Inhibition of Human Aldose Reductase-Like Protein (AKR1B10) by α- and γ-Mangostins, Major Components of Pericarps of Mangosteen

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A human member of the aldo-keto reductase (AKR) superfamily, AKR1B10, was recently identified as both diagnostic marker and therapeutic target in the treatment of several types of cancer. In this study, we have examined AKR1B10 inhibition by five xanthone derivatives, components of pericarps of mangosteen, of which α- and γ-mangostins show potential anti-cancer properties. Among the five xanthones, γ-mangostin was found to be the most potent competitive inhibitor (inhibition constant, 5.6 nM), but its 7-methoxy derivative, α-mangostin, was the second potent inhibitor (inhibition constant, 80 nM). Molecular docking of the two mangostins in AKR1B10 and site-directed mutagenesis of the putative binding residues revealed that Phe123, Trp220, Val301 and Gln303 are important for the tight binding of γ-mangostin, and suggested that the 7-methoxy group of α-mangostin impairs the inhibitory potency by altering the orientation of the inhibitor molecule in the substrate-binding site of the enzyme.

Key words aldo-keto reductase 1B10; aldose reductase; mangostin; xanthone; anti-cancer property; molecular docking

A human member of the aldo-keto reductase (AKR) superfamily, AKR1B10, is a reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reductase, which was originally identified as a human aldose reductase (AKR1B1)-like protein that is up-regulated in hepatocellular carcinomas.[1] Over-expression of AKR1B10 has been also observed in other tumors, such as smokers' non-small cell lung carcinomas,[2] uterine carcinomas,[3] cholangiocarcinomas,[4] pancreatic carcinoma,[5] and breast cancer.[6] The silencing of the AKR1B10 gene results in growth inhibition of cancer cells[5–8] and hepatocellular carcinoma xenografts in mice,[9] and its elevated expression in turn promotes proliferation of cancer cells,[10,11] indicating that the enzyme participates in tumor development. Due to its high catalytic efficiency towards aliphatic aldehydes, retinals and isoprenyl aldehydes,[1,6–8,12,13] the roles suggested for AKR1B10 in cell carcinogenesis and tumor development are detoxification of cytotoxic carbonyls derived from lipid peroxidation,[6–8] decrease in retinoic acid synthesis,[12] and modulation of protein prenylation.[6,11,13] In addition, AKR1B10 is suggested to promote fatty acid synthesis in liver cancer cells by blocking the ubiquitin-dependent degradation of acetyl CoA carboxylase.[8] Thus, this enzyme has been recognized not only as a potential diagnostic and/or prognostic marker, but also as a potential therapeutic target for the prevention and treatment of the above types of cancer. However, because of the high structural similarity between AKR1B10 and AKR1B1 that plays distinct roles in glucose and prostaglandin metabolism,[14,15] the selective inhibition of AKR1B10 over AKR1B1 is required for the development of treatments targeting the types of cancer linked to AKR1B10.

Synthetic and natural compounds that show inhibitory effects on AKR1B10 have been reported, as reviewed by Matsunaga *et al.*[11] Some inhibitors suppress the proliferation of AKR1B10-overexpressing cancer cells,[10,11,16] and as such a therapeutic method to reprogram and eliminate human solid hepatic tumor cells with an acyclic retinoid and AKR1B10 inhibitor has been recently developed.[17] Among the inhibitors, plant products, curcumins[18] and triterpenoids,[16] are relatively potent and act as selective inhibitors of AKR1B10, and their inhibitory activities are believed to be responsible for their anti-cancer properties. In order to elucidate the relationship between plant-derived compounds and their anti-cancer properties, we have examined the inhibitory potencies of five xanthones against AKR1B10 and AKR1B1. The inhibitors are constituents of the pericarp of mangosteen (*Garcinia mangostana* Linn) and were reported to show anti-cancer effects.[19,20] Since γ-mangostin was found to be a more potent inhibitor of AKR1B10 than α-mangostin, their binding modes in the active site of the enzyme were also investigated by docking simulation and site-directed mutagenesis of the binding-site residues of AKR1B10.

MATERIALS AND METHODS

Isolation of Xanthones α- and γ-Mangostins, mangostinone, 1,5-dihydroxy-2-isoprenyl-3-methoxyxanthone (1,5-DIMX) and 1,7-dihydroxy-2-isoprenyl-3-methoxyxanthone (1,7-DIMX) were isolated from the pericarps of mangosteen as reported previously.[21] The xanthones showing >98% purity were obtained by recrystallization. They were dissolved in polyethylene glycol to make a concentration of 2 mM as a stock solution and diluted with methanol to appropriate concentrations before use.

Preparation of Recombinant Enzymes Wild-type AKR1B10 with the N-terminal 6-His tag,[13] and its mutant forms (Gln114Thr, Phe123Ala, Lys125Leu, Trp220Tyr, Val301Leu, Gln303Ser, Ser304Ala, and Val301Leu/Gln303Ser)[10,18,22] were expressed in *Escherichia coli* BL21 (DE3) pLysS cells transformed with expression plasmids harboring their cDNAs, and purified to homogeneity, as described

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previously. The Trp112Phe mutant enzyme of AKR1B10 was prepared by site-directed mutagenesis[18] using a sense primer (5′-CTA TCT TAT TCA CTT TCC ACA GGG ATT CA-3′) and the corresponding antisense primer, expressed in the *E. coli* cells, and purified to homogeneity as described above. The recombinant AKR1B1 and human aldehyde reductase (AKR1A1) were prepared and purified to homogeneity as described previously.[23]

Assay of Enzyme Activity The reductase and dehydrogenase activities of the enzymes were determined at 25°C by measuring the rate of change in NADPH absorbance (at 340 nm) and fluorescence (at 455 nm with an excitation wavelength of 340 nm), respectively.[13] The IC_{50} values for inhibitors for AKR1B1 and AKR1B10 were determined in the reaction mixture that consisted of 0.1 M potassium phosphate, pH 7.4, 0.1 mM NADPH, 0.2 mM pyridine-3-aldehyde (approximately $15\times K_m$ concentration) and enzyme in a total volume of 2.0 mL. In the assay for AKR1A1 activity, 10 mM D-glucuronate was used as the substrate. Kinetic studies in the presence of inhibitors were carried out in both pyridine-3-aldehyde reduction and $NADP^+$-linked geraniol oxidation over a range of five substrate concentrations ($0.2–5\times K_m$) at a saturating concentration of coenzyme. The IC_{50} and K_i values are expressed as the means of at least three determinations.

Molecular Modeling and Energy Minimization The atomic coordinates for AKR1B10 (PDB code: 1ZUA)[24] were obtained from the RCSB Protein Data Bank. The structure was prepared using the Maestro (Schrodinger, LLC) software package Version 8.5, as described previously.[13] In order to eliminate any bond length and bond angle biases, the ligand (α-mangostin or γ-mangostin) was subjected to a full minimization prior to the docking. The docking calculations were performed using the program Glide 5.0[25] on a Linux workstation under the conditions described previously.[13] Figures were generated using *PyMOL* (DeLano Scientific).

RESULTS AND DISCUSSION

Inhibitory Potency and Selectivity of Mangostins The structures of the xanthones evaluated in this study are shown in Fig. 1. Among them, γ-mangostin with 7-hydroxy group most potently inhibited the reductase activity of AKR1B10 ($IC_{50}=0.018\,\mu$M), and α-mangostin with 7-methoxy group was the second potent inhibitor ($IC_{50}=0.16\,\mu$M). Mangostinone, 1,7-DIMX and 1,5-DIMX, which lack the 8-isoprenyl group of the mangostins, showed less inhibitory potencies of AKR1B10. In contrast to the moderate inhibition by 1,7-DIMX with 1,7-dihydroxy groups, no significant inhibition was observed with 10 μM 1,5-DIMX with 1,5-dihydroxy groups. The

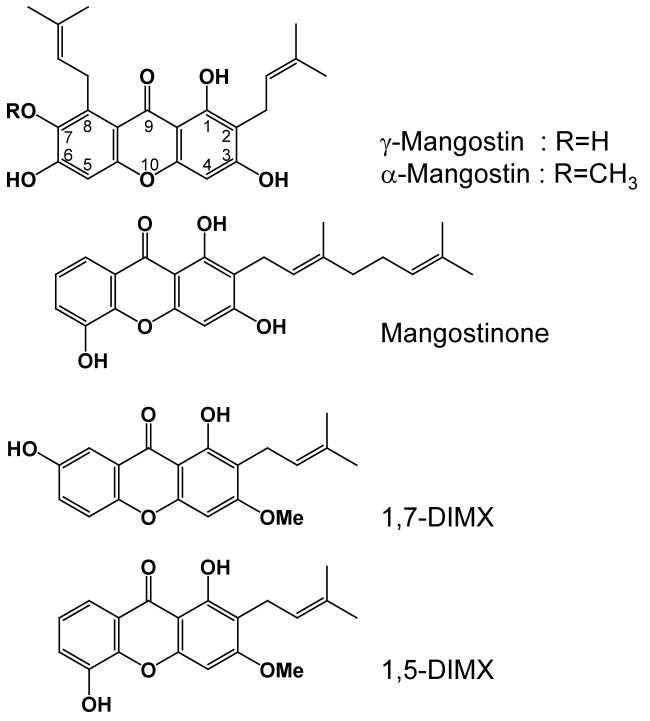

Fig. 1. Structures of Xanthones Evaluated in This Study

structure–activity relationship of these xanthones suggests the importance of the 7-hydroxy group for the binding to the enzyme. Since mangostinone does not possess the 7-hydroxy group, its moderate inhibition may result from interactions of the 2-geranyl and/or 3-hydroxy groups of this xanthone with amino acid residues of the enzyme.

The inhibition patterns of α- and γ-mangostins were noncompetitive with respect to pyridine-3-aldehyde in the reduction reaction by AKR1B10, and were competitive with respect to geraniol in the dehydrogenation reaction (K_i values were 80±11 and 5.6±0.7 nM, respectively). The different inhibition patterns in the forward and reverse reactions have been reported for other AKR1B10 inhibitors,[10,13,16,18,22] including tolrestat which binds to the substrate-binding site in the crystal structure of the enzyme-$NADP^+$-inhibitor ternary complex.[24] Thus, like the known inhibitors α- and γ-mangostins may bind to the substrate-binding site of AKR1B10.

The IC_{50} and K_i values for γ-mangostin are higher compared to the synthetic AKR1B10 inhibitors, (*Z*)-2-(4-methoxyphenylimino)-7-hydroxy-*N*-(pyridin-2-yl)-2*H*-chromene-3-carboxamide (PHPC)[26] and 3-(4-hydroxy-2-methoxyphenyl)acrylic acid 3-(3-hydroxyphenyl)propyl ester,[10] but are lower than

Table 1. Effects of Xanthones on Reductase Activities of AKR1B10, AKR1B1 and AKR1A1

Inhibitors	IC_{50} (μM)			Selectivity[a]	
	AKR1B10	AKR1B1	AKR1A1	1B1/1B10	1A1/1B10
γ-Mangostin	0.018±0.003	0.29±0.03	1.7±0.1	16	94
α-Mangostin	0.16±0.01	4.1±0.6	>10[b]	25	>63
Mangostinone	0.29±0.01	3.3±0.2	7.9±0.5	11	27
1,7-DIMX	0.85±0.05	3.8±0.2	6.5±0.1	4	8
1,5-DIMX	>10[b]	>10[b]	>10[b]	—	—

a) Selectivity is expressed as a ratio of AKR1B1/AKR1B10 or AKR1A1/AKR1B10. *b*) Inhibition percentages are less than 20% at 10 μM.

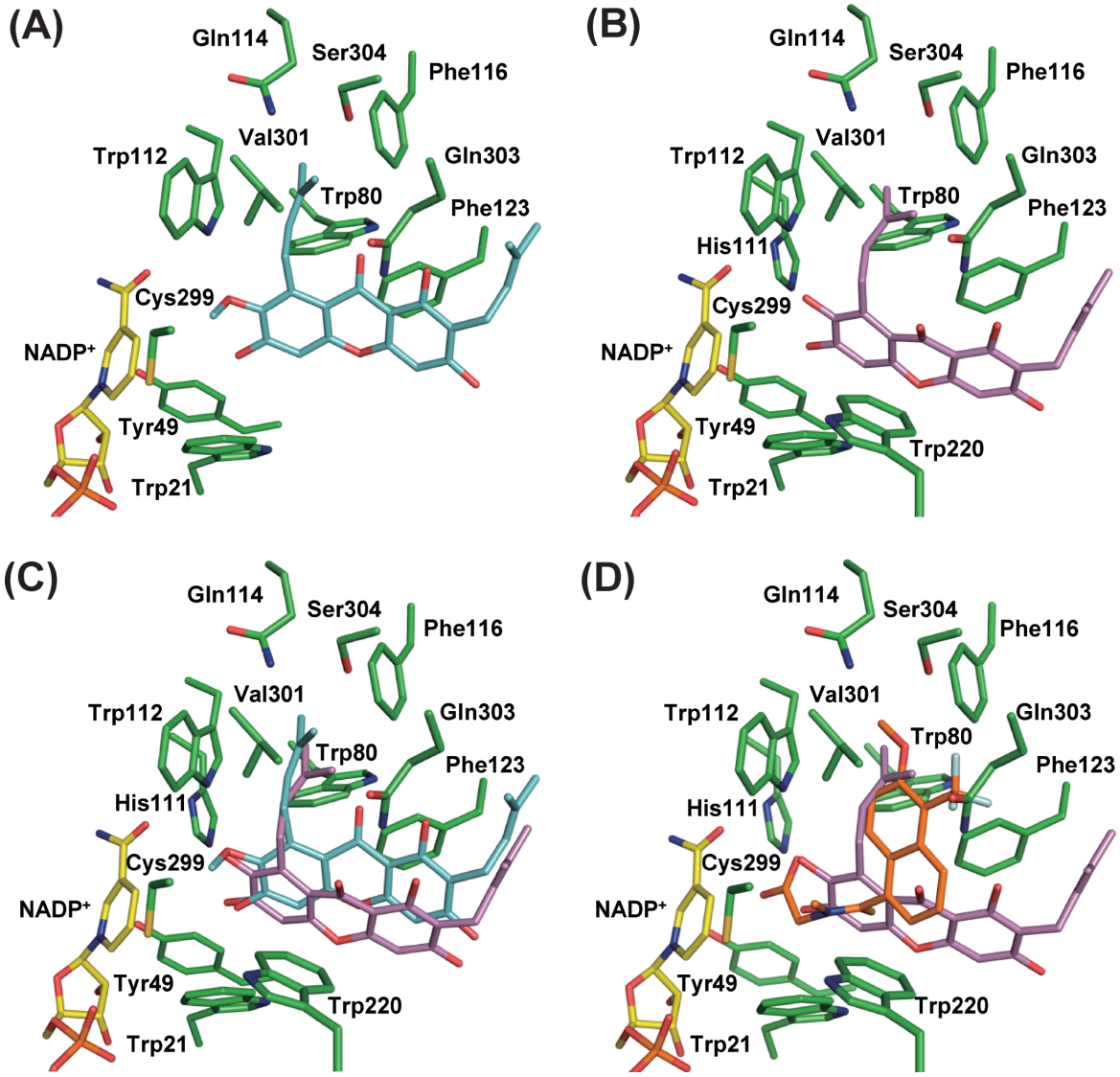

Fig. 2. Binding Models of α-Mangostin and γ-Mangostin in the $NADP^+$ Complex of AKR1B10

(A) α-Mangostin (sky-blue)-docked model. (B) γ-Mangostin (pink)-docked model. (C) Difference in orientations of α-mangostin and γ-mangostin, in which α-mangostin was superimposed onto the γ-mangostin-docked model. (D) Comparison of the binding modes between γ-mangostin and tolrestat. Tolrestat (orange) in the crystal structure of AKR1B10[24] was superimposed onto the γ-mangostin-docked model. The nicotinamide ribose portion of $NADP^+$ (yellow) is depicted with the residues within 4.0 Å from the mangostins, of which some residues (Lys125 and Gly129) are not shown for clarity.

Table 2. Effects of Mutations of AKR1B10 on K_i Values for α-Mangostin and γ-Mangostin

Enzyme	α-Mangostin[a]		γ-Mangostin[a]	
	K_i (nM)	Mu/Wt	K_i (nM)	Mu/Wt
Wild type	80±11	—	5.6±0.7	—
Trp112Phe	57±6	0.7	4.4±0.4	0.8
Gln114Thr	120±7	1.5	15±2.7	2.6
Phe123Ala	349±19	4.4	51±5.8	9.1
Lys125Leu	169±21	2.1	6.2±1.5	1.1
Trp220Tyr	52±3	0.7	17±2.0	3.0
Val301Leu	68±7	0.9	23±5.9	4.2
Gln303Ser	99±13	1.2	6.0±0.4	1.1
Ser304Ala	123±14	1.5	5.7±0.7	1.0
Val301Leu/Gln303Ser	176±14	2.2	38±1.7	6.8

a) The inhibition patterns of the two mangostins in the $NADP^+$-linked geraniol dehydrogenase were all competitive with respect to the substrate. Mu/Wt represents the ratio of the K_i value for the mutant enzyme to that for the wild-type enzyme.

those of other inhibitors, natural products (bile acids,[13] oleanolic acid,[16] curcumins,[18] flavonoids[22] and prostaglandin A_1[27]) and drugs (AKR1B1 inhibitors, anti-inflammatory agents and fibrates[22,28]). Although α-mangostin was less potent, its K_i value is lower than that of a xanthone-like inhibitor, 9-methyl-2,3,7-trihydroxy-6-fluorone (0.2 μM),[29] whose major structural difference from α-mangostin is the lack of the two isoprenyl groups. This also supports the contribution of the isoprenyl group(s) to the inhibitory potency of the two mangostins.

The inhibitory effects of the xanthones on the structurally and functionally related enzymes, AKR1B1 and AKR1A1, were examined, in order to evaluate their inhibitory selectivity (Table 1). The xanthones other than 1,5-DIMX inhibited AKR1B1 and AKR1A1, but their inhibitory potencies for the two enzymes were low compared to those for AKR1B10. α-Mangostin showed the highest selectivity to AKR1B10, followed by γ-mangostin. The inhibitory selectivity ratios of the two mangostins are higher than those of PHPC,[26] 9-methyl-2,3,7-trihydroxy-6-fluorone,[29] AKR1B1 inhibitors and flavonoids,[22] although they are low compared to other AKR1B10 inhibitors.[10,13,16,18]

Comparison of the Binding Modes of α- and γ-Mangostins in AKR1B10 The affinity of γ-mangostin to AKR1B10 was 14-fold higher than that of α-mangostin despite their structural difference only in the 7-substituent, hydroxy or methoxy group. The underlying structural reasons for the high affinity of γ-mangostin were examined by constructing models of docked γ- and α-mangostins in the AKR1B10-$NADP^+$ complex (Figs. 2A, B). In both models, the two mangostins occupied the substrate-binding site of the enzyme, in which their 7-substituted groups were in close proximity to the catalytically important residue (Tyr49) and Trp112, and the 8-isoprenyl chain was surrounded by hydrophobic residues (Trp80, Trp112, Phe116 and Val301). There were differences in the orientations of the other parts of the two mangostins, as shown in the superimposed structures of the models of α-mangostin and γ-mangostin (Fig. 2C). In the α-mangostin-docked model (Fig. 2A), its 9-carbonyl and 1-hydroxy groups were within hydrogen-bonding distance with the side chain of Gln303 (3.3, 3.1 Å, respectively), and its aromatic ring hydrophobically interacted with the side chain of Phe123. In addition to these interactions, a hydrophobic interaction was observed between Trp220 and the aromatic ring of γ-mangostin in this mangostin-docked model (Fig. 2B). The 6-hydroxy group of α-mangostin formed a hydrogen-bond with the indole nitrogen of Trp21, whereas that of γ-mangostin interacted with His111 and its 10-oxygen was within hydrogen-bonding distance with the indole nitrogen of Trp21.

The binding mode of γ-mangostin docked in AKR1B10 is also different from the crystallographically determined binding of tolrestat[24] (Fig. 2D), which almost equally inhibits both AKR1B10 and AKR1B1.[13] Among the inhibitor-binding residues predicted by the present docked models, Gln114, Lys125, Val301, Gln303 and Ser304 of AKR1B10 are different from the corresponding residues (Thr, Leu, Leu, Ser and Cys, respectively) in AKR1B1. Previous site-directed mutagenesis studies showed that Gln114, Val301, Gln303 and/or Ser304 are important for binding of several AKR1B10 inhibitors.[10,16,18,22,29] To investigate the participation of these residues in the binding and selectivity of α- and γ-mangostins, we prepared mutant AKR1B10s, in which the four residues, except for Ser304, were replaced with the corresponding residues in AKR1B1. Ser304 and Phe123 were replaced by Ala, and Trp112 and Trp220 were mutated to smaller aromatic residues, Phe and Tyr, respectively. The affinity (K_i value) for α-mangostin was influenced only by the single Phe123Ala and double Val301Leu/Gln303Ser mutations, whereas that for γ-mangostin was greatly impaired by the mutations Phe123Ala, Trp220Tyr, Val301Leu and Val301Leu/Gln303Ser (Table 2). The results support the different orientations of the two mangostins in the enzyme (Fig. 2). The above four residues may play important roles for the tight binding of γ-mangostin, and since Phe123 and Trp220 of the four residues are conserved in AKR1B1, they are likely responsible for the decrease in selectivity of γ-mangostin for AKR1B10 compared to α-mangostin. Although the structural reason for high selectivity of α-mangostin still remains unknown, the present study has revealed that the small chemical differences in the 7-substitute, hydroxy or methoxy group, between the two mangostins significantly affect their binding affinity by altering the orientation of the inhibitors.

Besides their anti-cancer effects,[19,20] α- and γ-mangostins exhibit multifunctional properties, such as antioxidant, anti-inflammatory, antiallergic, antibacterial and antiviral activities, *via* modulation activities and expression of multiple target proteins.[30] Such target proteins include fatty acid synthase,[31] cytochrome P450s,[32] 12-lipoxygenase,[33] acidic sphingomyelinase,[34] Ca^{2+}-ATPase,[35] aromatase,[36] human immunodeficiency virus (HIV)-1 protease,[37] Ca^{2+}- and cAMP-dependent protein kinases,[38] inhibitor-κB kinase,[39] and cyclooxygenases 1 and 2.[40] The IC_{50} values of α-mangostin and γ-mangostin for the known target enzymes are 0.58–33 μM[31–38] and 0.8–10 μM,[36–40] respectively, which are higher than those of the two mangostins for AKR1B10. To our knowledge, the IC_{50} value (0.018 μM) of γ-mangostin for AKR1B10 is the lowest among those of the two mangostins for other enzymes reported to date. Although the two mangostins exhibit anti-cancer properties through various mechanisms,[19,20] the potent inhibition of AKR1B10 particularly by γ-mangostin may contribute to its anti-tumor action in cancers, in which the enzyme is highly expressed.

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