1 and AKR1B1 activity in the direction of aldehyde reduction was routinely measured in 1 ml total volume of 0.1 M sodium phosphate buffer pH 7.0 with 10 mM glyceraldehyde and 0.1 mM NADPH. Enzyme activity was determined by following changes in NADPH concentration at 340 nm, $\epsilon = 6.2 \text{ mM}^{-1} \text{ cm}^{-1}$.

Enzyme kinetic studies

Initial velocity studies were conducted in the buffer described above at 25°C. Michaelis constants for substrates and cofactors and k_{cat} values were determined by nonlinear regression analysis of the initial rate data using the ENZFITTER program (Elsevier-Biosoft).

Flexible docking

Flexible docking of glutathione to AKR1A1 and AKR1B1 was performed using the AutoDock 3.0 software suite from Scripps Research Institute (32). The crystal structure of human AKR1B1 (1ADS.pdb) was modified to accommodate the docking (33). The coordinates of polar hydrogens were added as predicted by Sybyl 6.6 using torsional minimization. Partial charges were assigned from united Kollman dictionary charges and all substrate and ordered water atoms were removed. Inhibitor structure was predicted from GA conformational search followed by BFGS minimization in Sybyl. Inhibitor partial charges were assigned according to the Gasteiger-Huckel method. For human AKR1A1, the coordinates (1CWN.pdb) of porcine aldehyde reductase (34) were used to construct a homology model of AKR1A1.

Results and Discussion

Substrate Properties of AKR1A1 and AKR1B1 with Aldehydes Implicated in the Development of Diabetic Complications

The catalytic efficiencies k_{cat}/K_m of AKR1A1 and AKR1B1 with the reactive aldehydes shown in figure 1 are summarized in Table I. The k_{cat}/K_m values for AKR1B1 are 5-100 fold greater for AKR1B1 than for AKR1A1, with the highest difference observed with MeG, which appears to be the preferred substrate of AKR1B1. There is a marked increase in k_{cat}/K_m for any of the substrates compared to formaldehyde, raising the question of the role of the groups attached to the reactive aldehyde functional group. To address this question, the catalytic efficiencies of AKR1A1 and AKR1B1 were compared using a series of 2-carbon and 3-carbon aldehydes, summarized in Table II. For both the 2-carbon and 3-carbon series, k_{cat}/K_m values increase markedly if the 2-position of

Table I. AKR1A1 and AKR1B1 – Catalyzed Reduction of Reactive Aldehydes

<i>Substrate</i>	<i>k_{cat} / K_m (M⁻¹ min⁻¹)</i>	
	<i>AKR1A1</i>	<i>AKR1B1</i>
formaldehyde	2.1 x 10 ¹	2.2 x 10 ³
glyoxal	1.6 x 10 ⁴	3.0 x 10 ⁵
methylglyoxal	1.8 x 10 ⁵	1.8 x 10 ⁷
xylosone	1.1 x 10 ⁵	5.3 x 10 ⁵
3-deoxyxylosone	5.4 x 10 ⁵	2.1 x 10 ⁶
glucosone	8.5 x 10 ⁴	5.5 x 10 ⁵
3-deoxyglucosone	5.8 x 10 ⁵	2.6 x 10 ⁶
4-hydroxynonenal	– ^a	4.6 x 10 ⁶

Adapted in part from data in References 26, 28, 30

^a Low solubility precluded determination of kinetic parameters

the aldehyde is an oxidized carbon. Thus, glycoaldehyde, glyoxal and glyoxylate are much better substrates than acetaldehyde, and acrolein, glyceraldehyde and MeG are much better substrates than propionaldehyde. This difference is even greater than suggested by the k_{cat}/K_m values when one considers that the aldehyde functional groups of these aldehydes are highly hydrated compared to acetaldehyde and propionaldehyde,

leaving the concentration of free aldehyde that is the actual substrate of AKR1A1 and AKR1B1 very low. For example, MeG is 99.8% hydrated in solution (35). If corrected for this factor, the catalytic efficiency of AKR1B1 with MeG as substrate would approach a diffusion limited value.

Table II. AKR1A1 and AKR1B1 -- Catalyzed Reduction of 2-Carbon and 3-Carbon Aldehydes

Substrate	$k_{cat} / K_m (M^{-1} min^{-1})$	
	AKR1A1	AKR1B1
$CH_3-\overset{\overset{O}{\parallel}}{C}-CH$	5.1×10^1	1.7×10^3
$HOCH_2-\overset{\overset{O}{\parallel}}{C}-CH$	3.6×10^4	9.5×10^5
$HC-\overset{\overset{O}{\parallel}}{C}-\overset{\overset{O}{\parallel}}{C}-CH$	1.6×10^4	3.0×10^5
$HO_2C-\overset{\overset{O}{\parallel}}{C}-CH-\overset{\overset{O}{\parallel}}{C}-CH$	3.3×10^5	6.7×10^4
$CH_3-CH_2-\overset{\overset{O}{\parallel}}{C}-CH$	2.2×10^2	6.5×10^4
$CH_2=CH-\overset{\overset{O}{\parallel}}{C}-CH$	2.0×10^4	1.1×10^6
$HOCH_2-\underset{\underset{OH}{ }}{CH}-\overset{\overset{O}{\parallel}}{C}-CH$	1.6×10^5	7.5×10^6
$CH_3-\overset{\overset{O}{\parallel}}{C}-\overset{\overset{O}{\parallel}}{C}-CH$	1.8×10^5	1.8×10^7

The results in Tables I and II support the conclusion that AKR1A1 and AKR1B1 show broad specificities for reactive aldehydes that are highly hydrated and that these aldo-keto reductases appear to be designed to recognize the aldehyde functional group with limited sensitivities to the rest of the substrate, as long as the 2-position is an oxidized carbon. This preference for

aldehydes with oxidized carbons at the 2-position can be ascribed in part to the H-bonding roles of residues H112 and H110 in AKR1A1 and AKR1B1, respectively, in dictating substrate specificity (36).

Substrate Specificity of Reduced and Oxidized AKR1B1

AKR1B1, unlike AKR1A1, has an active site cysteine residue (C298) that is sensitive to redox conditions. Simply dialyzing AKR1B1 in the absence of cofactor will produce an oxidized form that can be converted back into the reduced form by treatment with DTT (24,24). The substrate properties of oxidized AKR1B1 differ markedly from those of reduced AKR1B1, as shown in Table III. Additional forms of AKR1B1 involving nitrosation or glutathionylation of C298 can be formed, each with unique kinetic properties (37,38). In general, any modification of AKR1B1 will decrease k_{cat}/K_m values for any of the substrates. The physiological significance of the redox-sensing activity of AKR1B1 is not known. By comparison, AKR1A1 does not exhibit these properties, suggesting that AKR1A1 may be the more important of these reductases for detoxication of reactive aldehydes under conditions of oxidative stress that may compromise the activity of AKR1B1.

Table III. Substrate Properties of Reduced and Oxidized AKR1B1

<i>Substrate</i>	<i>k_{cat} / K_m (M⁻¹ min⁻¹)</i>	
	<i>AKR1B1 (reduced)</i>	<i>AKR1B1 (oxidized)</i>
glucose	9.1 x 10 ²	— ^a
glyceraldehyde	7.5 x 10 ⁶	1.9 x 10 ⁵
methylglyoxal	1.8 x 10 ⁷	6.6 x 10 ⁴

^a Glucose is essentially not a substrate of oxidized AKR1B1

Adapted in part from data in Reference 27

Role of Glutathione in AKR1A1 and AKR1B1-Catalyzed Reactions

Glutathione reacts non-enzymatically with MeG to form the hemithioacetal. Thus intracellular MeG is an equilibrium mixture of free and hydrated MeG along with the glutathione-MeG hemithioacetal (figure 2). AKR1B1 is able to catalyze the reduction of the aldehyde functional group of MeG to produce

acetol and of the ketone functional group of the hemithioacetal to produce the hemithioacetal of lactaldehyde, which can then form lactaldehyde (39). Thus, with MeG as substrate AKR1B1 is both an aldehyde and a ketone reductase. Both lactaldehyde and acetol can be reduced further to propanediol, catalyzed by AKR1B1, again demonstrating the ability of AKR1B1 to function as a ketone reductase.

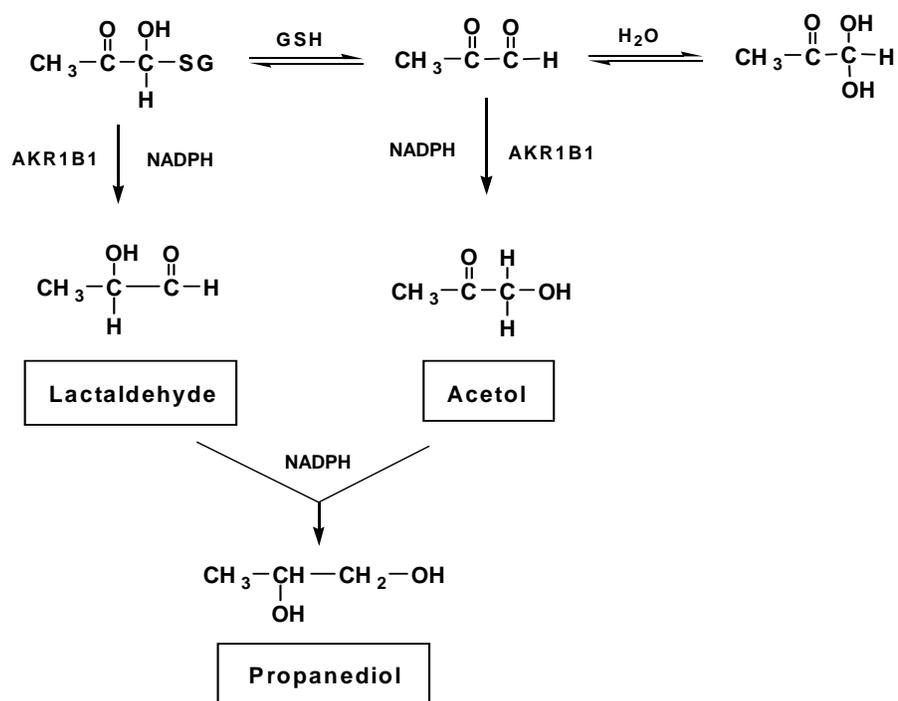


Figure 2. Role of glutathione in the AKR1B-catalyzed reduction of methylglyoxal (adapted from Reference 38).

The known ability (37) of AKR1B1 to catalyze the reduction of the Michael adducts of glutathione and α,β -unsaturated aldehydes is in agreement with the suggestion of a glutathione binding site at the active site of AKR1B1. The presence of a glutathione binding site near the active site of both AKR1A1 and AKR1B1 is supported by molecular modeling (figure 3).

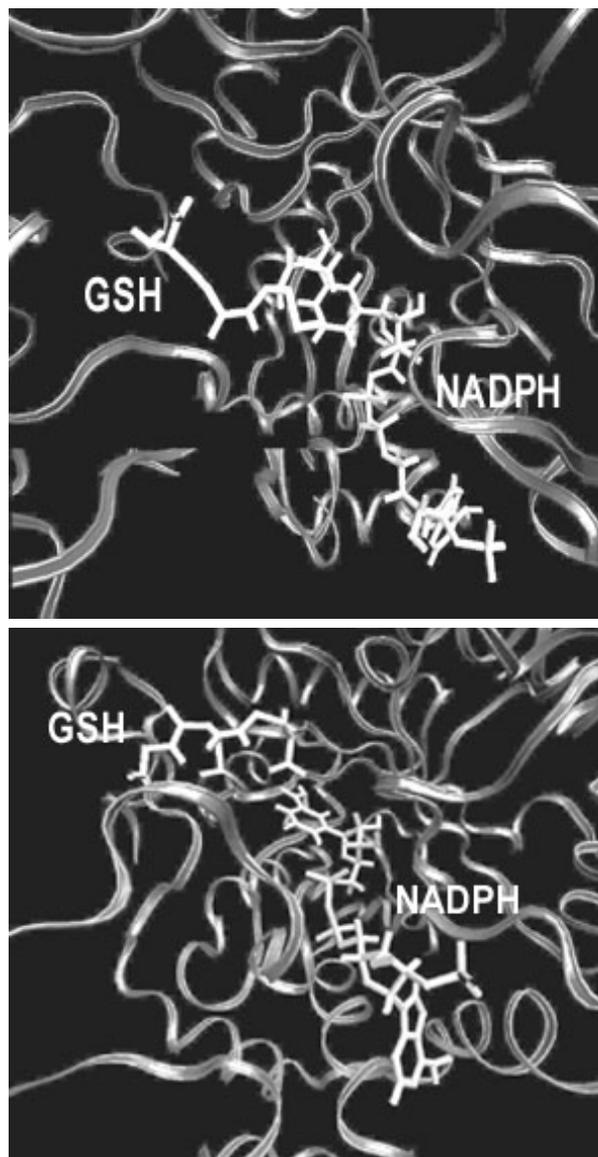
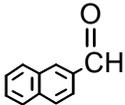
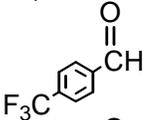
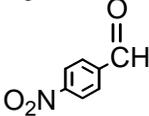


Figure 3: Molecular modeling identified GSH binding sites in AKR1A1 (top) and AKR1B1.

AKR1A1 and AKR1B1-catalyzed reduction of hydrophobic aldehydes

The reduction of aldehydes catalyzed by AKR1A1 and AKR1B1 is not limited to small reactive aldehydes. Hydrophobic aldehydes, including aldehydes that do not possess an oxidized 2-carbon, can also be reduced efficiently (Table IV). In this case, the large substrate binding pocket of AKR1A1 and AKR1B1 provides the binding energy for substrate recognition (28,30).

**Table IV. AKR1A1 and AKR1B1 --
Catalyzed Reduction of Hydrophobic Aldehydes**

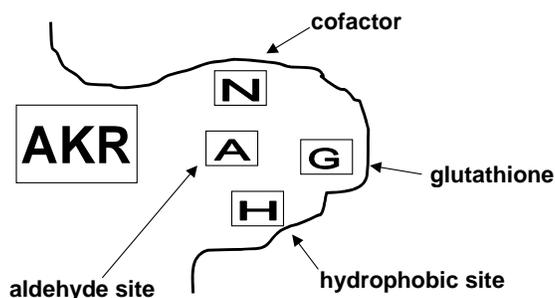
<i>Substrate</i>	$k_{cat} / K_m (M^{-1} min^{-1})$	
	<i>AKR1A1</i>	<i>AKR1B1</i>
	3.7×10^5	1.0×10^8
	2.1×10^6	1.1×10^8
	1.3×10^7	5.3×10^7

Adapted in part from data in Reference 28

Summary

In summary, AKR1A1 and AKR1B1 exhibit broad specificities for reduction of aldehydes and in some cases ketones, derived from a versatile active site (figure 3) that can accommodate reactive aldehydes with oxidized carbons at the 2-position, hydrophobic substrates, and selected glutathione adducts of aldehydes. This is consistent with the suggestion that a major function of AKR1A1 and AKR1B1 is detoxication of aldehydes.

Aldehyde Detoxication Functions of AKR1A1 and AKR1B1



- 1) **A site** – small reactive aldehydes are substrates
- 2) **N site** – NADPH is the preferred cofactor
- 3) **G site** – glutathione adducts can be substrates
- 4) **H site** – hydrophobic aldehydes are substrates

Figure 4: Summary of the active site regions of AKR1A1 and AKR1B1 that provide broad ability to catalyze the reduction of aldehydes

Acknowledgement: This work was supported by grants from the Juvenile Diabetes Foundation (1-1999-535) and National Institutes of Health (EY13695).

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