Reactivity of Horse Liver Alcohol Dehydrogenase with 3-Methylcyclohexanols†

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ABSTRACT: The specificity of horse liver alcohol dehydrogenase for cyclohexanol and its 3-methyl derivatives was investigated by stopped-flow and initial velocity kinetic studies. The (1*S*,3*S*)-3-methylcyclohexanol was 7 times more reactive (V/K_m) than cyclohexanol, whereas the (1*R*,3*R*)-3-methylcyclohexanol was at least 1000 times less reactive than its enantiomer. Computer simulation of the transient reaction of NAD^+ and the cyclohexanols catalyzed by the enzyme suggests that the rate of transfer of hydrogen from the alcohol to NAD^+ is increased with the 1*S*,3*S* isomer. Modeling of the three-dimensional structure of the ternary complex of the enzyme suggests that the 1*S*,3*S* isomer should only bind in a productive, reactive mode, whereas the 1*R*,3*R* isomer would bind predominantly in a nonproductive, inhibitory mode.

Horse liver alcohol dehydrogenase has a large substrate-binding pocket and a broad specificity for alcohols. From steady-state kinetic studies and X-ray crystallography, the size and shape of the active site has been characterized by "diamond lattice" or "cubic space" models (Prelog, 1964; Dutler & Brändén, 1981; Irwin & Jones, 1976; Jones & Jakovac, 1982; Lemière et al., 1982; Horjales & Brändén, 1985). These models are useful for predicting which compounds are substrates for the enzyme for organic synthesis and for understanding the physiological activity (Jones & Beck, 1976). Nevertheless, quantitative data on the rate constants of interconversion of stereochemically defined substrates should be determined and correlated with the size and shape of the pocket. Furthermore, the consequence of binding of substrates in nonproductive modes should be evaluated. All of the models currently proposed assume that the substrates, in general cyclohexanols, bind in just one mode.

In particular, we have examined the effect of the 3-methyl group on the reaction of cyclohexanols. Computer modeling suggests that (1*S*,3*S*)-3-methylcyclohexanol should fit well into the active site in a productive mode (Figure 1A). In contrast, the 1*R*,3*R* isomer is predicted to be a poor substrate (Horjales & Brändén, 1985; Graves et al., 1965; Van Osselaer et al., 1978). Our model building suggests that this isomer would bind best in an inhibitory, nonproductive mode, with the hydrogen that should be transferred to NAD^+ pointing away from the nicotinamide ring (Figure 1B). It does not appear likely that 3-methylcyclohexanol could rotate in the active-site pocket, as was proposed for the *p*-bromobenzyl alcohol complex that was defined by X-ray crystallography (Eklund et al., 1982).

Experimental Procedures

Crystalline horse liver alcohol dehydrogenase was obtained from Boehringer-Mannheim. The enzyme was freed of ethanol by gel filtration, and the enzyme was assayed in a standard procedure (Plapp, 1970). The concentration of active sites of enzyme was determined by titration with NAD^+ and pyrazole (Theorell & Yonetani, 1963). At 30 °C, the enzyme had 3.5 units/mg. The coenzymes $LiNAD^+$ and Na_2NADH were obtained from Boehringer-Mannheim.

Cyclohexanol and cyclohexanone were obtained from Eastman Kodak and redistilled before use. Cyclohexanol-d_{12} was purchased from Merck, Sharp and Dohme. Aldrich Chemical Co. provided (±)-3-methylcyclohexanone (97%), (3*R*)-(+)-3-methylcyclohexanone (98%), $LiAlH_4$, and L-Selectride (lithium tri-*sec*-butylborohydride, 1 M, in tetrahydrofuran).

The (1*S*,3*S*)-3-methylcyclohexanol was prepared with a coupled substrate recycling system (Van Osselaer et al., 1978). The reaction mixture contained 0.4 M ethanol, 40 μM NAD^+, 0.1 M (±)-3-methylcyclohexanone, and 0.12 μN liver alcohol dehydrogenase in 0.4 M tris(hydroxymethyl)aminomethane hydrochloride, pH 8.5, buffer. The reaction proceeded at 30 °C for 22 h, which yielded 30% conversion to the 3-methylcyclohexanol. The products were extracted into diethyl ether, dried with $MgSO_4$, concentrated by evaporation, and purified on a silica column (E. Merck LiChroprep Si 60, 40–63 μm) developed with hexane–ethyl acetate (70:30) with detection by refractive index.

The (1*R*,3*R*)-3-methylcyclohexanol was prepared by reduction of (*R*)-(+)-3-methylcyclohexanone with L-Selectride according to the method of Brown and Krishnamurthy (1972). The reaction was stopped by the addition of NaOH and H_2O_2, and the product was purified as above for the 1*S*,3*S* isomer.

The purities of the 3-methylcyclohexanols were monitored by using gas chromatography (5% Carbowax 20M, 60/80 Carbopak B column, 6 ft × 2 mm i.d., glass, Supelco) with a Varian Model 3740 chromatograph, with N_2 flow of 4.5 cm^3/s at 122 °C. Cyclohexanol eluted at 8.8 min, 3-methylcyclohexanone at 10.8 min, and 3-methylcyclohexanol at 17.2 min. NMR spectra were determined with the 360-MHz Bruker instrument with 20 mM compounds in D_2O. The alcohols appeared to be homogeneous. Nevertheless, the L-Selectride reduction gives 94.5% trans products (Brown & Krishnamurthy, 1972), and the predicted ratio of products should be 92.61% 1*R*,3*R*, 5.39% 1*S*,3*R*, 1.89% 1*S*,3*S*, and 0.11% 1*R*,3*S*. During purification on the silica column, the diastereoisomers should be separated, but the 1*R*,3*R* isomer might still contain 2.00% of the 1*S*,3*S* isomer.

Kinetic studies were carried out with a Cary 118C spectrophotometer interfaced to a Data Translation A/D board in an IBM PC/XT. Initial velocities were determined by a fit of the data to a straight line or a parabolic function using FORTRAN programs. The buffer was 33 mM sodium phosphate, containing 0.25 mM sodium ethylenediaminetetraacetate (EDTA), pH 8, at 30 °C. Stopped-flow experiments used a custom instrument designed by Dr. David P. Ballou with a Kinetic Instruments flow device and a Dur-

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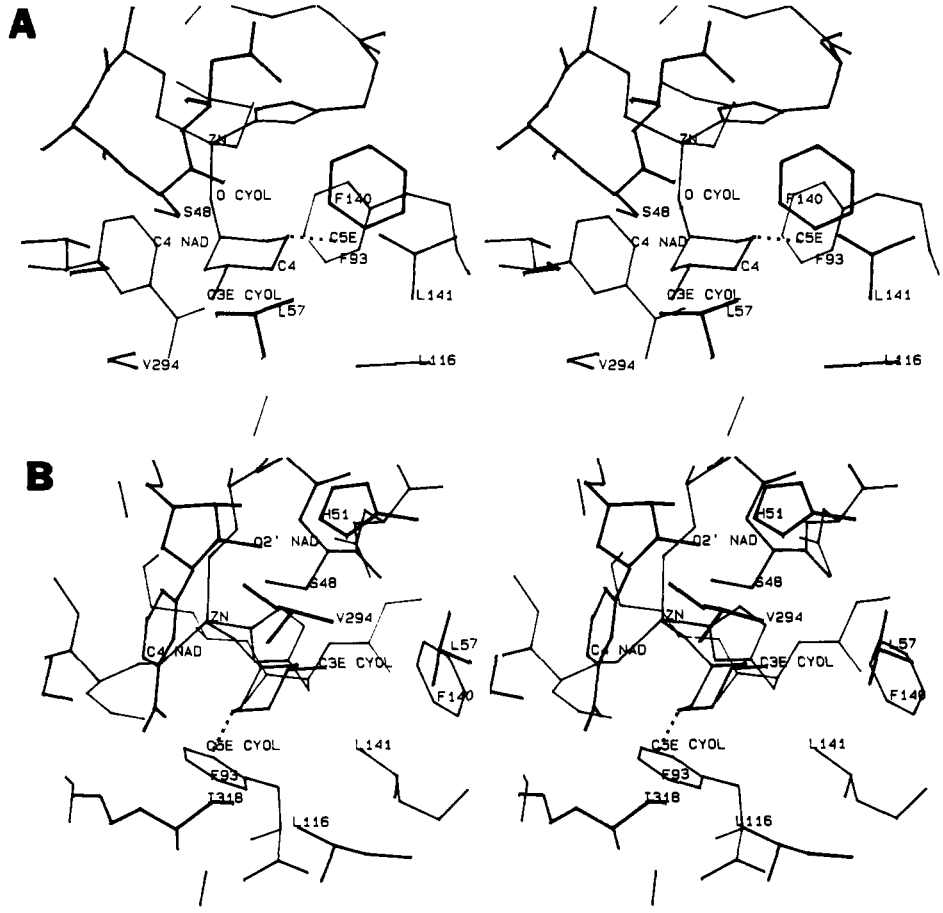

FIGURE 1: Stereo diagrams of the models for binding of 3- (or 5-) methylcyclohexanols in the active site of horse liver alcohol dehydrogenase. The coordinates for the enzyme and NAD^+ were obtained from the refined structure of the enzyme–NAD^+–*p*-bromobenzyl alcohol complex (Eklund et al., 1982). (A) Productive binding mode of (1*S*,3*S*)-3-methylcyclohexanol with the 1-hydroxyl group in the axial position ligated to the zinc and the 3-methyl group (C3E CYOL) in the equatorial position, making favorable contacts with the side chains of Val-294 and Leu-57. This mode of binding was described by Horjales and Brändén (1985) as "allowed". Indicated by the dotted line is a methyl group (C5E) that would represent how the (1*R*,3*R*)-3-methylcyclohexanol presumably would have to bind if it were to be oxidized. The model shows that the distance to CD1 of Leu-141 would be too close (2.4 Å) to allow favorable binding. (B) Nonproductive, inhibitory binding mode of (1*R*,3*R*)-3-methylcyclohexanol. The C1 hydrogen is pointed away from the C4 of the nicotinamide ring. The 3-methyl group (C3E CYOL) makes favorable contacts with the side chains of Val-294 and Leu-57. The dotted line indicates a methyl group (C5E CYOL) that would represent how (1*S*,3*S*)-3-methylcyclohexanol could bind in the nonproductive mode; however, the contact is too close (2.3 Å) to CE2 of Phe-93.

Table I: Kinetic Constants for Cyclohexanols, Cyclohexanones, and Reference Substrates[a]

	stopped flow[b]			steady state[c]			
substrate	conc (mM)	K_m (mM)	k_H (s^{-1})	conc (mM)	K_m (mM)	V (s^{-1})	V/K_m
cyclohexanol	5–40	27	640	0.4–8	0.86	9.6	11
cyclohexanol-d_{11}	5–40	14	160				
(1*R*,3*R*)-3-methylcyclohexanol	0.5–8		0.25[d]	1–8	2.0	0.16	0.08
(1*S*,3*S*)-3-methylcyclohexanol	0.5–8	5.7	1040	0.05–0.8	0.096	7.0	73
ethanol	1.2–20	5.3	270	0.1–4.9	0.32	7.3	23
cyclohexanone	10–80	78*	170*	0.2–8	5.3	26	4.9
(3*R*)-3-methylcyclohexanone	10–30	10*	3.2*	1–9	5.0	0.64	0.13
(±)-3-methylcyclohexanone	10–50	41	36	0.2–8	9.4	20	2.1
acetaldehyde	0.5–20	2.6	570	1–5	0.28	120	430

[a] The kinetic constants were determined in 33 mM sodium phosphate buffer, pH 8, at 30 °C. Standard errors of fits to a hyperbola were less than 20%, except for the values marked with an asterisk, which were 30–90%. [b] k_H is the maximum rate for the transient burst. K_m is the apparent Michaelis constant. [c] V is the turnover number. [d] There was no observed burst reaction.

rum-Gibson monochromator interfaced to an IBM/AT computer with OLIS programs for data analysis. The instrument dead time was 1.3 ms. A typical reaction contained final concentrations of 10 μN enzyme, 2 mM LiNAD or 0.1 mM Na_2NADH, and varied concentrations of substrates. For reduction of cyclohexanones, 20 mM pyrazole was added to trap the enzyme–NAD^+ complex so that only a single turnover was observed.

Steady-state data were evaluated by using the FORTRAN programs of Cleland (1979). The kinetic simulation program, KINSIM, and an automatic fitting routine, FITSIM, were used to obtain rate constants for the overall enzymatic reaction (Barshop et al., 1983; Zimmerle et al., 1987).

RESULTS

The steady-state kinetic constants for the cyclohexanols and cyclohexanones are summarized in Table I. All of these substrates showed normal Michaelis–Menten behavior over the range of concentrations investigated. Excessively high concentrations were avoided as substrate activation or inhibition could occur due to formation of enzyme–NADH–alcohol complexes (Dalziel & Dickinson, 1966; Cook & Cleland,

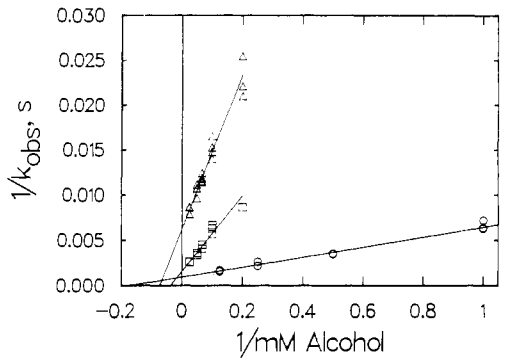

FIGURE 2: Substrate saturation curves from stopped-flow studies with cyclohexanols. The first-order rate constants for the transient phase of oxidation by 10 μN enzyme and 2 mM NAD^+ and varied concentrations of the alcohols, as given in Table I, are plotted in double-reciprocal form. The lines are calculated from the HYPER program (Cleland, 1979). Cyclohexanol-d_{11} (△); cyclohexanol (□); (1*S*,3*S*)-3-methylcyclohexanol (○).

1981). The transient rates for oxidation of the alcohols or reduction of the ketones were also determined with varied concentrations of substrate. As shown in Figure 2, the apparent exponential rate constant for the "burst" reaction depended upon substrate concentration; extrapolation to saturating substrate concentration gave the maximal rate constant.

The data in Table I illustrate some important features about the substrate specificity of the enzyme. As previously described, the enzyme has an essentially ordered mechanism with either ethanol or cyclohexanol (Dalziel & Dickinson, 1966; Ainslie & Cleland, 1972). The turnover number with cyclohexanol or ethanol is controlled by the rate of dissociation of the enzyme–NADH complex rather than by the hydrogen-transfer reaction. Thus, one should compare reactivities of different substrates with the enzyme with the V/K_m parameter, which is the overall bimolecular rate constant for reaction. The results show that cyclohexanol is almost as reactive as ethanol. In contrast, the 3-methylcyclohexanol derivatives show greatly different reactivities. (1*S*,3*S*)-3-Methylcyclohexanol was 7 times better and the 1*R*,3*R* isomer was 140 times less reactive than the corresponding unsubstituted cyclohexanol. The reactivity of the 1*R*,3*R* isomer reported in Table I is probably an upper limit for the actual activity, since the synthetic method might have given 2% of the 1*S*,3*S* isomer as a by-product.

In agreement with previous results (Graves et al., 1965; Dutler & Brändén, 1981), (3*R*)-3-methylcyclohexanone was a poorer substrate than the (3*S*)-3-methylcyclohexanone found in the racemic mixture. In general, these cyclohexanones were poorer substrates than acetaldehyde, whereas the cyclohexanols were better substrates than ethanol.

In order to describe the complete kinetic mechanism for reaction of the enzyme with the cyclohexanols, the rate constants for binding and dissociation of NAD^+ and NADH were determined in the stopped flow under the conditions used for the other experiments (Table II). The binding of NAD^+ to the enzyme is accompanied by an isomerization (Plapp et al., 1986), and the rate constants can be determined by stopped-flow techniques (Sekhar & Plapp, 1988).

The rate constants for oxidation of cyclohexanols were estimated by fitting the progress curves for the transient reaction with KINSIM and FITSIM. Typical experimental results and the simulated curves are presented in Figure 3. In general, the data could be simulated readily, but the magnitudes of the rate constants were, in several cases, quite sensitive to the particular values chosen for the enzyme concentration, the magnitudes of the fixed rate constants, and the concentrations estimated

Table II: Rate Constants for Binding of NAD^+[a] and NADH[b]

$$E \underset{k_{-1}}{\overset{k_1[NAD^+]}{\rightleftharpoons}} E{\cdot}NAD^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} F{\cdot}NAD^+ \qquad E{\cdot}NADH \underset{k_{-6}[NADH]}{\overset{k_6}{\rightleftharpoons}} E$$

$k_1 = 8 \times 10^7\ M^{-1}\ s^{-1}$; $k_2 = 1600\ s^{-1}$	$k_6 = 8.6\ s^{-1}$
$k_{-1} = 6 \times 10^4\ s^{-1}$; $k_{-2} = 61\ s^{-1}$	$k_{-6} = 1.3 \times 10^7\ M^{-1}\ s^{-1}$

[a] The rate constants were obtained from the FITSIM computer simulation program with data from the stopped-flow instrument. One syringe contained 20 μN enzyme and the other syringe a solution of NAD^+ (0.2–2 mM) and pyrazole (1–8 mM) in systematically varied concentrations. The change in absorbance at 300 nm was observed. The standard errors of the fitted parameters were less than 30%. The value determined for pyrazole binding to the F·NAD^+ complex was $1.2 \times 10^5\ M^{-1}\ s^{-1}$, and the rate constant for dissociation of pyrazole was assumed to be $0.01\ s^{-1}$. The apparent rate constant for NAD^+ binding is expressed as $k_{on} = k_1k_2/(k_1 + k_2)$ and the rate of dissociation as $k_{off} = k_{-1}k_2k_{-2}/(k_{-1} + k_2)(k_2 + k_{-2})$. [b] The rate constant for dissociation of NADH was determined by mixing 20 μN enzyme and 20 μM NADH in one syringe with 2 mM NAD^+ and 20 mM pyrazole from another syringe. The change in absorbance at 355 nm was fitted to a first-order equation. The association rate constant was determined by mixing 17 μN enzyme with varied concentrations of NADH (17–77 μM); the observed first-order rate constant was directly proportional to the concentration of NADH.

Table III: Rate Constants for Reaction of Cyclohexanols[a]

$$F{\cdot}NAD^+ \underset{k_{-3}}{\overset{k_3[alcohol]}{\rightleftharpoons}} E{\cdot}NAD^+{\cdot}alcohol \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E{\cdot}NADH{\cdot}ketone \underset{k_{-5}[ketone]}{\overset{k_5}{\rightleftharpoons}} E{\cdot}NADH$$

rate constant	cyclohexanol	cyclohexanol-d_{11}	(1*S*,3*S*)-3-methylcyclohexanol
k_3, $M^{-1}\ s^{-1}$	3.2×10^5	4.4×10^5	1.0×10^6
k_{-3}, s^{-1}	550	3000	4000
k_4, s^{-1}	190	94	2000
k_{-4}, s^{-1}	170	63	700
k_5, s^{-1}	60	67	310
k_{-5}, $M^{-1}\ s^{-1}$	5.6×10^5	9.4×10^5	3.2×10^6

[a] The rate constants were obtained from the FITSIM simulations of stopped-flow results of the oxidation by NAD^+ with varied concentrations of alcohols as listed in Table I. Standard errors of the fits were usually less than 20% for k_4, k_{-4}, and k_5 but were 30–50% for k_3, k_{-3}, and k_{-5}, which also indicates the range of values from various simulations.

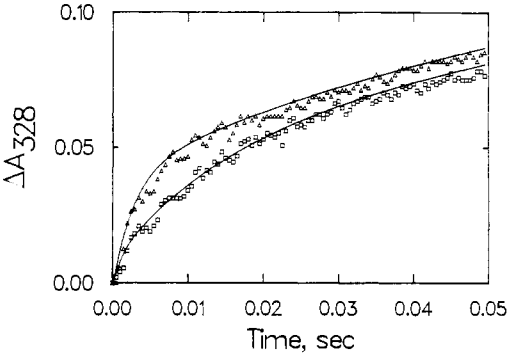

FIGURE 3: Simulation of transient phase of oxidation of cyclohexanol in the stopped-flow instrument. Cyclohexanol (15 mM) reacting with 8.8 μN enzyme and 2 mM NAD^+ (△); (1*S*,3*S*)-3-methylcyclohexanol (0.5 mM) reacting with 7.4 μN enzyme and 2 mM NAD^+ (□). The smooth lines are the simulated results, and the points are observed. The rate constants are listed in Table III.

for the beginning of the reaction observed after the dead time. Nevertheless, many of the rate constants appeared to be sensitive to the data and were descriptive of the system. The values calculated for V_1/K_b from the rate constants (Plapp, 1973) agreed closely with the experimentally determined values. Table III summarizes the values obtained for three different cyclohexanols. Kinetic constants calculated for the

Table IV: Kinetic Constants for Steady-State Reaction of Cyclohexanol and Cyclohexanone[a]

NAD^+ + cyclohexanol	NADH + cyclohexanone
$V_1 = 5.5\ s^{-1}$	$V_2 = 39\ s^{-1}$
$K_a = 4.0\ \mu M$	$K_q = 9.0\ \mu M$
$K_b = 260\ \mu M$	$K_p = 13$ mM
$K_{ia} = 43\ \mu M$	$K_{iq} = 0.6\ \mu M$
$K_{ib} = 16$ mM	K_{ip} (large)
$K_bK_{ia}/K_a = 3.9$ mM	$K_pK_{iq}/K_q = 2.2$ mM

[a] The constants were determined by product inhibition studies in 33 mM sodium phosphate buffer, pH 8.0, containing 0.25 mM EDTA, at 30 °C, and corrected for nonsaturation by the fixed substrate on the assumption of the ordered Bi-Bi mechanism (Plapp, 1970). Subscripts a, b, p, and q represent constants for NAD^+, cyclohexanol, cyclohexanone, and NADH, respectively. K_x are Michaelis constants, and K_{ix} are product inhibition constants. The standard errors were less than 15% of the values, except for K_{ib}, which was 30%. K_{ip} was not determined since the inhibition pattern of cyclohexanone against cyclohexanol was competitive.

Table V: Relative Reactivities of 3- (or 5-) Methylcyclohexanols

cyclohexanol substituent (OH axial)	stereochemistry	classification	kinetic data: cyclohexanol oxidation	kinetic data: cyclohexanone reduction
3 axial	1*S*,3*R*	F,[a] F[b]	ND[c]	0.46,[e] 5.1,[f] 0.18[g]
5 axial	1*R*,3*S*	F,[a] F[b]	ND[c]	0,[e] 0.18[g]
3 equatorial	1*S*,3*S*	A,[a] A[b]	73[d]	102,[e] 276,[f] 12.9[g]
5 equatorial	1*R*,3*R*	B2,[a] H[b]	0.08[d]	0.046,[e] 0.14,[f] 0.1[g]

[a] Assignment by direct diamond lattice: F, forbidden; A, allowed; B2, boundary (Horjales & Brändén, 1985). [b] Assigned by kinetic studies in solution: F, forbidden; A, allowed; H, hindered (Irwin & Jones, 1976). [c] ND, not determined. [d] V/K_m kinetic results from Table I. [e] Kinetic results in terms of ratios of reduction products (Graves et al., 1965). [f] Rates of alcohol production (Van Osselaer et al., 1980). [g] Rates of alcohol production (Dutler & Brändén, 1981).

ordered Bi-Bi mechanism with the rate constants in Tables II and III agreed within a factor of about 2 with the constants determined by product inhibition studies (Table IV).

Discussion

The magnitudes of the rate constants for oxidation of cyclohexanol and its derivatives generally support the predictions of the diamond lattice models deduced from the rates of reduction of cyclohexanones (Table V). However, the kinetic data for the 1*R*,3*R* alcohol suggest that the (3*R*)-methyl is a "forbidden" substitution rather than "boundary" or "hindered" as indicated by models. The models, of course, assume that the cyclohexanols bind in only one mode, which has the hydrogen to be transferred to the NAD^+ in a productive position, as in Figure 1A. Our objective is to explain why the (3*S*)-methyl group enhances reactivity 7-fold as compared to a hydrogen and why the 1*S*,3*S* isomer is 1000 times more reactive than its enantiomer.

As shown in Figure 1A, the (3*S*)-methyl group should make favorable contacts with the enzyme. These interactions could facilitate binding or formation of the transition state, effectively increasing V/K_m, the specificity constant. The rate constants in Table III suggest that the increased reactivity is due to increased rates of hydrogen transfer in the ternary complex, k_4. We think that the differences for step 4 are probably significant since the deuteriated cyclohexanol had slower rates. Some smaller effects are seen on the rates of binding, step 3, and larger effects are evident on step 5, the binding of the cyclohexanone, but the errors (or variability) of the simulation were larger.

An enhanced reactivity of 7-fold due to one methyl group is consistent with observations that one methylene unit improves hydrophobic binding interactions by about 2-fold (Hansch et al., 1972) and that changing a methionine to leucine in the substrate-binding pocket of yeast alcohol dehydrogenase increased reactivity 10-fold (Ganzhorn et al., 1987). In the latter case, there was a 2-fold increase in binding and a 5-fold increase in the hydrogen-transfer rate.

If the 1*R*,3*R* isomer is a substrate, it presumably must bind with its 3-methyl group "back" in the pocket, with contacts that are too close to the side chain of Phe-93 to allow the protein structure to remain undistorted or the substrate to bind in a good, productive mode. Of course, the 3-methylcyclohexanone reacts to produce this isomer but at a rate that is less than 1% of the rate at which the enantiomer is produced. One must assume that the alcohol can also be oxidized. Inspection of the structure of the enzyme–substrate complex suggests that a more favorable binding mode for the 1*R*,3*R* isomer would have the methyl group in the "front" of the pocket, in about the same position as that predicted for the enantiomer. This complex would have the structure shown in Figure 1B. The essential difference between the productive and nonproductive modes is that the cyclohexanol has been rotated in the active site so that the C_1 hydrogens are pointing in opposite directions. We assume that the hydrogen must point toward the NAD^+ for hydrogen transfer.

It is significant that the most stable binding mode of *p*-bromobenzyl alcohol in an active enzyme-substrate complex determined by X-ray crystallography has the hydrogen pointing away from the nicotinamide ring (Eklund et al., 1982). However, it appears that the benzyl alcohol can easily rotate in the active site to form a productive binding mode. The interactions of the protein with the substrate are not very different in the two binding modes, as the substrate-binding pocket is almost a barrel. In the case of 3-methylcyclohexanol, however, rotation is much less likely to occur because of the protruding methyl group.

Thus, we suggest that the 1*R*,3*R* isomer binds predominantly in the nonproductive mode shown in Figure 1B. The dissociation constant for this interaction is about 2 mM, as determined by using the compound as an inhibitor against oxidation of ethanol. This is about the same magnitude as the binding constants calculated for the cyclohexanols from the simulation results in Table III. Although the 1*R*,3*R* isomer could bind in an inhibitory mode and decrease the maximum velocity of oxidation of this isomer (bound in the productive mode), K_m is decreased proportionately, and thus the magnitudes of V/K_m reported in Tables I and V are not affected. The conclusion, nevertheless, is that alternative modes of binding of alcohols should be considered when one is mapping out the active site of an enzyme and correlating kinetic data with the structure.

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CORRECTIONS

Spectroscopic Investigations of Bovine Lens Crystallins. 2. Fluorescent Probes for Polar–Apolar Nature and Sulfhydryl Group Accessibility, by Usha P. Andley, Jack N. Liang, and Bireswar Chakrabarti*, Volume 21, Number 8, April 13, 1982, pages 1853–1858.

Pages 1853 and 1856. In the Abstract and under Results, the k_q value for the acrylamide quenching of the major component of AEDANS-labeled β_H-crystallin, which can be calculated from the slope of the line in Figure 4, should read 2.7×10^8 M^{-1} s^{-1}.

Effects of the Phenylalanine-22 → Leucine, Glutamic Acid-49 → Methionine, Glycine-234 → Aspartic Acid, and Glycine-234 → Lysine Mutations on the Folding and Stability of the α Subunit of Tryptophan Synthase from *Escherichia coli*, by A. M. Beasty, M. R. Hurle, J. T. Manz, T. Stackhouse, J. J. Onuffer, and C. R. Matthews*, Volume 25, Number 10, May 20, 1986, pages 2965–2974.

Page 2969. The equation for F_{app} should read

$$F_{app} = \frac{K_{NI}(Z + K_{IU})}{1 + K_{NI} + K_{NI}K_{IU}}$$

Although this equation was printed incorrectly in the paper, the data presented were calculated correctly according to the equation given here.

Synergism in Folding of a Double Mutant of the α Subunit of Tryptophan Synthase, by M. R. Hurle, N. B. Tweedy, and C. R. Matthews*, Volume 25, Number 21, October 21, 1986, pages 6356–6360.

Page 6358. The equation for F_{app} should read

$$F_{app} = \frac{K_{NI}(Z + K_{IU})}{1 + K_{NI} + K_{NI}K_{IU}}$$

Although this equation was printed incorrectly in the paper, the data presented were calculated correctly according to the equation given here.

Kinetics and Mechanism of Transitions Involving the Lamellar, Cubic, Inverted Hexagonal, and Fluid Isotropic Phases of Hydrated Monoacylglycerides Monitored by Time-Resolved X-ray Diffraction, by Martin Caffrey, Volume 26, Number 20, October 6, 1987, pages 6349–6363.

Page 6349. The address given in the byline is the author's present address. The study was carried out while the author was associated with the Section of Biochemistry, Molecular and Cell Biology, 249 Clark Hall, Cornell University, Ithaca, New York 14853.

Comparisons of Redox Kinetics of Methemerythrin and μ-Sulfidomethemerythrin. Implications for Interactions with Cytochrome b_5, by Linda L. Pearce, Ronald E. Utecht, and Donald M. Kurtz, Jr.*, Volume 26, Number 26, December 29, 1987, pages 8709–8717.

Page 8716. In column 1, the sentence beginning on line 1 should read as follows: In order to calculate this ratio, we need not only the appropriate reduction potentials (Table I) but also an estimate of the ratio $[k_{11}(Mb)/k_{11}(Hr)]^{1/2}$.