The C-Terminal Loop of Aldehyde Reductase Determines the Substrate and Inhibitor Specificity†

Oleg A. Barski,‡ Kenneth H. Gabbay,‡,§ and Kurt M. Bohren*,‡

Molecular Diabetes & Metabolism Section, Departments of Pediatrics and Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Received August 7, 1996⊗

ABSTRACT: Human aldehyde reductase has a preference for carboxyl group-containing negatively charged substrates. It belongs to the NADPH-dependent aldo-keto reductase superfamily whose members are in part distinguished by unique C-terminal loops. To probe the role of the C-terminal loops in determining substrate specificities in these enzymes, two arginine residues, Arg308 and Arg311, located in the C-terminal loop of aldehyde reductase, and not found in any other C-terminal loop, were replaced with alanine residues. The catalytic efficiency of the R311A mutant for aldehydes containing a carboxyl group is reduced 150−250-fold in comparison to that of the wild-type enzyme, while substrates not containing a negative charge are unaffected. The R311A mutant is also significantly less sensitive to inhibition by dicarboxylic acids, indicating that Arg311 interacts with one of the carboxyl groups. The inhibition pattern indicates that the other carboxyl group binds to the anion binding site formed by Tyr49, His112, and the nicotinamide moiety of $NADP^+$. The correlation between inhibitor potency and the length of the dicarboxylic acid molecules suggests a distance of approximately 10 Å between the amino group of Arg311 and the anion binding site in the aldehyde reductase molecule. The sensitivity of inhibition of the R311A mutant by several commercially available aldose reductase inhibitors (ARIs) was variable, with tolrestat and zopolrestat becoming more potent inhibitors (30- and 5-fold, respectively), while others remained the same or became less potent. The catalytic properties, substrate specificity, and susceptibility to inhibition of the R308A mutant remained similar to that of the wild-type enzyme. The data provide direct evidence for C-terminal loop participation in determining substrate and inhibitor specificity of aldo-keto reductases and specifically identifies Arg311 as the basis for the carboxyl-containing substrate preference of aldehyde reductase.

Aldehyde reductase (EC 1.1.1.2) catalyzes the NADPH-dependent reduction of a variety of biogenic and xenobiotic aldehydes to their corresponding alcohols. Aldehyde reductase is a 35 kDa cytosolic protein and a member of the aldo-keto reductase superfamily of proteins (Bohren et al., 1989). The members of this protein family share many common structural and functional characteristics, namely an α/β barrel tertiary structure, a NADPH cofactor which is enfolded by a mobile loop that varies among the different members, an active site located at the C terminus of the barrel, and a catalytic mechanism which involves recruitment of a tyrosine residue as the proton donor (Rondeau et al., 1992; Wilson et al., 1992; Harrison et al., 1994; El-Kabbani et al., 1995; Tarle et al., 1993; Bohren et al., 1994; Barski et al., 1995). The conserved tyrosine residue is uniformly involved in a hydrogen-bonding network with an underlying conserved pair of lysine and aspartic acid residues, and a conserved histidine residue was shown to direct substrate orientation at the active site in both aldose and aldehyde reductases (Bohren et al., 1994; Barski et al., 1995).

The C-terminal loops of the aldo-keto reductases are unique for each member and differ drastically in length and amino acid composition. For instance, the C-terminal loop of aldehyde reductase is markedly different in length and composition from those of all other members of the superfamily, with an additional stretch of nine amino acid residues (Bohren et al., 1989; Bruce et al., 1994). Studies in aldose and aldehyde reductase have suggested that the C-terminal loop is critical for catalytic efficiency and substrate and inhibitor specificity (Bohren et al., 1992). Deletion of the C-terminal loop in aldose reductase severely decreases the catalytic efficiency for uncharged substrates (e.g., DL-glyceraldehyde, D-glucose) but has no effect on a charged substrate such as D-glucuronate (Bohren et al., 1992), suggesting that the C-terminal loop in this enzyme has no specificity for charged substrates.

Aldehyde reductase, on the other hand, is notable for its preference for substrates having a negatively charged carboxyl group such as glucuronate, succinic semialdehyde, and *p*-carboxybenzaldehyde (Branlant & Biellmann, 1980; Wermuth, 1985). Although the structural basis for this preference is not known, chemical modification and inhibition studies with dicarboxylic acids suggested the existence of an anion recognition site which was tentatively identified as an arginine residue (Branlant et al., 1981). On the basis of these results, we investigated the two arginine residues in the C-terminal loop of aldehyde reductase to determine their role in the binding of carboxyl-containing substrates. We present the results of site-directed mutagenesis studies of Arg308

† This work was supported by NIH Grant EY11018 (K.H.G.), grants from the Juvenile Diabetes Foundation (O.A.B., K.H.G., and K.M.B.), and the Harry B. and Aileen B. Gordon Foundation (K.H.G.).

* Corresponding author: Kurt M. Bohren, Department of Pediatrics, Baylor College of Medicine, 6621 Fannin MC3-2351, Houston, TX 77030. Phone: (713) 770-3759. Fax: (713) 770-3766. E-mail: kbohren@mbcr.bcm.tmc.edu.

‡ Department of Pediatrics.

§ Department of Cell Biology.

⊗ Abstract published in *Advance ACS Abstracts,* November 1, 1996.

S0006-2960(96)01974-5 CCC: $12.00

and Arg311 and identify Arg311 as the residue responsible for the binding of a distal carboxyl group of certain substrates and inhibitors. These studies provide direct evidence for the role of the C-terminal loop in determining substrate and inhibitor specificities in the aldo-keto reductase superfamily.

MATERIALS AND METHODS

Materials. Alrestatin was obtained from Ayerst Laboratories, and tolrestat was obtained from Wyeth-Ayerst. Sorbinil and zopolrestat (Pfizer), ponalrestat (ICI), FK366 (Fujisawa), and AL1576 (Alcon) were kindly provided by colleagues and collaborators. Succinic semialdehyde was from Aldrich, and 2,3-dimethylsuccinic acid was from Fluka. All other chemicals, including substrates and dicarboxylic acid inhibitors, were purchased from Sigma.

Expression and Purification of Aldehyde Reductase Mutants. Site-directed mutagenesis of the wild-type aldehyde reductase, insertion of constructs into the pET vector and their overexpression in *Escherichia coli*, and protein purification were performed as previously described (Barski et al., 1995). The oligonucleotides containing the mismatches to produce R308A and R311A mutations were (5′ to 3′) GTGGATGGGAAGGCAGTCCCAAGGG and AAGAGAGTCCCAGCGGATGCAGGGC, respectively. The mutations were verified by sequencing the cDNA. SDS−polyacrylamide gel electrophoresis and isoelectric focusing were performed using the Phastsystem (Pharmacia).

Enzyme Assays and Kinetic Analysis. Enzyme assays were routinely performed at 25 °C in 100 mM phosphate buffer containing 0.1 mM EDTA[1] at pH 7.0, except inhibition by dicarboxylic acids, which was measured in 50 mM MOPSO/0.1 mM EDTA. For determination of kinetic constants (k_{cat} and K_m), the NADPH concentration was held constant at 0.2 mM, while that of the substrate varied. Kinetic constants were calculated by fitting the Michaelis−Menten function directly in the hyperbolic form to the data with an unweighted least-squares analysis using Sigmaplot for Windows, version 2.0. To obtain k_{cat} values, a molecular mass of 36.5 kDa was used for aldehyde reductase, and the protein concentration was determined by the Bradford method[2] (BioRad protein determination kit). To determine the inhibitor concentration causing a 50% inhibition (IC_{50}), the aldehyde concentration giving a maximum activity was held constant while that of the inhibitor varied. IC_{50} was calculated by fitting the data to $v_i = V_0/(1 + I/IC_{50})$, where V_0 is the velocity in the absence of an inhibitor.

Nucleotide Binding. Nucleotide binding to the aldehyde reductase mutants was determined in 5 mM phosphate buffer at pH 7.0 and 20 °C by measuring the quenching of enzyme fluorescence resulting from cofactor binding as described in our previous paper (Barski et al., 1995). Data were treated according to Stinson and Holbrook (1973) to determine the dissociation constant, K_d, and the total active site concentration.

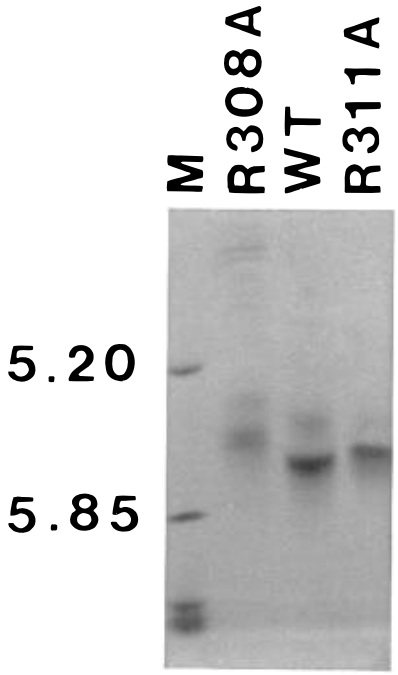

FIGURE 1: Isoelectric focusing of wild-type aldehyde reductase and its mutants. One microgram of each protein was applied in the middle of precast 4−6.5 IEF gel (Pharmacia), and the gel was stained with Coomassie Brilliant Blue R. Standards are β-lactoglobulin A (5.20) and bovine carbonic anhydrase B (5.85).

RESULTS AND DISCUSSION

Purification and Physical Properties of the Mutant Proteins. Expression of the R311A mutant gave a yield of ∼15 mg per 3 L of culture, while the yield of the R308A mutant was lower (∼5 mg). Wild-type and R311A aldehyde reductase preparations were apparently homogeneous as judged from SDS−polyacrylamide gel electrophoresis. Due to the low yield, the R308A mutant protein preparation contained minor impurities (<5%) that did not significantly affect our kinetic results. IEF analysis shows some charge heterogeneity which is very common with human aldose reductase as well (Bohren et al., 1991). The isoelectric point of the R308A mutant is shifted −0.12 pH unit in comparison to that of the wild-type enzyme, while the R311A exhibited about one-half of that shift (Figure 1). The clear difference in the observed shifts between the two mutants, although small, suggests that the Arg308 is directed to the outside of the protein molecule and is exposed to the solvent, while Arg311 is at least partially buried and may face the active site cavity. The calculated theoretical shift in the isoelectric point of the protein (Genetics Computer Group, 1994) due to the elimination of the positive charge of one arginine residue from the aldehyde reductase sequence is −0.18 pH unit.

The similarity in the nucleotide binding constants of the wild-type enzyme and the two mutants indicates that there is no gross perturbation in the overall mutant protein structures. The K_d values for NADPH and $NADP^+$, respectively, are 0.071 and 0.36 μM for the wild-type enzyme, 0.042 and 0.41 μM for the R311A mutant, and 0.10 and 1.18 μM for the R308A mutant. These dissociation constants for the two mutants maintain the ∼5−10-fold tighter binding of NADPH as compared to that of $NADP^+$ and differ only slightly from those of the wild-type enzyme constants.

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; MOPSO, 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid; ARI, aldose reductase inhibitor.

[2] Active site titration yielded less protein on a molar basis than the Bradford method by a factor of 2.4 ± 0.3. The kinetic parameters are nevertheless based on the Bradford method, using gammaglobulin, as a standard in order to be consistent with other publications (Barski et al., 1995).

Table 1: Kinetic Constants of Wild-type and Mutant Aldehyde Reductase[a]

	wild type (WT)	R311A	WT/R311A ratio	R308A	WT/R308A ratio
DL-glyceraldehyde					
k_{cat}	1.3	1.5	0.84	1.8	0.71
K_m	1.7	2.2	0.76	2.3	0.72
k_{cat}/K_m	786	717	1.1	795	1.0
D-glucuronate					
k_{cat}	2.7	0.92	2.9	4.1	0.64
K_m	4.2	364	0.011	7.5	0.56
k_{cat}/K_m	637	2.5	252	554	1.1
succinic semialdehyde					
k_{cat}	3.7	2.1	1.79	4.9	0.77
K_m	0.17	14.1	0.012	0.20	0.84
k_{cat}/K_m	22186	149	149	24425	0.91
p-nitrobenzaldehyde					
k_{cat}	5.1	4.2	1.2	6.7	0.77
K_m	0.16	0.38	0.42	0.25	0.63
k_{cat}/K_m	32025	11060	2.9	26306	1.2
p-carboxybenzaldehyde					
k_{cat}	3.7	2.5	1.45	6.8	0.54
K_m	0.038	4.0	0.01	0.11	0.35
k_{cat}/K_m	97534	642	152	63170	1.54

[a] k_{cat} is expressed in s^{-1}, K_m in mM, and k_{cat}/K_m in s^{-1} M^{-1}. Standard errors are ≤10% for k_{cat} and k_{cat}/K_m and ≤20% for K_m.

Kinetic Properties of the Mutant Enzymes. The kinetic constants of the wild-type aldehyde reductase and the R308A and R311A mutants are summarized in Table 1. Three out of five substrates used in this study, namely D-glucuronate, succinic semialdehyde, and *p*-carboxybenzaldehyde, have a negatively charged carboxyl group at the other end of the molecule from the aldehyde group undergoing catalytic transformation. The Michaelis constants for these negatively charged substrates increased greatly (approximately 100 times) in the R311A mutant, resulting in a 150−250-fold loss of catalytic efficiency (k_{cat}/K_m). However, these constants did not change for substrates such as DL-glyceraldehyde and *p*-nitrobenzaldehyde which do not contain negatively charged groups. In contrast, the kinetic parameters of the R308A mutant enzyme do not exhibit any significant change with either type of substrate and are very similar to that of the wild-type enzyme.

These results indicate that Arg311 binds the terminal negative charge of substrates containing a carboxyl group by forming a salt link. This conclusion is consistent with the fact that the catalytic efficiency is affected mainly by the increase in K_m (83−100-fold), while the k_{cat} decreased ∼3-fold for D-glucuronate and <2-fold for succinic semialdehyde and *p*-carboxybenzaldehyde. Altered substrate binding affinity due to interactions occurring at a site distant from the catalytic site would be expected to affect K_m and not k_{cat} (Menger, 1992). In fact, the K_m values for the uncharged substrates did not change in the R311A mutant, indicating that the catalytic chemistry of this mutant was unaltered. Thus, Arg311 plays an important role in defining one aspect of the substrate specificity of aldehyde reductase, namely its preference for negatively charged substrates.

Inhibition by Dicarboxylic Acids. Certain dicarboxylic acids are known to be inhibitors of both aldose and aldehyde reductase, although with much greater potency toward aldehyde reductase (Branlant, 1982a,b). The degree of inhibition of aldehyde reductase by dicarboxylic acids depends on the distance between the two carboxyl groups and the hydrophobic character of the carbon chain (Branlant, 1982a). The possible interaction of dicarboxylic acid inhibitors with the two arginines contained in the C-terminal loop of aldehyde reductase was investigated using a series of such compounds. In preliminary studies, glutaric acid was used to confirm the mechanism of inhibition by dicarboxylic acids. It is a noncompetitive inhibitor with respect to glyceraldehyde in the forward reaction, while the inhibition pattern was competitive with respect to benzyl alcohol as a substrate in the reverse reaction (results not shown). Such an inhibition pattern is characteristic of aldose and aldehyde reductase inhibitors and is consistent with the fact that inhibitors bearing at least one negative charge bind preferentially to the $NADP^+$ form of the enzyme to form dead-end complexes (Ehrig et al., 1994; Barski et al., 1995).

The effect of the R308A and R311A mutations on the inhibitory potency of saturated unsubstituted dicarboxylic acids of various chain lengths was investigated. Dicarboxylic acids with a chain length from three (malonic acid) to eight (suberic acid) carbon atoms were used. The IC_{50} values obtained in the presence of 4 mM DL-glyceraldehyde as a substrate are presented in Table 2. For the wild-type enzyme, the inhibitory potency progressively increases with increasing chain length from malonic (336 mM) to glutaric acid (2.1 mM) without any further change in potency occurring with longer chain length (suberic acid, 1.7 mM). The R311A mutant has a substantial decrease in affinity for dicarboxylic acid inhibitors as compared to the wild-type enzyme. The IC_{50} ratio of R311A/wild type reaches a maximum of 23 with glutaric acid (Table 2), suggesting that among all acids tested it interacts most strongly with Arg311. The systematic investigation of the correlation between the length of saturated linear dicarboxylic acids and inhibitory potency allowed us to "measure" the distance between the anion binding site (formed by the C4N of $NADP^+$, the hydroxyl of Tyr49, and the Nϵ of His112) where one carboxyl group binds and the positive charge of the Arg311 which binds the other carboxyl group. Thus, the length of glutaric acid with three methylene groups separating its two carboxyl groups approximates the distance between the anion binding site at the bottom of the active site pocket and the positive charge of Arg311 in aldehyde reductase.

Using energy-minimized structures obtained from CORINA (Sadowski & Gasteiger, 1993), the distances between

Table 2: Inhibition of Aldehyde Reductase and Its Mutants by Dicarboxylic Acids (IC_{50}, mM)[a]

dicarboxylic acid	wild type	R311A	R311A/WT	R308A	R308A/WT
malonic (1 CH_2)	336	–[b]	–	155	0.46
succinic (2 CH_2)	85	122	1.4	81.6	0.95
glutaric (3 CH_2)	2.11	48.3	22.8	2.83	1.34
adipic (4 CH_2)	1.89	17.4	9.2	5.02	2.65
pimelic (5 CH_2)	1.61	21.4	13.4	3.88	2.42
suberic (6 CH_2)	1.73	9.62	5.6	5.75	3.33
dimethylsuccinic	0.0106	0.251	23.7	0.0129	1.22
tetramethyleneglutaric	0.138	4.59	33.3	0.417	3.02

[a] Measurements were performed with 4 mM DL-glyceraldehyde and 0.2 mM NADPH in 50 mM MOPSO buffer at pH 7.0. Standard errors were less than 20%. [b] 10% activation was observed at 90 mM malonic acid.

the carboxyl groups in succinic and glutaric acids were calculated. The longest distance between the two carboxyl oxygen atoms is 6.3 Å in succinate and 7.5 Å in glutarate. The shortest distance is 4.8 Å in both compounds. Taking into account an average length for the salt-link bond of ~3 Å, the distance between the anion binding site and the positive charge of Arg311 is estimated to be around 10 Å. The R308A mutation does not significantly affect inhibition by dicarboxylic acids (Table 2), with the maximal IC_{50} ratio of the R308A and the wild-type enzymes being 3.3 with suberic acid. Thus, Arg308 does not participate in either substrate or inhibitor binding.

Role of Hydrophobic Groups in Aldehyde Reductase Inhibition. Substituted dicarboxylic acids are better inhibitors of aldehyde reductase than unsubstituted ones presumably due to additional stabilization of the enzyme−inhibitor complex caused by hydrophobic interactions of the side chains with hydrophobic residues in the active site pocket. Dimethylsuccinic and tetramethyleneglutaric acids are the examples used in this study. IC_{50}s for these two acids in the wild-type enzyme are at least 1 order of magnitude smaller than those for succinic and glutaric acids (Table 2). As with the unsubstituted acid inhibitors, the R308A mutation did not lead to any substantial changes in the IC_{50} of these compounds, while the R311A mutant exhibited IC_{50}s 20−30 times higher than those of the wild-type enzyme. Thus, Arg311−COO^- interaction is important for binding of substituted as well as unsubstituted dicarboxylic acids. The importance of bulk in an inhibitor is nicely illustrated by the R311A mutation which had a huge effect on the binding of dimethylsuccinic acid but no effect on the simpler succinic acid. Since the carboxyl groups of dimethyl succinate (6.2 Å apart) are not further apart than in succinate, it is suggested that the interaction of the hydrophobic bulk with hydrophobic residues on the enzyme slightly shortens the distance between the $NADP^+$ anion well and the positive charge of Arg311 by causing the C-terminal loop to move closer to the active site pocket. Movement of a short segment (residues 298−303) of the C-terminal domain, away from the pocket, was reported in an aldose reductase−zopolrestat complex upon binding of this inhibitor (Wilson et al., 1993).

Inhibition by Aldose Reductase Inhibitors (ARIs). Kinetic and binding studies previously showed that several commercially developed ARIs inhibit aldose and aldehyde reductase by binding to the E−$NADP^+$ form of the enzymes (Ehrig et al., 1994; Barski et al., 1995). Table 3 shows that the inhibitory potency of various ARIs is barely affected by the R308A mutation, while significant changes occur in both directions with the R311A mutant when compared with the wild-type enzyme. Tolrestat and zopolrestat inhibit the R311A mutant with greater potencies with IC_{50}s that are lower by 30- and 5-fold, respectively, while the inhibitory potencies for FK366 and ponalrestat are decreased with IC_{50}s that are higher by 2- and 5-fold, respectively. These opposite directional effects seen with these inhibitors suggest possible steric interactions of the Arg311 side chain with some of the inhibitor molecules. Such interactions may hamper (tolrestat and zopolrestat) or enhance (FK366 and ponalrestat) binding in the wild-type aldehyde reductase. Mutation of Arg311 to alanine (a much less bulky residue than arginine) improves the occupancy of some inhibitors in the active site pocket (tolrestat and zopolrestat). That some inhibitors are affected in the opposite direction by the R311A mutation while others are not affected at all suggests that the C-terminal loop has more complex interactions with these inhibitors and is thus a more important participant in the active site pocket than hitherto appreciated.

Table 3: Inhibition of Aldehyde Reductase by ARIs (IC_{50}, μM)[a]

inhibitor	wild type	R311A	R311A/WT	R308A	R308A/WT
AL1576	0.062	0.093	1.50	0.036	0.58
tolrestat	0.72	0.025	0.035	0.51	0.71
ponalrestat	4.6	30.1	6.54	13.0	2.83
sorbinil	5.4	2.4	0.44	2.7	0.50
FK366	14.5	28.0	1.93	27.6	1.90
zopolrestat	27.0	5.1	0.19	50.5	1.87
alrestatin	148	119[b]	0.80	67.3	0.45

[a] Measurements were performed with 3 mM DL-glyceraldehyde and 0.2 mM NADPH in 100 mM phosphate buffer at pH 7.0. Standard errors were less than 15%. [b] 40% standard error.

The present work identifies Arg311, present in the C-terminal loop, as the residue that binds the negatively charged ω-carboxyl group of substrates and is therefore the basis for the preference of aldehyde reductase for carboxyl-containing substrates. This residue also interacts with the negatively charged carboxyl group of dicarboxylic acid inhibitors and is unique to this member of the aldo-keto reductase superfamily. These results demonstrate that the C-terminal loop of aldehyde reductase directly interacts with the substrates and inhibitors, with the diversity of responses suggesting considerable mobility of the entire loop. Such interactions have broad implications for the future design of pharmaceutical effectors specifically targeted to the various members of the aldo-keto reductase superfamily.

NOTE ADDED IN PROOF

After submission of this paper for publication, we found that the crystallographic coordinates of the human aldehyde reductase apoenzyme determined by El-Kabbani et al. (1994) were recently disembargoed and deposited in the Brookhaven

Protein Data Bank (2ALR.pdb). The crystal structure confirms our conclusions about the location of the two arginine residues and the approximate distance from Arg311 to the anion binding site. Indeed, Arg311 is located within the active site cavity and is partially buried in the cavity wall. The Arg308 residue is directed to the outside of the enzyme molecule and is fully exposed to the solvent. The distance between the solvent-exposed amino group of Arg311 and the estimated location (we approximated the position of the nicotinamide in the apoenzyme structure) of the anion binding site is ~11 Å. This corresponds well with our estimate of 10 Å for the distance between the anion binding site and the positive charge of Arg311 which we obtained using dicarboxylic acid inhibitors of progressively increasing lengths. The close values of the distances derived from our biochemical experiments that characterize the holoenzyme and the crystal structure of the apoenzyme suggest that the active site of the apoenzyme is not significantly different from that of the holoenzyme. The crystal coordinates for the latter structure continue to be embargoed by the authors (El-Kabbani et al., 1995). A figure derived from the apoenzyme crystallographic coordinates shows the aldehyde reductase apoenzyme active site pocket and the location of the two arginine residues with respect to the anion binding site. This figure is available as Supporting Information and may also be viewed and/or downloaded at our web site (http://www.bcm.tmc.edu/pedi/md&m/Biochem8.html).

SUPPORTING INFORMATION AVAILABLE

One figure showing the aldehyde reductase apoenzyme active site pocket and the location of two arginine residues, available in electronic form only via the Internet. See the current masthead page for accessing information.

REFERENCES

Barski, O. A., Gabbay, K. H., Grimshaw, C. E., & Bohren, K. M. (1995) *Biochemistry 34*, 11264−11275.

Bohren, K. M., Bullock, B., Wermuth, B., & Gabbay, K. H. (1989) *J. Biol. Chem. 264*, 9547−9551.

Bohren, K. M., Page, J. L., Shankar, R., Henry, S. P., & Gabbay, K. H. **(**1991**)** *J. Biol. Chem. 266*, 24031−24037.

Bohren, K. M., Grimshaw, C. E., & Gabbay, K. H. (1992) *J. Biol. Chem. 267*, 20965−20970.

Bohren, K. M., Grimshaw, C. E., Lai, C.-J., Harrison, D. H., Ringe, D., Petsko, G. A., & Gabbay, K. H. (1994) *Biochemistry 33*, 2021−2032.

Branlant, G. (1982a) *Eur. J. Biochem. 121*, 407−411.

Branlant, G. (1982b) *Eur. J. Biochem. 129*, 99−104.

Branlant, G., & Biellmann, J.-F. (1980) *Eur. J. Biochem. 105*, 611−621.

Branlant, G., Tritsch, D., & Biellmann, J.-F. (1981) *Eur. J. Biochem. 116*, 505−512.

Bruce, N. C., Willey, D. L., Coulson, A. F. W., & Jeffery, J. (1994) *Biochem. J. 299*, 805−811.

Ehrig, T., Bohren, K. M., Prendergast, F. G., & Gabbay, K. H. (1994) *Biochemistry 33*, 7157−7165.

El-Kabbani, O., Green, N. C., Flynn, T. G., & DeLucas, L. J. (1994) *Acta Crystallogr. D50*, 859−868.

El-Kabbani, O., Judge, K., Ginell, S. L., Myles, D. A. A., Delucas, L. J., & Flynn, T. G. (1995) *Nat. Struct. Biol. 2*, 687−692.

Genetics Computer Group (1994) *Program Manual for the Wisconsin Package*, Version 8, September 1994, 575 Science Dr., Madison, WI 53711.

Harrison, D. H., Bohren, K. M., Ringe, D., Petsko, G. A., & Gabbay, K. H. (1994) *Biochemistry 33*, 2011−2020.

Menger, F. M. (1992) Biochemistry 31, 5368−5373.

Rondeau, J.-M., Tete-Fâvier, F., Podjarny, A., Reyman, J.-M., Barth, P., Biellmann, J.-F., & Moras, D. (1992) *Nature 355*, 469−472.

Sadowski, J., & Gasteiger, J. (1993) *Chem. Rev. 93*, 2567−2581.

Stinson, R. A., & Holbrook, J. J. (1973) *Biochem. J. 131*, 719−728.

Tarle, I., Borhani, D. W., Wilson, D. K., Quiocho, F. A., & Petrash, J. M. (1993) *J. Biol. Chem. 268*, 25687−25693.

Wermuth, B. (1985) in *Enzymology of Carbonyl Metabolism 2: Aldehyde Dehydrogenase, Aldo/Keto Reductase, and Alcohol Dehydrogenase*, pp 209−230, Alan R. Liss, Inc., New York.

Wilson, D. K., Bohren, K. M., Gabbay, K. H., & Quiocho, F. A. (1992) *Science 257*, 81−84.

Wilson, D. K., Tarle, I., Petrash, J. M., & Quiocho, F. A. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 9847−9851.

BI9619740