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All in the family: aldose reductase and closely related aldo-keto reductases

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Abstract. Aldose reductase catalyzes the first step in the polyol pathway and is thought to be involved in the pathogenesis of diabetic complications. In addition to polyol synthesis, aldose reductase may have multiple other activities that intersect with signal processing and oxidative defense mechanisms. Multiple aldose reductase-like proteins have been discovered to have structures and catalytic properties that broadly overlap those of aldose reductase. This chapter will summarize new insights into properties and functions of aldose reductase and closely related members of the aldo-keto reductase enzyme superfamily.

Key words. Aldo-keto reductase; diabetes; cancer; adrenal gland; vas deferens; eye.

Introduction

Aldose reductase (AR) catalyzes the NADPH-dependent conversion of glucose to sorbitol, the first step in polyol pathway of glucose metabolism (fig. 1). The pathway is completed by sorbitol dehydrogenase, which catalyzes the NAD-linked oxidation of sorbitol to fructose. Thus, the polyol pathway results in conversion of glucose to fructose with stoichiometric utilization of NADPH and production of NADH. Under normal glycemic conditions, only a small fraction of glucose is metabolized through the polyol pathway, as the majority is phosphorylated by hexokinase, and the resulting product, glucose-6-phosphate, is utilized as a substrate for glycolysis or pentose phosphate metabolism. However, in response to chronic hyperglycemia, glucose flux through the polyol pathway is markedly increased and may account for up to 33% of glucose utilization in some tissues [1]. Galactose is also a substrate for the polyol pathway, but the corresponding keto sugar is not produced because sorbitol (polyol) dehydrogenase is incapable of oxidizing galactitol [2–3]. Interest in the polyol pathway was stimulated by the observation that sorbitol levels were markedly increased in target tissues associated with diabetic complications [4, 5]. If polyol accumulation, either directly or indirectly, plays a role in the pathogenesis of diabetic complications, the catalyst of the first step in polyol synthesis would be an obvious and attractive therapeutic target for drug inhibitors. Thus, much effort has been focused on gaining a better understanding of the catalyst, AR, and in developing therapeutic strategies to block sorbitol synthesis in diabetic tissues. The goal of this review is to summarize new findings on AR, particularly in light of new insights from cell and molecular biology studies. In addition, aldose reductase

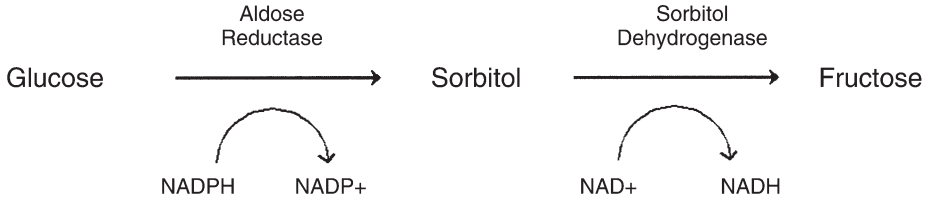

Figure 1. The polyol pathway of glucose metabolism. AR catalyzes the NADPH-dependent reduction of glucose to sorbitol. Sorbitol dehydrogenase oxidizes sorbitol to fructose in an NAD^+-linked reaction.

will be discussed in the context of its similarities and differences with structurally related members of the aldo-keto reductase (AKR) enzyme superfamily.

Structural and functional features of AR

Most organisms contain a large number of structurally related enzymes that catalyze the pyridine nucleotide-dependent reduction of carbonyl groups. Examples include aldose and aldehyde reductases, which are responsible for NADPH-linked formation of alcohol products from aldo and keto sugars, and aldehydic functional groups of aromatic and aliphatic hydrocarbons [6–8], hydroxysteroid dehydrogenases, which catalyze steroid conversion and detoxification of polycyclic aromatic hydrocarbons [9], and prostaglandin synthases [10]. Because of overlapping substrate and coenzyme specificity, a generally broad pattern of tissue distribution and similarities in subunit mass, there are many cases where identical gene products having been assigned different names and inferred functions. For example, the same protein has been purified and studied by different investigators as AR, 20α hydroxysteroid dehydrogenase and prostaglandin synthase [10–12]. Primary structural data produced from cloning and genome sequencing efforts over recent years have made it possible to minimize if not eliminate the apparent redundancy in enzyme nomenclature. These enzymes are now categorized into functionally and evolutionarily related groups based on their genetic origins [13, 14]. Thus, the superfamily of AKRs is organized into a hierarchy of families (<40% amino acid identity with other families) and subfamilies (>60% identity among constituent members). A web site created to post currently recognized members of the AKR superfamily may be viewed at www.med.upenn.edu/akr/

AR may be considered the prototypical enzyme of the AKR superfamily. The enzyme comprises 315 amino acid residues and folds into a β/α-barrel structural motif with the catalytic active site situated in the barrel core [15, 16]. The nucleotide cofactor binds in an extended conformation at the top of the β/α barrel, with the nicotinamide ring projected down in the center of the barrel and pyrophosphate straddling the barrel lip. The reaction mechanism of AR in the direction of aldehyde reduction follows a sequential ordered mechanism in which NADPH binds first, followed by aldehyde substrate binding (fig. 2). Binding of NADPH induces a conformational change (E•NADPH → E*•NADPH) that involves hinge-like movement of a surface loop so as to cover a portion of the coenzyme in a fashion similar to a safety belt. The alcohol product is formed by transfer of the *pro*-R hydride of NADPH to the *re* face of the substrate's carbonyl carbon. Following release of the alcohol product, a second conformation change is required (E*•$NADP^+$ → E•$NADP^+$) in order to release the oxidized coenzyme. Elegant kinetic studies showed that the conformation change required for $NADP^+$ release represents the rate-limiting step in the direction of aldehyde reduction [17–19]. As the rate of coenzyme release limits the catalytic rate, it can be seen that perturbation of interactions that stabilize coenzyme binding can have dramatic effects on V_{max} [20] (see below).

Location of the C-4 of the nicotinamide ring at the base of the hydrophobic cavity defined the enzyme's active site. Three residues within a suitable distance of the C-4 were identified as the potential proton donor, including Tyr-48, His-110 and Cys-298. Evolutionary, thermodynamic and molecular modeling considerations, which implicated Tyr-48 as the proton donor, were borne out by the results of mutagenesis studies [16, 21, 22]. A hydrogen-bonding interaction between the phenolic hydroxyl group of Tyr-48 and the ammonium side chain of Lys-77 is thought to help to facilitate hydrogen transfer [16]. Lys-77 is in turn salt linked to carboxylate of Asp-43. This general arrangement is conserved among other AKR subfamily enzymes whose structures have been solved, including AR/AKR1A1 [23], 3α-hydroxysteroid dehydrogenase AKR 1C9 [24], FR-1 AKR1B8 [25] and CHO reductase AKR1B9 [26]. While the overall structural features of the AKR family members are well conserved, subtle differences near the C-terminal domain are thought to be responsible for differences in substrate specificity among closely related enzymes (see below).

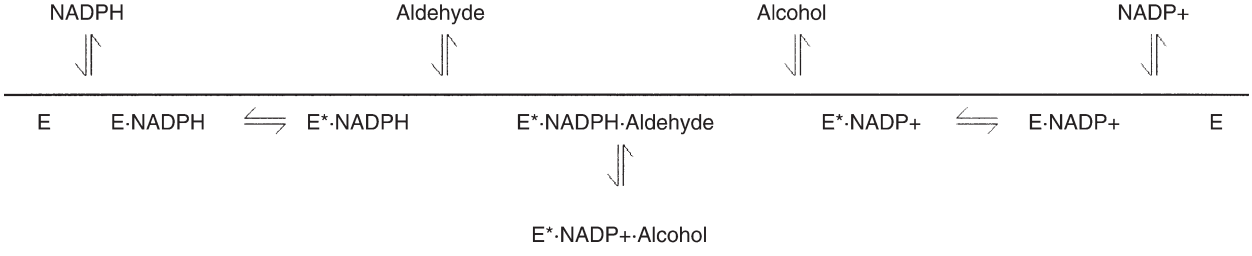

Figure 2. AR follows a sequential ordered reaction mechanism. A large conformational change involving movement of a loop akin to a safety belt occurs after binding of NADPH to free enzyme (E•NADPH → E*•NADPH) and prior to release of $NADP^+$ (E*•$NADP^+$ → E•$NADP^+$).

General kinetic features

AR has been isolated and studied from a variety of species and tissues. Placenta and muscle are typical human tissue sources for enzyme purification [27–29], although enzyme levels may be highest in the adrenal gland [30]. Kinetic and structural properties appear to be consistent regardless of tissue source. Since recombinant AR produced in *Escherichia coli*-based bacterial expression systems is essentially identical to the native enzyme purified from tissues or cultured cells [31–34], most studies now utilize the recombinant enzyme for biochemical, kinetic and structural studies.

AR exhibits a broad substrate specificity, reducing the aldehyde group of aldoses, aliphatic and aromatic aldehydes, and to a lesser extent keto groups, from aromatic and aliphatic ketones. Major endogenous substrates linked to metabolic diseases are glucose and galactose, which are converted to sorbitol and galactitol, respectively. Reactive sugars and trioses derived from aldohexoses, including methylglyoxal, glucosone and deoxyglucosone, are excellent substrates for AR [35]. Indeed, methylglyoxal is one of the best physiological substrates identified so far [36]. Several investigators have noted a marked preference of AR for hydrophobic substrates, in apparent contrast to what one would expect for an enzyme associated with metabolic disturbances involving aldo sugars [28, 37]. Endogenous substrates with marked hydrophobic character have been identified recently, including steroids and precursors [12, 38–40], norepinephrine catabolites [41], retinal isomers [42] and lipid-derived aldehydes [43].

In relative terms, glucose is a poor substrate for AR. The apparent K_m value for glucose has been reported by various groups to be in the range of 50–200 mM, well above normal physiological levels [29, 34] but not unreasonable considering that the reducible (acyclic carbonyl species) form of glucose represents less than 0.1 % of the substrate population. Nevertheless, the catalytic efficiency of aldose reductase is approximately four orders of magnitude lower for D-glucose than for lipid-derived aldehydes, including 4-hydroxy-2-nonenal [35, 44]. The poor apparent kinetic efficiency of AR with glucose suggests that the enzyme is maximally active only when intracellular hexose concentrations rise to abnormally high levels. That glucose is an endogenous substrate of AR has been convincingly demonstrated in a variety of experimental settings. In diabetic animals, sorbitol production correlates with tissue levels of AR – low levels of sorbitol accumulate in the diabetic mouse lens, which contains low levels of AR [45]. In contrast, rat and gerbil lenses, which are endowed with an abundance of AR, accumulate pathological levels of polyols when exposed to high sugar levels [46]. Transgenic overexpression of the AR gene causes polyol accumulation in targeted tissues following diabetes induction [47, 48]. The opposite effect is obtained when the AR gene is knocked out; sorbitol production in the kidney is essentially eliminated in AR-null mice [49]. On the basis of these observations, there seems little doubt that AR is an in vivo catalyst of polyol synthesis.

Inhibitor binding

Structural studies of the AR holoenzyme complexed with drugs revolutionized our understanding of interactions that occur to stabilize inhibitors in the enzyme active site [50–52]. Most tight-binding AR inhibitors (ARIs) have a polar group, usually a carboxylate, tethered from a hydrophobic core contributed by one or more ring structures. Inhibitors bind with their polar head groups oriented close to the pyridine ring, usually forming hydrogen bonding interactions involving residues Tyr-48, His-110 and Tyr-111. Extensive hydrophobic interactions between inhibitors and residues that line the deep hydrophobic active site cavity help to stabilize the ternary enzyme-coenzyme-inhibitor complex. Structural details of inhibitor binding and interactions will be addressed in greater length in other chapters in this Multi-author review.

Posttranslational regulation of AR activity

Conflicting reports have been made on whether AR activity is subject to posttranslational regulation, and in particular whether the enzyme is activated in diabetic tissues. Several investigators have reported evidence for an activated form of AR, distinguishable from the native enzyme form by differences (typically increases) in K_m for aldehyde substrates and by a marked reduction in sensitivity to ARIs [53–57]. Of potential physiological importance, levels of the activated form were found to be elevated in diabetic tissues [53]. Activated enzyme forms are generally less susceptible to inhibition by ARIs, which suggests that the therapeutic effectiveness of such drugs would be compromised if a large fraction of the enzyme underwent conversion to the activated form.

Hints that disulfide-mediated posttranslational modifications could play a role in enzyme activation came from studies which demonstrated that activation was coincident with a loss of reactive thiol groups, presumably through formation of a disulfide bond [58, 59]. Treatment of the AR apoenzyme with thiol-modifying agents such as iodoacetic acid (IAA) or *N*-ethylmaleimide (NEM) causes an increase in K_m for most substrates and a decrease in sensitivity to inhibition by ARIs. These functional changes are largely prevented if the enzyme is complexed with NADP(H) prior to treatment with modifying agents. Sequencing of peptides containing cysteine side chains covalently modified by treatment of the enzyme with iodoacetic acid revealed that Cys-298 was

a highly reactive cysteine group [60]. Crystallography studies revealed that of the six cysteine residues in human AR, three (namely Cys-80, Cys-298 and Cys-303) were accessible to solvent in the binary enzyme-NADPH complex [61]. Of these, only Cys-298 was located in sufficient proximity to the active site to be a candidate catalytic side chain. Conversion of Cys-298 to serine (C298S) or alanine (C298A) resulted in an enzyme form that was resistant to modification with reagents that are known to cause functional changes in enzyme activity [34, 62]. Cys-298 is also a site for thiolation of AR by oxidized glutathione [63]; glutathiolated AR is catalytically inactive, most likely due to blockade of the catalytic site due to steric interference and interactions between the glycyl carboxylate of glutathione and His-110 at the AR active site [63]. Proximity of Cys-298 to the active site has been demonstrated by affinity-labeling studies [38]. For example, treatment of AR with an alkylating substrate analog, 16α-bromoacetoxyprogesterone, resulted in a time- and concentration-dependent loss of enzyme activity. However, a C298S mutant was insensitive to inactivation by this affinity-labeling reagent [38]. 4-Hydroxy-2-nonenal modifies AR predominantly at the Cys-298 position, resulting in an enzyme form with reduced sensitivity to AR inhibitors [43].

Recent evidence suggests that nitric oxide (NO), acting through a posttranslational mechanism, could be a regulator of AR activity. Treatment of AR with NO donors such as diethylamine NONOate causes a time- and concentration-dependent increase in activity by a mechanism consistent with direct nitrosation of the enzyme at a single site [64, 65]. Mutagenesis studies revealed that the likely site of NO-modification was at Cys-298. Rather than activate, *S*-nitroso-glutathione (GS-NO) causes inhibition of the enzyme by a mechanism that most likely involves formation of a mixed disulfide. Electrospray mass spectroscopic analysis suggests that the mixed disulfide is formed between glutathione and Cys-298 [66]. These results are consistent with other studies which showed that formation of a mixed disulfide between glutathione and AR (at position Cys-298) results in loss of enzyme activity [63]. Results from animal studies agree with the in vitro experiments carried out with purified recombinant proteins. Treatment of rat aortic tissues with NO donors, which generate *S*-nitroso-glutathione, leads to a loss of AR activity and a marked reduction in sorbitol synthesis [64, 67, 68].

As summarized above, several lines of evidence point to Cys-298 as an important regulatory site on AR. When complexed with NADP, AR is less susceptible to modification by several different agents, including glutathione, NO and 4-hydroxy-2-nonenal (HNE). This suggests that the cysteine side chain is involved with stabilizing the coenzyme in such a way that the reactive thiol group becomes inaccessible. Due to tight binding affinity, most endogenous AR will be complexed with NADP(H) in vivo. Therefore, the enzyme should be susceptible to modification through Cys-298 only during a catalytic cycle when nucleotide exchange occurs. Therefore, endogenous regulators that act through Cys-298 should be most effective when substrate levels rise to a sufficient level to stimulate catalytic turnover.

AR in metabolism and signaling

Current evidence suggests that AR contributes to metabolic imbalances associated with diabetes and its complications in the eye and peripheral nervous system [69]. While it is generally accepted that AR-mediated pathogenesis is dependent on chronically elevated ambient hexose levels, such as in diabetes mellitus and galactosemia, we still do not know what beneficial role(s) AR fulfills in the cell when hexose levels are normal. AR gene expression is widespread, as evidenced by the presence of gene transcripts in a large number of human tissue libraries constructed for analysis by expressed sequence tag (EST) or serial analysis of gene expression (SAGE) techniques. Interested readers can browse to the UniGene Cluster Hs.75313 *Homo sapiens* at http://www.ncbi.nlm.nih.gov/UniGene/ for a current listing of complementary DNA (cDNA) sources in which AR is represented. That AR gene expression is so broadly distributed suggests that the enzyme might function physiologically as a general housekeeping enzyme under normal conditions. In addition, new evidence points to a potential role for AR in cytokine-mediating signaling processes. The following sections will summarize new information on potential physiological roles for AR in euglycemia.

Osmotic regulation

The kidney is one of the richest tissue sources of AR, with most of the enzyme localized in the medullary portion [70], from which quantities of the enzyme have been isolated for biochemical study [71–73]. Sorbitol is a nonperturbing osmolyte in kidney inner medullary cells, where its synthesis, along with other osmolytes such as inositol, glycerophosphorylcholine and betaine, functions to counterbalance the hyperosmotic extracellular fluids during antidiuresis [74–76]. The functional consequence of AR expression in kidney medulla was made clear when it was discovered that exposure of a line of renal papillary epithelial cells to increased extracellular osmolarity stimulated an increase in intracellular sorbitol [77]. Studies showed that AR gene expression in a variety of different types of tissue culture cells, in addition to kidney-derived cells, is strongly induced under hyperosmotic conditions [78–81]. Transcriptional induction

leads to an accumulation of enzyme protein and sorbitol synthesis [82, 83]. To explore the relative importance of AR-mediated sorbitol synthesis to osmoregulation, a gene knockout approach has been taken. Kidneys of AR knockout mice are morphologically normal; however, these mice are unable to concentrate their urine to normal levels [49, 70]. At this point, it is not clear why the absence of AR (and presumably its metabolic product sorbitol) leads to a defect in water resorption, as sorbitol constitutes only ~2% of the total osmolality of the wild-type mouse kidney [49]. Aida and co-workers have speculated that elevated serum and urinary levels of the divalent cations calcium and magnesium observed in AR-null mice may play a role, but a mechanism linking divalent cations and diabetes insipidus is as yet undetermined [70]. Other factors associated with water resorption, such as response to the antidiuretic hormone arginine vasopressin (AVP) or function of aquaporin 2, have been ruled out as factors in the mechanism of the diabetes insipidus phenotype [49]. It is interesting to note that while ablation of the AR gene results in this defect in kidney function in mice, no such phenotype has been reported when animal models from other species (rat) are treated with ARIs. This suggests either that ARIs are not sufficiently effective to reduce sorbitol levels enough to cause a defect in urinary concentration, or that other mechanisms, not present in the mouse kidney, are able to functionally compensate for the loss of AR activity.

Detoxification

While AR is thought to play a major role in the synthesis of sorbitol as an osmolyte in the kidney medulla, its distribution among tissues unaffected by extracellular osmotic stress suggests an alternate metabolic role. In addition, the marked hydrophobic nature of the active site is unusual for an enzyme thought to be involved with metabolism of aldo sugars. While the catalytic preference of AR for hydrophobic substrates, and sensitivity to inhibition by fatty acids, was observed in early studies [37], approximately 3 decades passed before in vitro studies showed that HNE was a probable endogenous substrate [35, 84]. HNE is derived from the oxidation of arachidonic acid and in most cells is converted to a glutathione conjugate. Remarkably, AR is able to reduce the free aldehyde as well as its glutathione conjugate [85–87]. Recent studies have shown that AR is the major oxidoreductase responsible for conversion of HNE to HNE in the heart [43, 88]. Other byproducts of oxidative stress, such as methylglyoxal, which is a potent protein crosslinking agent, and 3-deoxyglucosone [89], have been shown to be excellent substrates for AR [36, 90]. These studies suggest that AR could fulfill a role as an oxidative defense protein. In addition to its ability to detoxify lipid-derived aldehydes, AR may be involved in detoxification of environmental toxins such as acrolein [91]. Treatment of tumor cells with an ARI enhances the cytotoxic effects of some anticancer drugs such as cisplatin and doxorubicin, which suggests that AR is involved in the metabolic inactivation of these drugs or their by products [92]. Grimshaw has offered compelling arguments that kinetic and thermodynamic properties endow AR with unique abilities to function as an NADPH-dependent AKR [93]. The large, accommodative active site cavity also makes the enzyme suitable to react with a broad range of potentially toxic aromatic and aliphatic aldehydes [16].

Protein kinase C activation

Mounting evidence suggests that AR interacts in some way with signaling cascades involving protein kinase C (PKC). Activation of the diacyl glycerol (DAG)-PKC pathway is thought to be a key factor in the pathogenesis of vascular complications of diabetes [94]. Cultured human kidney mesangial cells demonstrate elevated synthesis of transforming growth factor (TGF-β) when exposed to high glucose (33 mM). Treatment of human kidney mesangial cells with epalrestat, a potent ARI, prevents the glucose-induced activation of PKC. Direct enzyme measurements showed that epalrestat prevented the appearance of membrane-associated (activated) PKC [95]. In a different experimental setting, Ramana et al. [96] evaluated the effect of AR inhibition on tissue necrosis factor (TNF-α)-mediated rat vascular smooth muscle cells (VSMC) growth. They demonstrated that induction of nuclear factor kappa B (NF-κB) by TNF-α in the rat aorta can be blocked by ARIs. Blockade of NF-κB (inhibitor of NF-κB) activation was associated with a reduction in Iκ-Bα phosphorylation and a reduction in NF-κB translocation from cytoplasm to nucleus. PKC activation, which occurs downstream of TNF-α receptor activation, was also blocked by ARIs. In concordance with work carried out in mesangial cells, these results suggest that AR, either directly or through an enzymatic product, may be an obligatory requirement for activation of PKC. Direct activation of PKC by the phorbol ester PMA was not inhibited by an ARI, indicating that the inhibitory effect was taking place upstream of PKC. This effect is not limited to vascular cells, as essentially the same effects of AR ablation were observed in a transformed lens epithelial cell line, HLE-B3 [97]. Possible interactions between PKC-β and the polyol pathway were observed also in a study of rat aortic smooth muscle cells. Glucose-induced cellular hyperproliferation and PKC-β activation were effectively inhibited by epalrestat and the PKC-β inhibitor LY333531 [98]. Intriguing new evidence suggests that AR itself may be a direct substrate of PKC. Treatment of cells with potent stimulators of PKC such as bryostatin-1 resulted in phosphorylation of AR. Studies with recombinant AR

demonstrated that the PKCα isoform was the most efficient, while βII and ε isoforms were also able to phosphorylate AR [99]. The functional consequences of AR phosphorylation are not yet known.

Genetic complexity of AR and related enzymes

Multiplicity of AKR1B genes

Nucleotide sequencing of complementary DNA libraries and clones constructed from human, rat and mouse tissues have provided direct evidence that the AKR1B subfamily of AKRs contains several proteins with very high structural similarity to AR. A list of the genes and their corresponding GenBank accession numbers are shown in table 1. Two human AKR1B genes, encoding AR (AKR1B1) and small intestine reductase/AR-like protein 1 (AKR1B10), are located within about 68 kilobases of each other in opposite orientations on chromosome 7q35. Each gene is organized into 10 exons, with the AR gene covering 17.1 kilobases and the HSIR/ARL-1 gene covering almost 14 kilobases (fig. 3). Similarly, the two rat AKR1B genes, AR and AR-like protein, are located on chromosome 4. The mouse genome contains three transcriptionally active AKR1B genes: AR, FR-1 and MVDP. They are tandemly arrayed on chromosome 6 in a region syntentic to human chromosome 7q35 (fig. 3). Primary structures of the selected AKR1B polypeptides are shown in figure 4. It can be readily appreciated that within a given species, there is a high degree of amino acid identity over most of the sequence. Major observations that led to the identification of the newly recognized AKR1B genes are summarized in the following sections.

FR-1

Winkles was the first to recognize that expression of some AKRs can be induced by mitogens [100]. Differential display analysis of murine fibroblasts following treatment with fibroblast growth factor-1 identified an anonymous transcript that was upregulated with delayed-early kinetics. Full-length cDNA clones corresponding to this transcript showed a high degree of similarity to AR (see fig. 4). The encoded protein, designated FR-1 (FGF regulated 1), is 70% identical to human AR. Mitogen-induction of FR-1 gene expression required de novo protein synthesis, which indicates that immediate early proteins are required for transcriptional induction and/or transcript stabilization. The time course of FR-1 protein accumulation in growth factor-stimulated fibroblasts was somewhat delayed, indicating that the protein may be associated with cell cycle progression [101]. FR-1 gene transcripts were readily detected in many adult tissues by Northern blot or RNase protection experiments; these included heart, ovary and testis [100, 102]. Liver-specific FR-1 expression levels showed marked developmental regulation, as high levels were observed in the newborn liver, while no transcripts were detected in the adult. Unlike AR, the FR-1 gene is not induced by hyperosmolarity [101]; this is not surprising since the FR-1 promoter region does not contain one or more osmotic response elements (OREs) like those found associated with the AR gene [103, 104]. To date, surprisingly little is known about transcriptional regulation of the FR-1 gene, and in particular what elements of the mitogen signaling cascade interact with the FR-1 gene promoter to effect upregulation of gene transcription and/or messenger RNA (mRNA) stabilization.

Kinetic study of FR-1 demonstrated that the encoded protein is a functional AKR [105]. Like AR, FR-1 was found

Table 1. Entrez accession numbers for AKR1B subfamily members.

AKR nomenclature	Common name	Species	Protein	Genomic	Genbank	RNA
1B1	aldose reductase	human	NP_001619	NT_007933	BC000260	NM_001628
1B3	aldose reductase	mouse	NP_033788	NT_039341	BC021655	NM_009658
1B4	aldose reductase	rat	NP_036630	NW_043747	M60322	NM_012498
1B5	aldose reductase	bovine	AAA30370		M31463	M31463
1B7	major vas deferens protein (MVDP)	mouse	NP_033861	NT_039341	J05663	NM_009731
1B8	fibroblast growth factor induced-1 (FR-1)	mouse	NP_032038	NT_039341	U04204	NM_008012
1B9	chinese hamster ovary reductase	hamster	AAC53199	Not available	U81045	CGU81045
1B10	small intestine reductase/aldose reductase-like protein 1	human	NP_064695	NT_007933	BC008837	NM_020299
1B13	aldose reductase-like protein 1	rat	NP_775159	NW_043747	AJ277957	NM_173136

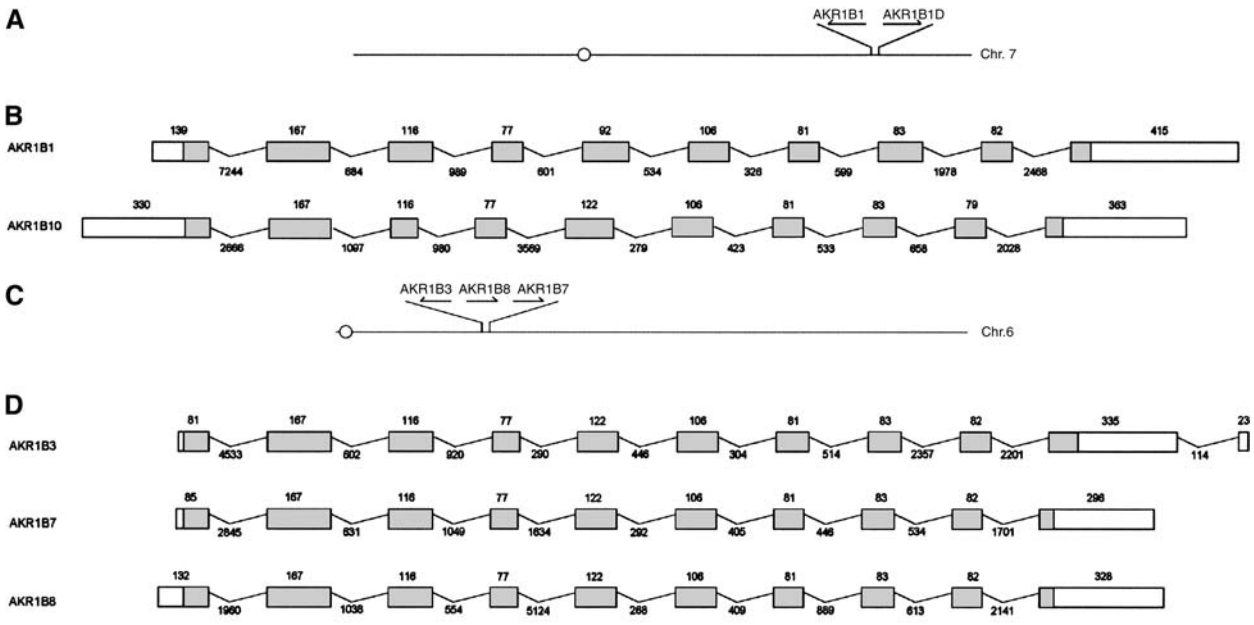

Figure 3. Structural organization of genes encoding selected AKR1B subfamily members. Filled boxes are coding regions within exons. Unfilled portions are noncoding exon sequences.

A

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                *         20         *         40         *         60         *         80
AKR1B1  : MASRLLLNNGAKMPILGLGTWKSPPGQVTEAVKVAIDVGYRHIDCAHVYQNENEVGVAIQEKLREQVVKREELFIVSKLW :  80
AKR1B10 : MATFVELSTKAKMPIVGLGTWKSPLGKVKEAVKVAIDAGYRHIDCAYVYQNEHEVGEAIQEKIQEKAVKREDLFIVSKLW :  80

                *        100         *        120         *        140         *        160
AKR1B1  : CTYHEKGLVKGACQKTLSDLKLDYLDLYLIHWPTGFKPGKEFFPLDESGNVVPSDTNILDTWAAMEELVDEGLVKAIGIS : 160
AKR1B10 : PTFFERPLVRKAFEKTLKDLKLSYLDVYLIHWPQGFKSGDDLFPKDDKGNAIGGKATFLDAWEAMEELVDEGLVKALGVS : 160

                *        180         *        200         *        220         *        240
AKR1B1  : NFNHLQVEMILNKPGLKYKPAVNQIECHPYLTQEKLIQYCQSKGIVVTAYSPLGSPDRPWAKPEDPSLLEDPRIKAIAAK : 240
AKR1B10 : NFSHFQIEKLLNKPGLKYKPVTNQVECHPYLTQEKLIQYCHSKGITVTAYSPLGSPDRPWAKPEDPSLLEDPKIKEIAAK : 240

                *        260         *        280         *        300         *        320
AKR1B1  : HNKTTAQVLIRFPMQRNLVVIPKSVTPERIAENFKVFDFELSSQDMTTLLSYNRNWRVCALLSCTSHKDYPFHEEF---- : 316
AKR1B10 : HKKTAAQVLIRFHIQRNVIVIPKSVTPARIVENIQVFDFKLSDEEMATILSFNRNWRACNVLQSSHLEDYPFDAEY---- : 316
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B

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                *         20         *         40         *         60         *         80
AKR1B3 : MASHLELNNGTKMPTLGLGTWKSPPGQVTEAVKVAIDLGYRHIDCSQVYQNEKEVGVALQEKLKEQVVKRQDLFIVSKLW :  80
AKR1B7 : MATFVELSTKAKMPLVGLGTWKSSPGQVKEAVKAAIDAGYRHIDCAYVYHNENEVGEAIQEKIKENAVKREDLFIVSKLW :  80
AKR1B8 : MATFVELSTKAKMPIVGLGTWKSPPNQVKEAVKAAIDAGYRHIDCAYAYCNENEVGEAIQEKIKEKAVQREDLFIVSKLW :  80

                *        100         *        120         *        140         *        160
AKR1B3 : CTFHDKSMVKGAFQKTLSDLQLDYLDLYLIHWPTGFKPGPDYFPLDASGNVIPSDTDFVDTWTAMEQLVDEGLVKTIGVS : 160
AKR1B7 : ATFFEKSLVKKAFDNTLSDLKLDYLDLYLVHWPQGFQAGNALLPKDNKGKVLLSKSTFLDAWEAMEELVDQGLVKALGIS : 160
AKR1B8 : PTCFEKKLLKEAFQKTLTDLKLDYLDLYLIHWPQGLQPCKELFPKDDQGRILTSKTTFLEAWEGMEELVDQGLVKALGVS : 160

                *        180         *        200         *        220         *        240
AKR1B3 : NFNPLQIERILNKPGLKYKPAVNQIECHPYLTQEKLIEYCHSKGIVVTAYSPLGSPDRPWAKPEDPSLLEDPRIKAIAAK : 240
AKR1B7 : NFNHFQIERLLNKPGLKHKPVTNQIESHPYLTQEKLIQYCQSKGIAVTAYSPLGSPDRPYAKPEDPVVMEIPKIKEIAAK : 240
AKR1B8 : NFNHFQIERLLNKPGLKHKPVTNQVECHPYLTQEKLIQYCHSKGISVTAYSPLGSPDRPSAKPEDPSLLEDPKIKEIAAK : 240

                *        260         *        280         *        300         *        320
AKR1B3 : YNKTTAQVLIRFPIQRNLVVIPKSVTPVRIAENLKVFDFEVSSEDMATLLSYNRNWRVCALMSCAKHKDYPFHAEV----- : 316
AKR1B7 : HKKTVAQVLIRFHVQRNVVVIPKSVTPSRIQENLQVFDFQLSEEDMAAILSFNRNWRACDLLDARTEEDYPFHEEY----- : 316
AKR1B8 : HEKTSAQVLIRFHIQRNVVVIPKSVTPSRIQENIQVFDFQLSDEEMATILSFNRNWRACLLPETVNMEEYPYDAEY----- : 316
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Figure 4. Alignment of AKR1B primary structures. Amino acid identities are highlighted. (*A*) Human AKR1B1 (AR) and AKR1B10 (small intestine reductase/AR-like protein 1]; (*B*) mouse AKR1B3 (AR), AKR1B7 (MVDP, major vas deferens protein), AKR1B8 (FR-1, FGF regulated-1).

to catalyze the NADPH-dependent reduction of a variety of aliphatic and aromatic aldehydes, including the reactive lipid-derived aldehyde, HNE. FR-1 had negligible reactivity with aldoses and sugar ketoses, indicating that these compounds are unlikely to be in vivo substrates. Although FR-1 differs from AR in ability to produce polyols, the enzymes share sensitivity to inhibition by some ARIs. FR-1 is sensitive to the carboxylic acid inhibitors tolrestat and zopolrestat, as evidenced by IC_{50} values of approximately 134 nM and 71 nM, respectively [105].

Given their similarities in primary structure but differences in ability to reduce aldoses, it was of interest to determine the three-dimensional structure of the FR-1 holoenzyme in hopes of identifying a structural basis for the measurable differences in substrate specificity. The structure of FR-1 in complex with NADPH and zopolrestat revealed striking similarity to the human AR holoenzyme-inhibitor complex, particularly in terms of the binding interactions that stabilize the inhibitor at the enzyme active site [25, 50]. While the overall architecture and hydrophobicity of the active site cavity are well conserved between the AR and FR-1 ternary complexes, differences in the position of a segment of residues at the carboxy terminus creates a slightly larger opening which could potentially accommodate larger substrates. This portion of the carboxy-terminal region appears to be the most divergent among AKR1B subfamily members. However, there is as yet no evidence that differences in substrate specificity among individual enzymes is conferred by this region. Due to the lack of reactivity measured with glucose or galactose, it seems unlikely that FR-1, acting as an AR, could contribute to the pathogenesis of diabetic complications through synthesis of polyols. Clearly, the favorable kinetic constants with lipid-derived aldehydes indicate that FR-1 could function as an oxidative defense enzyme [105]. In addition, the high level of FR-1 expression in the adrenal gland, testis and ovary suggests that the protein could be involved with other metabolic pathways which are yet to be identified [102].

MVDP

Epithelial cells that line the vas deferens secrete an assortment of proteins that serve to enhance the survival of sperm. In a search for androgen-dependent vas deferens proteins, Taragnat and co-workers found a major protein species, designated the major vas deferens protein (MVDP), that accounts for over 40% of soluble protein in the vas deferens luminal fluid [106]. cDNA cloning studies revealed that MVDP is a 316-amino acid polypeptide with 82% sequence identity to FR-1 (fig. 4) and 70% identity to human AR [107].

Expression of MVDP in epithelial cells of the vas deferens appears to be highly restricted by species, as transcripts in rat, rabbit and human vas deferens are not detectable by Northern blotting [108]. MVDP gene transcripts are also found at high levels in the zona fasciculata of the mouse adrenal cortex [102, 109], suggesting a possible physiological role in steroidogenic tissues [109]. Using a sensitive RNase protection assay, Lau and coworkers found moderate levels of MVDP transcripts in the mouse intestine and eye, but these were far lower than in the adrenal and vas deferens [102].

Since the zona fasciculata is the major site of corticosteroid synthesis in the adrenal gland, it is interesting to speculate on whether MVDP could participate in steroid biosynthesis or its regulation. Evidence in favor of this possibility was provided by recent studies which showed that MVDP, besides having a generally broad specificity toward aldehyde-containing substrates, efficiently reduces the aldehyde group of isocaproaldehyde, which is a toxic byproduct of steroid biosynthesis [109]. By differentially controlling AR and MVDP gene expression levels in adrenocortical Y1 cells, Martinez and coworkers demonstrated that MVDP and not AR is the major reductase for isocaproaldehyde and HNE [109].

While the primary structures of AR and MVDP are highly conserved, some functional properties of the enzymes are quite distinct. For example, MVDP shows less preference than AR or FR-1 for NADPH over NADH as the reducing cofactor. Nonetheless, the catalytic efficiency of MVDP, using aldehydes from several structural classes, is higher with NADPH than with NADH [109]. Using isocaproaldehyde as substrate, MVDP is less sensitive than AR to several ARIs, including sorbinil, imiristat and tolrestat [40, 109]. Further study will be necessary to determine whether this is a general trend or whether the relatively diminished inhibitor sensitivity is substrate dependent. MVDP is essentially inactive with glucose and galactose, which indicates that it does not function as a source of polyol synthesis in vivo.

Expression of MVDP is strongly influenced by androgens. Castration causes a marked reduction in the abundance of this protein, but postsurgical treatment with testosterone brings about recovery to normal levels. Run on transcription experiments showed that androgen-regulated changes in MVDP levels are controlled at the level of gene transcription [110]. To get at functional elements in the MVDP promoter, transgenic studies using MVDP promoter constructs driving chloramphenicol acetyltransferase (CAT) expression were carried out. The region at –1804 to –510 (relative to the transcription start site) is necessary for expression in vas deferens, whereas the region spanning –510 to +41 is necessary for adrenal expression [109, 111]. Significantly, CAT expression levels were markedly increased when the transgene construct included sequences derived from an intragenic region spanning introns 1 and 2 and exon 2. Thus, an intragenic enhancer is required for full transcriptional response. Of two probable androgen response elements (AREs) contained

within the –1804/+41 promoter region, only the proximal ARE located in the –510 to +41 region is responsible for androgen responsiveness in the adrenal gland. Studies using transient transfections showed that mutation of the proximal ARE abolished androgen induction [112].

HSIR/ALR-1

Identification of AR-like genes, such as FR-1 [100], MVDP [107] and CHO reductase [113], in the mouse and guinea pig, respectively, stimulated a search for orthologous genes in human tissues. One such gene, designated human small intestine reductase (HSIR), was identified by polymerase chain reaction (PCR) amplification from multiple tissue sources. Sequence alignment showed that the primary structure of HSIR (AKR1B10) is remarkably similar to human AR, with 70% sequence identity. Based on a blot analysis of multiple tissue RNAs, gene transcript levels of HSIR closely parallel those of AR, with a particular abundance being found in the adrenal gland [114]. Independently, Cao and co-workers found an allelic variant of the same gene in a low-stringency hybridization screen of cDNA library constructed from human hepatocellular carcinoma tissue [115]. Designated AR-like protein 1 (ALR-1), the gene product is essentially identical to HSIR. Northern blotting studies showed high transcript levels in small intestine and colon, with substantial but relatively lower levels in adult liver and thymus (the adrenal gland was not examined). Preliminary kinetic studies showed that HSIR/ALR-1 (AKR1B10) is similar to AR in its utilization of a broad range of aldehyde substrates, including aromatic and aliphatic aldehydes and sensitivity to some ARIs [42, 115].

Tumor-associated AKRs

AR-like proteins are becoming increasingly recognized as tumor-associated proteins [116–119]. Comparison of normal and hepatocellular carcinoma-derived proteins by two-dimensional electrophoresis revealed a rat AR-like protein (rALP-1) that was significantly elevated in carcinomas induced by the nitroso compounds MNU (*N*-methyl-*N*-nitrosourea), DENA (diethylnitrosamine) and NNM (*N*-nitrosomorpholine) but not in NAF (nafenopin). Sequence analysis and expression studies using the rALP-1 cRNA (complementary RNA) demonstrated nonidentity with rat lens AR (AKR1B4). However, sequence analysis alone was not sufficient to establish whether rALP-1 is orthologous to other characterized mammalian AR-like proteins such as MVDP (AKR1B7), FR-1 (AKR1B8) or HSIR/HARL-1 (AKR1B10) [117]. Enhanced expression of rat AR (AKR1B4) in normal fetal liver and adult liver tumors but not in normal adult liver is consistent with the notion that genes expressed in hepatocellular carcinomas reflect a profile characteristic of fetal liver. In a screen of human liver tumor samples, Cao and co-workers identified a human AR-like protein, designated ARL-1, that appears to be orthologous to rALP-1 from Zeindl-Eberhart's studies in rat [115]. Based on Northern blot analysis, the human ARL-1 (AKR1B10) was found to be abundantly expressed in ~54% of 24 tumor samples examined, whereas AR (AKR1B1) was upregulated in about 30%. Scuric and co-workers also identified this gene in a differential display study of upregulated genes in human hepatocellular carcinoma [120].

Recent studies suggest that AR or possibly closely related enzymes of the AKR1B subgroup may influence the effectiveness of chemotherapeutic drugs. Hyndman and Flynn identified HSIR (AKR1B10) as the apparent human ortholog to a reductase previously identified in CHO as a factor responsible for resistance to a cytotoxic aldehyde [121, 122]. Transcripts for AR and HSIR/ARL-1 are frequently elevated in transformed cell lines derived from a variety of tissues. However, transcripts from HSIR/ARL-1 appear to be more frequently induced in comparison with AR.

The cytotoxic response of HeLa cells to doxorubicin and cisplatin appears to be modulated in part by AR [123]. Activation of the extracellular signal-regulated kinase (ERK) pathway, which is an early event characteristic of HeLa cells stimulated to undergo apoptosis following treatment with doxorubicin or cisplatin, was enhanced when cells were co-treated with the ARI EBPC [ethyl-1-benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate]. Thus, AR inhibition enhanced ERK activation and resulted in a more pronounced cytotoxic response to these chemotherapeutic drugs. Studies with an MEK inhibitor showed that the ARI effect was upstream of ERK. While the role of AR in this process is not yet clear, the authors speculate that the enzyme might impact on the metabolism or transport of doxorubicin or cisplatin. Similar to the case with CHO reductase-mediated resistance to aldehyde cytotoxicity, a correlation between drug sensitivity and AR gene expression in liver tumors provides strong evidence for a direct effect of AR on drug resistance [116]. Cells induced to overexpress AR are more resistant to the cytotoxic effects of daunorubicin in comparison with control cells or with induced cells simultaneously treated with an AR inhibitor [124]. While studies have not conclusively shown whether this effect is due to the effects of one or more AKRs that share sensitivity to ARIs, the evidence clearly points to a potential role for this group of enzymes in regulating the effectiveness of some chemotherapeutic agents.

Conclusion and perspective

Diabetes mellitus is recognized as a leading cause of new cases of blindness, and is associated with increased risk

for painful neuropathy, heart disease and kidney failure. Many theories have been advanced to explain mechanisms leading to diabetic complications, including accelerated protein glycation, altered signaling involving PKC, excessive oxidative stress and stimulation of glucose metabolism by the polyol pathway. While it is highly unlikely that any one mechanism can explain pathogenesis associated with chronic hyperglycemia, a better understanding of each potential point of imbalance is likely to uncover possible entry points for therapeutic intervention. Drugs designed for a precise purpose often produce unintended effects, and ARIs are probably no exception. The challenge is to learn from experience by gaining a better understanding of the playing field: What pathways are the unexpected targets of ARIs, and do they offer new strategies for intervention?

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