Probing the Active Site of Human Aldose Reductase

SITE-DIRECTED MUTAGENESIS OF ASP-43, TYR-48, LYS-77, AND HIS-110*

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Structural models of human aldose reductase complexed with NADPH have revealed the apposition of C4 of the nicotinamide ring with tyrosine 48 and histidine 110, suggesting that either of these residues could function as the proton donor in the reaction mechanism. Tyrosine 48 is also part of a hydrogen-bonding network that includes lysine 77 and aspartate 43. In order to study the potential catalytic roles of these 4 residues, we evaluated the kinetic properties of mutants containing structurally conservative replacements at these sites. Enzymatic activity was undetectable when Tyr-48 was mutated to phenylalanine (Y48F) although affinity for NADPH was unchanged. In contrast, a mutant containing asparagine substituted for His-110 (H110N) was characterized by an almost 80,000-fold increase in $K_{m}$, but only about a 14-fold reduction in $k_{cat}$ measured with $d$-glyceraldehyde. Modest changes in catalytic properties were observed in the mutant containing aspartate 43 substituted with asparagine (D43N): $K_{m}$ for aldehyde substrates was elevated up to 17-fold, and $k_{cat}$ decreased less than 16-fold. However, the $K_{G(NADP)}$ values for D43N were about 5 times higher than those for wild type. Mutant enzyme containing methionine substituted for lysine 77 (K77M) was up to 1,460-fold less active than the wild type. These results are consistent with Tyr-48 acting as the acid-base catalyst in human aldose reductase and confirm the importance of Asp-43, Lys-77, and His-110 to the structure and function of the active site.

Aldose reductase (alcohol:NADP$^+$ oxidoreductase, EC 1.1.1.21) catalyzes the NADPH-dependent reduction of a variety of aldehyde substrates to their corresponding alcohols. Although the normal physiologic role(s) for aldose reductase has not been established in most mammalian tissues in which aldose reductase is found, the action of aldose reductase has been linked to the development of certain long-term complications of diabetes mellitus such as cataract, retinopathy, nephropathy, and neuropathy (reviewed in Ref. 1). Administration of aldose reductase inhibitors has been shown to delay onset or substantially prevent these complications in experimental animals (1–4). These results have sparked intense study of the enzyme since the treatment of diabetic patients with aldose reductase inhibitors may represent a therapeutic route for preventing the onset or progression of diabetic complications in patients with chronic hyperglycemia.

Aldose reductases from various human and animal tissues have been purified and extensively studied. The enzyme is a monomer with a calculated molecular mass of about 35,800 Da. Amino acid and nucleotide sequence comparisons of aldose reductases from several mammalian species have revealed their evolutionary relatedness with a family of oxidoreductases and structurally related proteins including steroid dehydrogenases (5, 6), prostaglandin synthases (7), lens crystallins (8), and xenobiotic metabolizing enzymes (9). Although heterogeneity has been described for aldose reductases extracted from different tissue donors (10) and from diabetic as compared to non-diabetic tissues (11), cDNA cloning studies have shown that aldose reductase expressed in different human tissues is the product of a single gene (12).

Despite some controversy over the kinetic mechanism of aldose reductase, the recent steady state and pre-steady state kinetic studies by Kubisessi et al. (13) have established that, at pH 7.0 and with $d$-glyceraldehyde as the aldehyde substrate, the enzyme follows a sequential ordered mechanism in which NADPH binds before the aldehyde substrate and NADP$^+$ is released after the alcohol product. A conformational change in the enzyme occurs both upon NADPH binding and prior to release of NADP$^+$. The latter isomerization is reported to be the rate-limiting step in the forward reaction (13, 14). The physical basis for this isomerization was recently identified in crystal structures of aldose reductase complexed with NADPH (15, 16), as compared to the structure lacking NADPH (17), to be a hinge motion of the loop of residues Gly-213 to Leu-227 which helps to hold the coenzyme in place. Two other members of the aldoketo oxidoreductase superfamily, aldehyde reductase (18–20) and 3α-hydroxysteroid dehydrogenase (21), have also been shown to obey a sequential ordered reaction mechanism similar to that of aldose reductase.

Until recently, very little was known about the nature of the catalytic center or any other structural features that may be important for this enzyme's function. Despite numerous chemical modification studies to determine the potential involvement of arginine, histidine, cysteine, or lysine residues in the catalytic mechanism, no conclusive results have been obtained (22–24). The only exception to the above were the chemical modi-
fication studies of human psoas muscle aldose reductase, where the involvement of lysine 262 in NADPH binding was demonstrated by affinity labeling with 6-iodo-d-glucopyranos-4-ulose 5'-diphosphate (25). With the advent of crystal structures for porcine (17) and human (15, 16) aldose reductases, together with systems to overexpress cloned human aldose reductase in Escherichia coli (26), systematic and rational site-directed mutagenesis studies to probe specific features of the enzyme's structure and function are now possible.

The catalytic mechanism of aldose reductase is thought to be relatively simple, involving a stereospecific transfer of the pro-R hydride from C4 of the nicotinamide (27) to the substrate carbonyl carbon atom and protonation of the substrate carbonyl oxygen atom by an enzyme functional group. The identity of the proton donor in this mechanism could not be ascertained from the crystal structures, although Tyr-48 was considered the most likely candidate based on its proximity to the hydride-donating nicotinamide C4 atom of NADPH and its hydrogen-bonding interaction with other residues likely to facilitate its function as a proton donor (15). The possibility that His-110 could be the proton donor was also considered, but since it is found in a predominantly hydrophobic area, an environment not considered conducive to the formation of a positively charged imidazolium group, it was deemed less likely (15). In the present study, we individually replaced Tyr-48 and His-110 with Phe and Asn, respectively, by site-directed mutagenesis in anticipation that the resulting mutants (Y48F and H110N) would reveal which one of these amino acid residues is the true proton donor. We also constructed point mutants at two other locations in the enzyme, namely at Asp-43 (resulting in the D43N mutant) and Lys-77 (K77M mutant), because these two residues participate in a hydrogen-bonding network linking the nicotinamide ribose 2'-hydroxyl group with the hydroxyl of Tyr-48.

MATERIALS AND METHODS

Site-directed Mutagenesis—Human placental aldose reductase cDNA (containing a silent mutation introduced to abolish an internal NcoI site at a position corresponding to codon 145) was excised from pBLUE-SCRIPT II SK(−) phagemid (Stratagene) by digestion with HindIII and NcoI. Plasmid pHuALR2–1 was created by ligating the resulting 1354-bp fragment with pMON20,400, a derivative of the plasmid containing the M13 origin of replication and provision of a lytic particle. This plasmid was linearized with HindIII and purified on hydroxylapatite column chromatography (26). Oligonucleotide primers used to construct mutant forms are shown in Table I. Mutagenic primers were synthesized and purified as described previously (26). Clones containing the intended mutation were identified by nucleotide sequence analysis across the mutation site. Integrity of the entire coding sequence of the mutants was then confirmed by the same sequencing procedure using a set of eight internal sequencing primers.

Expression in E. coli and Purification—Plasmids encoding WT and mutant aldose reductases were transfected into E. coli strain JM101 (30), and their respective protein products were expressed and purified under conditions described previously (26), except that cultures were maintained in the presence of ampicillin (50 μg/ml) rather than spectinomycin. Briefly, the enzyme was extracted from host cells by osmotic shock and purified by ammonium sulfate fractionation under conditions described previously (26), except that cultures were maintained in the presence of ampicillin (0.25–1.5 μg/ml) to a 1-cm path length quartz cuvette containing mutant or WT aldose reductase (0.25–1.5 μg/ml) at pH 7.0 at 15°C, to a final volume of 2 or 3 ml. Glycyltryptophan standard solution was used to correct for dilution and internal filter effects. All subsequent steps were performed at room temperature.

Enzyme Assays—During purification, WT aldose reductase activity was monitored spectrophotometrically at 340 nm using 5 μm D-glycer-aldehyde and 0.15 mM NADPH as substrate and coenzyme, respectively (35). Kinetic constants for the WT enzyme were determined in a triple buffer system consisting of 25 mM MES, 25 mM potassium phosphate, and 90 mM Tris at pH 7.0 and 0.15 mM NADPH and variable amounts of the appropriate substrate. For the mutant enzymes, assays were performed in the same triple buffer system, pH 5.0–10.1. Enzyme concentrations in the assays were 0.03–0.3 mg/ml for the active site mutants and about 2 μg/ml for the WT enzyme. Apparent Michaelis constant, K<sub>m</sub> values were determined by fitting initial rate data obtained from duplicate assays to a rectangular hyperbola as described previously (26). At least two separate batches of each enzyme were analyzed.

RESULTS

Expression and Purification—The four newly constructed aldose reductase genes that code for D43N, Y48F, K77M, and H110N were overexpressed in E. coli strain JM101 shaker-flask cultures and purified to virtual homogeneity following the same procedure as described previously for the WT enzyme (26). All four mutant enzymes eluted upon chromatofocusing at positions, relative to other bacterial proteins, similar to that of the WT enzyme. On hydroxylapatite column chromatography, the mutant and WT enzymes exhibited similar elution profiles, appearing as single, distinct, protein peaks. Although the mutant enzymes exhibited reduced or no catalytic activity, their purification was easily followed by the appearance of a predominant, M<sub>r</sub> ~ 36,000, polypeptide on SDS-PAGE of material from chromatography fractions. All enzyme forms had the same mobilities on SDS-polyacrylamide gels (Fig. 1A) and all are apparently homogeneous. A Western blot (Fig. 1B) confirmed that all enzyme forms cross-reacted with antibodies made against bovine lens aldose reductase (36). One-liter shaker flask cultures of WT, H110N, and Y48F typically yielded approximately 12 mg of pure protein. Cultures of D43N and K77M expression constructs typically yielded approximately 2–3 mg of protein per liter.

Steady-state Kinetic Properties of the Mutant Enzymes—All

TABLE 1

| Mutant* | Oligonucleotide |
|---------|----------------|
| D43N    | 5'-TGUGCCAAGTGTGCG-3' |
| Y48F    | 5'-GTCATGCGACAGCAATGCCG-3' |
| K77M    | 5'-GCAACCACCACTGCAGGT-3' |
| H110N   | 5'-GGCCGATATTAAGTTGAG-3' |

*Abbreviations are defined in Footnote 1.

The abbreviations used are: Y48F, Tyr-48→Phe; D43N, Asp-43→Asn; K77M, Lys-77→Met; H110N, His-110→Asn; WT, wild type.
four mutant enzymes exhibited dramatically altered kinetic behavior as compared to that of the WT enzyme. When evaluated with variable amounts of benzaldehyde, D-glyceraldehyde, L-glyceraldehyde, and p-nitrobenzaldehyde as substrates at pH values ranging from 5.4 to 10.1, the activity of the Y48F mutant was not detectable. Similarly, no activity was detectable in the reverse direction with glycerol or benzyl alcohol. This indicated a decrease in enzymatic activity exceeding 3 orders of magnitude relative to the WT enzyme. As a consequence of this dramatic decrease in activity, it was not possible to determine the $K_m$ and $k_{cat}$ values for this mutant using our assay system.

The other three mutants had significantly decreased, but measurable, enzymatic activities. Kinetic analysis of the H110N mutant using D-glyceraldehyde as the variable substrate was carried out at pH 5.4 (pH optimum for this mutant, data not shown). The apparent Michaelis constant ($K_m = 1.87 \pm 0.45$ nM) is nearly 80,000-fold higher and the apparent turnover number ($k_{cat} = 5.1 \pm 0.8$ min$^{-1}$) about 14-fold lower than those for the WT enzyme ($K_m = 23.8 \pm 0.55$ nM, $k_{cat} = 73.9 \pm 0.5$ min$^{-1}$). Therefore, the catalytic efficiency ($k_{cat}/K_m$) for this substrate was approximately $1.1 \times 10^8$-fold lower than WT. Kinetic constants using $p$-nitrobenzaldehyde, benzaldehyde, and L-glyceraldehyde as substrates could not be accurately determined because sufficiently high substrate concentrations could not be achieved in our assay system. No activity was detected in the reverse direction with glycerol or benzyl alcohol.

At pH 7.0, the specific activity of the K77M mutant (1.4 milliunits/mg and 2.6 milliunits/mg with D- and L-glyceraldehyde, respectively) is about 1,460- to 700-fold lower than that for the WT enzyme (specific activity of WT is 2,050 milliunits/mg and 1,800 milliunits/mg with D- and L-glyceraldehyde, respectively; 1 unit of enzyme oxidizes 1 μmol NADPH/min). Using our enzyme assay (see "Materials and Methods"), we could not achieve conditions under which saturating amounts of NADPH could be maintained during the entire course of the assay (25 μM K77M mutant concentration and 150 μM initial NADPH concentration), and thus steady-state kinetic constants could not be calculated. No activity was detect-

### Table II

**Apparent kinetic constants of wild type (WT) and aspartate 43 mutant (D43N) aldose reductases**

|                | WT* | D43N* | WT/D43N |
|----------------|-----|-------|---------|
| **D-Glyceraldehyde** |     |       |         |
| $K_m$ (μM)    | 66.9 ± 1.8 | 774 ± 74 | 0.086 ± 0.009 |
| $k_{cat}$     | 61.0 ± 0.6 | 4.00 ± 0.1 | 15.3 ± 0.4 |
| $k_{cat}/K_m$ (μM$^{-1}$ min$^{-1}$) | 912 ± 17 | 5.17 ± 0.4 | 176 ± 14 |
| **p-Nitrobenzaldehyde** |     |       |         |
| $K_m$ (μM)    | 7.97 ± 0.6 | 73.9 ± 5.7 | 0.096 ± 0.011 |
| $k_{cat}$     | 43.0 ± 1.0 | 5.65 ± 0.2 | 7.61 ± 0.32 |
| $k_{cat}/K_m$ (μM$^{-1}$ min$^{-1}$) | 6,080 ± 430 | 76.5 ± 4.5 | 79.5 ± 7.3 |
| **Benzaldehyde** |     |       |         |
| $K_m$ (μM)    | 57.3 ± 4.2 | 957 ± 111 | 0.060 ± 0.008 |
| $k_{cat}$     | 47.0 ± 1.1 | 5.65 ± 0.2 | 8.32 ± 0.35 |
| $k_{cat}/K_m$ (μM$^{-1}$ min$^{-1}$) | 820 ± 45 | 5.90 ± 0.5 | 139 ± 14 |
| **NADPH**     |     |       |         |
| $K_m$ (μM)    | 2.27 ± 0.2 | 11.2 ± 1.2 | 0.203 ± 0.028 |
| $k_{cat}$     | 70.1 ± 1.2 | 4.53 ± 0.1 | 15.5 ± 0.4 |
| $k_{cat}/K_m$ (μM$^{-1}$ min$^{-1}$) | 30.9 ± 2.6 | 0.40 ± 0.04 | 77.3 ± 10.1 |
| **Benzyl alcohol** |     |       |         |
| $K_m$ (μM)    | 3.08 ± 0.2 | 94.6 ± 7.6 | 0.033 ± 0.003 |
| $k_{cat}$     | 2.35 ± 0.1 | 0.92 ± 0.04 | 2.55 ± 0.16 |
| $k_{cat}/K_m$ (μM$^{-1}$ min$^{-1}$) | 763 ± 30 | 9.73 ± 0.4 | 78.4 ± 4.5 |

* All assays were performed at pH 7.0 as described under "Material and Methods" except where noted otherwise.
* All $k_{cat}$ values are min$^{-1}$.
* D-Glyceraldehyde was held constant at 10 mM.
* Determined at pH 8.2 with NADP+ held constant at 0.1–0.5 mM.
able with either benzyl alcohol or glycerol in the reverse direction.

The apparent kinetic constants for reduction and oxidation of various substrates by the D43N mutant are compared to those of the WT enzyme in Table II. The apparent $K_d$ values for the three substrates in the forward reaction were increased 10.5- to 16.7-fold when compared to the WT enzyme. The apparent turnover numbers ($k_{cat}$) for the various substrates were decreased by 7.6- to 15.3-fold when compared to the WT enzyme. Likewise, the apparent catalytic efficiencies ($k_{cat}/K_m$) for those substrates have dropped approximately 80- to 176-fold, compared to those of the WT enzyme. Similar changes were observed in the kinetic constants with NADPH as the variable substrate; $K_m$ increased 5-fold, $k_{cat}$ decreased over 15-fold, and the catalytic efficiency dropped 77-fold. In the reverse reaction with benzyl alcohol as the variable substrate, the kinetic constants have changed similarly; $K_m$ increased over 30-fold, $k_{cat}$ decreased 2.6-fold, and the catalytic efficiency dropped 78-fold.

**Table III**

Coenzyme Binding Affinities — The dissociation constants ($K_d$) for NADPH and NADP$^+$ with the various enzyme forms are shown in Table III. The $K_d$ values obtained for the WT enzyme are similar and about 5- to 6.5 times higher than that for the WT enzyme. The $K_d$ of Y48F is about the same as that for the WT. The $K_d$ of the H110N mutant could not be accurately determined with the present technique due to a large amount of fluorescence energy transfer to NADP (13) as evidenced by an intense emission band at 460 nm.

The $K_d$ of the WT enzyme is almost twice that for NADP. This ratio of dissociation constants is approximately maintained with the D43N mutant. However, there is an unexpected reversal of affinities of the K77M for the two forms of the enzyme. The K77M mutant has a 7.5-fold lower $K_d$ than the WT enzyme, $K_d$, being slightly lower than the $K_d$ of the WT enzyme. The Y48F mutant has an 8.8-fold higher $K_d$ than the WT enzyme. The $K_d$ of the H110N mutant is similar to the $K_d$ of the WT enzyme. The $\Delta F_{max}$, with NADPH as coenzyme, varied among the enzymes tested from 80% to 97% quenching of the initial fluorescence values. With NADP$^+$ as coenzyme, the $\Delta F_{max}$ ranged from 40% to 96% quenching.

**DISCUSSION**

Functional Roles of Active Site Residues — The identification of the proton donor in the catalytic mechanism of aldose reductase was not possible from the crystal structures (15, 16), although Wilson et al. (15) proposed that Tyr-48 was the most likely candidate based on sequence conservation among the members of the enzyme superfamily and an interaction (hydrogen bonding with Lys-77 and Asp-43) that is likely to depress its pK$_a$ (Fig. 2). The other amino acid residues that could potentially function as the proton donor are Cys-298 and His-110 since they, in addition to Tyr-48, are the only residues within a 5 to 6 Å radius from the nitrosoimide C4 that have side chains with the ability to donate a proton. We have recently ruled out Cys-298 as the proton donor since Ala (data not shown) and Ser (26) mutants at that position show robust reductase activity. Our kinetic data in the present paper are consistent with Tyr-48 being the proton donor in the catalytic mechanism. Replacement of Tyr-48 with Phe resulted in an essentially inactive mutant, with $\geq$5000-fold reduction in enzymatic activity as compared with that of the WT enzyme (sensitivity limit of assay system). On the other hand, replacement of His-110 with Asn results in a much smaller decrease in activity, as evidenced by a 14-fold decrease in $k_{cat}$.

This result may be somewhat surprising in light of the expected acidity of Tyr and His side chains: Tyr hydroxyl groups normally have a pK$_a$ of about 10 while His imidazolium side chains have pK$_a$ values in the 6-7 range. These pK$_a$ values would implicate His as the likely proton donor at physiological pH values. However, in light of the known crystal structure of aldose reductase, it seems reasonable that the acidity of Tyr-48 might be altered. Tyrosine 48 is part of a three-membered hydrogen-bonding network that also includes Lys-77 and Asp-43 (Fig. 2). Lysine 77 donates a hydrogen bond through its side chain amino group to Tyr-48 hydroxyl oxygen (3.19 Å distance between the N and O atoms). A similar interaction with 2 Lys residues at the active site of a Class C β-lactamase from *Citrobacter freundii* (37) appears to lower the pK$_a$ of Tyr-150, which acts as a general acid-base in the catalytic mechanism of this β-lactamase. The presence of adjacent positive charges has been shown to cause a substantial decrease in pK$_a$ values of tyrosine copolymers (38) and in phenol-containing crown ethers complexed with ammonium ions (39). A drop in specific activity of the aldose reductase K77M mutant by about 3 orders of magnitude reinforces the likely importance of Lys-77 in decreasing the pK$_a$ of Tyr-48 in aldose reductase. Mutation of Asp-43, farther from the tyrosine in the hydrogen-bonding network, has a lesser effect as would be expected in such a system.

The question still remains as to the function of His-110. Possible explanations for the 80,000-fold elevation in Km(o-glyceraldehyde) observed with the H110N mutant include an alteration in a binding interaction with the substrate and/or a larger structural rearrangement of the active site. Evidence in support of the rearrangement of the active site in the H110N mutant includes the unexpectedly large change in Km(o-glyceraldehyde) and the unusual fluorescence quenching pattern with NADPH.

Very little is known about functional group(s) involved in binding of substrates by aldose reductase. A functional group with a pK$_a$ of 7.5-7.7 was recently shown to be important in substrate binding of aldose reductase (40) in the reverse reaction, where kinetic studies have shown that the isomerization of $E^\prime$-NADPH $\rightarrow$ $E$-NADPH is not rate-limiting (13). While cysteine and histidine side chains would normally be expected to have a pK$_a$ in this range, Tyr-48 could also be included in this group due to its hydrogen-bonding interaction with Lys-77.

The $K_{d$NADPH} and $K_{d$NADP} values obtained for the WT enzyme are in good agreement with those reported previously by Kubisek et al. (13). Two points may be emphasized from the

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results of our coenzyme binding studies. Given the relatively minor changes in binding affinity for oxidized and reduced coenzymes, the changes observed in enzymatic activities for the D43N and Y48F mutants appear to be mainly a consequence of replacement of catalytically important residues and are not due to major structural changes inadvertently introduced by substituting specific residues. Second, most of the individual values for the dissociation constants are consistent with what one would predict from structural models of the WT enzyme. For example, Asp-43 is hydrogen-bonded to the 2'-hydroxyl of the nicotinamide ribose (15), so its replacement should have an equal effect on binding of either form of the coenzyme.

The D43N mutant is informative because numerous kinetic parameters can be measured relatively accurately. There appears to be good agreement between a 5-fold increase in $K_m(NADPH)$ and a 5.7-fold increase in $K_m(NADP^+)$, consistent with the position of Asp-43 near the 2'-hydroxyl of the nicotinamide ribose of NADPH. That mutation of Asp-43 affects both the $K_m$ and $k_{cat}$ of aldehyde and alcohol substrates in both catalytic directions supports its indirect involvement in substrate binding and catalysis. The chemical (hydride transfer) step is not rate-limiting in the catalytic mechanism of native aldose reductase (13, 14). Accordingly, the question arises as to whether the 7.6- to 15.5-fold decrease in $k_{cat}$ for the D43N mutant in the forward reaction results from a decrease in the rate of the demonstrated slow step in the kinetic mechanism, i.e. the isomerization of $E^+\cdot NADP^+$ to $E\cdot NADP^+$ (13, 14), or from a larger decrease in the rate of the hydride transfer step such that the chemical step now becomes rate-limiting. If the latter scenario is correct, such a significant drop in the rate of hydride transfer may mean that Asp-43 plays an important role in facilitating the interaction between Lys-77 and Tyr-48.

In summary, we know the location of the reducing hydride, the acidic proton, and grossly how the substrate carbonyl must be oriented between the two.

Both $ab$ initio (42–44) and semiempirical (45, 46) theoretical calculations on the hydride transfer reactions of dihydro- nicotinamides provide additional constraints. The atoms referred to below are nitrogen 1 (N1) and carbon 4 (C4) of the nicotinamide ring of NADPH, the pro-R hydrogen at C4 (H4R), the substrate carbonyl carbon (C) and oxygen (O), and the phenolic oxygen of the proton donor Tyr-48 (OH). The calculations indicate that in the transition state: 1) the nicotinamide ring puckers slightly toward the substrate, placing H4R in a pseudoaxial conformation; 2) the hydride transfers in a bent mode, such that C4-H4R-C angle is about 150°; 3) the hydride approaches the backside of the carbonyl, such that the H4R-C-O angle is about 110°; and 4) the C4-C distance is about 2.6 Å. Point 3 is in accord with studies of crystal structures wherein nucleophiles are closely apposed to carbonyl groups (47). All of these constraints, the substrate D-glyceraldehyde was modeled into the aldose reductase active site. The coordinates for D-glyceraldehyde were generated with MACROMODEL (4). The gross position of D-glyceraldehyde was first adjusted so that the aldehydic hydrogen pointed toward Trp-20, the glycol side chain (–CH(OH)CH2OH) pointed toward His-110, and the carbonyl oxygen atom pointed toward Tyr-48; this ensured that the re face of the carbonyl received the hydride from NADPH. Next, D-glyceraldehyde was translated to place C about 2.6 Å from C4, simultaneously satisfying the angular constraints mentioned above. C was also constrained to lie in the plane formed by N1, C4, and H4R. D-Glyceraldehyde was then rotated about C, to position O about 2.7 Å from OH. This rotation also ensured that the glycol side chain did not approach His-110 too closely. Finally, the D-glyceraldehyde torsion angles were adjusted to favorable staggered conformations, which also allowed the glycol side chain to point out of the active site toward the bulk solvent.
Fig. 3. Hypothetical model of α-glyceraldehyde in the active site of aldose reductase. α-Glyceraldehyde is shown modeled into the crystallographically determined aldose reductase active site (18), in a conformation suitable to accept a hydride ion from C4 of NADPH (residue 356) and a proton from OH of Tyr-48. The model of α-glyceraldehyde is labeled on the carbonyl (500GLYC) and includes all atoms. The aldehydic hydrogen of α-glyceraldehyde points toward the indole side chain of Trp-20; the glycol side chain points up out of the active site. The hydrogen-bonding network connecting the 2'-OH of nicotinamide ribose (residue 355), Asp-43 O′, Lys-77 N′, and Tyr-48 O′ is indicated (——).

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