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Oxidation and Reduction of 4-Hydroxyalkenals Catalyzed by Isozymes of Human Alcohol Dehydrogenase†

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ABSTRACT: 4-Hydroxyalkenals, natural cytotoxic products of lipid peroxidation, are substrates for human alcohol dehydrogenases (ADH). Class I and II ADHs reduce aliphatic 4-hydroxyalkenals with chain lengths of from 5 to 15 carbons at pH 7 with k_{cat} and K_m values comparable to simple aliphatic aldehydes of the same chain length. Class II is particularly effective in the reduction with k_{cat} values as high as 3300 min^{-1} for 4-hydroxyundecenal. Class III ADH is essentially inactive toward all of these substrates. The class I and II isozymes also catalyze the oxidation of the 4-hydroxy group at pH 10. However, during the reaction, an NAD^+-dependent irreversible partial inactivation of the $\alpha\beta_1$ isozyme is observed which is attributed, with the aid of computer graphics modeling, to selective modification of the α subunit. Both ethanol and 1,10-phenanthroline, known to compete with conventional substrates, instantaneously, reversibly, and competitively inhibit 4-hydroxyalkenal reduction and oxidation, indicating that 4-hydroxyalkenals bind at the same site as do conventional substates. The fact that the class II enzyme $\pi\pi$-ADH so far is found only in the liver and that the 4-hydroxyalkenals are the best substrates known for this isozyme suggest that it may play a significant role in cellular defenses in the conversion of the cytotoxic aldehydes to the less reactive alcohols.

The cytotoxicity of products of stimulated lipid peroxidation has been attributed to the formation of aldehydes, particularly 4-hydroxyalkenals (Benedetti et al., 1980; Esterbauer et al., 1981). A major toxic product of the peroxidation process, 4-hydroxy-2,3-*trans*-nonenal (Benedetti et al., 1980; Esterbauer et al., 1982), is formed from arachidonic acid contained in the polar phospholipids (Esterbauer et al., 1986). Smaller amounts of other toxic products such as alkanals, 2-alkenals, and other 4-hydroxyalkenals are also formed [for a review, see Esterbauer (1985)]. Among the many biological effects of 4-hydroxynonenal are high cytotoxicity to Ehrlich ascites tumor cells (Schauenstein et al., 1977) and *Salmonella typhimurium* (Marnett et al., 1985), lysis of erythrocytes (Benedetti et al., 1980); and facile reactivity with thiols such as glutathione (Esterbauer et al., 1975), cysteine (Esterbauer et al., 1976), and thiol groups of proteins (Esterbauer, 1982). Moreover, 4-hydroxyalkenals generally inhibit the activity of many enzymes as well as the synthesis of DNA and protein (Esterbauer et al., 1985), and mutagenicity has been demonstrated in the *Salmonella* tester strain TA104 (Marnett et al., 1985). Detoxication of hydroxyalkenals by conjugation with glutathione proceeds efficiently through a glutathione transferase pathway (Ålin et al., 1985; Jensson et al., 1986; Danielson et al., 1987), but the efficiency of a proposed alternative pathway, reduction by alcohol dehydrogenase, an activity demonstrated in the rat liver cytosol fraction (Esterbauer et al., 1985), has not been reported.

The substrate specificity established for the alcohol dehydrogenases would suggest that one or more of the many enzymes could efficiently catalyze 4-hydroxyalkenal reduction.

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In man, three classes of alcohol dehydrogenase (ADH),[1] all of which are present in liver, have been identified based primarily on substrate and inhibitor specificity. Those of class I, composed of dimeric combinations of three major subunits, α, β, and γ, are particularly effective in oxidizing ethanol and provide the major pathway for detoxication of beverage alcohol (von Wartburg et al., 1967). Class II ($\pi\pi$-ADH) is less effective (Bosron et al., 1980), and class III ($\chi\chi$-ADH) hardly oxidizes ethanol at all (Wagner et al., 1984). However, the enzymes do exhibit remarkable specificity toward metabolically important substrates and inhibitors. Thus, the γ-containing forms are singularly inhibited by testosterone and capable of oxidizing 3-hydroxysteroids (McEvily et al., 1988), $\pi\pi$-ADH has a redox-specific function in norepinephrine metabolism (Mårdh et al., 1986), and $\chi\chi$-ADH is the only ADH capable of oxidizing formaldehyde (unpublished results) in a glutathione-dependent reaction as reported for the class III enzyme by Koivusalo et al., (1989). These and other features have indicated that the large complement of ADH isozymes (>20) likely serve critical metabolic functions beyond oxidation of ethanol. Indeed, the fact that all enzymes are able to reduce long-chain aldehydes suggested that the isozymes may participate in the detoxication of 4-hydroxyalkenals.

In this study, purified isozymes of human alcohol dehydrogenase are shown to reduce 4-hydroxyalkenals under physiological conditions at rates comparable to those for the glutathione-dependent detoxication pathway, and specific enzymes exhibit preferences for particular aldehydes. Both the reduction of the aldehyde and the oxidation of the secondary 4-hydroxy group of a homologous series of 4-hydroxyalkenals are examined. The various ADH isozymes show differences in their specificity toward these natural cytotoxins, indicating a potential for isozyme-specific roles in the detoxication process.

Materials and Methods

Chemicals. 4-Hydroxyalkenals, prepared as described (Esterbauer & Weger, 1967; Esterbauer, 1971), were generously provided by Professor H. Esterbauer, University of Graz, Graz, Austria. All except 4-hydroxypentadecenal were stored at −20 °C in chloroform, which was evaporated under nitrogen immediately before use. The residue was dissolved in water except that 4-hydroxypentadecenal, which is sparingly soluble in water, was dissolved in acetonitrile (1.8 mg/mL). The concentrations of the substrates were determined spectrophotometrically at 224 nm assuming a molar absorptivity of 13 750 M^{-1} cm^{-1} (Esterbauer & Weger, 1967).

ADH Isozymes. Homogeneous human liver alcohol dehydrogenase isozymes were isolated, purified, and characterized as previously described (Wagner et al., 1983, 1984; Ditlow et al., 1984; Beisswenger et al., 1985).

Measurement of Kinetic Constants. ADH activities were determined spectrophotometrically by measuring the absorbance change at 340 nm. Reduction of 4-hydroxyalkenals was measured in 50 mM TES/0.21 mM NADH, pH 7.0. For most of the studies, a kinetic mixing device (Scopes & Holmquist, 1987) was used to determine kinetic constants of the ADH enzymes. In this system, equal volumes of substrate and enzyme are mixed and introduced into an 8-μL flow cell positioned in the beam of a diode array spectrophotometer (Hewlett-Packard 8451A) where the rate is recorded for up to 30 s. A complete set of v vs S data are determined in a few minutes entirely under computer control. The substrate concentration is varied automatically in a single mixing chamber version of the instrument. The enzyme solution in one syringe contained 0.42 mM NADH in 0.1 M TES, pH 7.0. The second syringe contained water, and the mixing chamber contained the substrate, a 4-hydroxyalkenal, and 3 μM rhodamine B, an internal standard used to quantitate substrate dilution. Up to 12 different substrate concentrations served to determine K_m and k_{cat} with the highest concentration at least 2-fold above K_m. With the enzyme kinetics software used, K_m and k_{cat} were calculated, and data were presented as Eadie–Hofstee, Lineweaver–Burk, and v versus S plots (Figure 1). Good agreement was noted between manual determinations made with a Varian 219 spectrophotometer and those obtained by the automatic system. Oxidation of 4-hydroxyalkenals was measured in 0.1 M glycine–NaOH, pH 10.0, and 2.5 mM NAD^+, at 25 °C on a Varian 219 spectrophotometer. Inhibition constants were calculated from Lineweaver–Burk plots determined at different inhibitor concentrations.

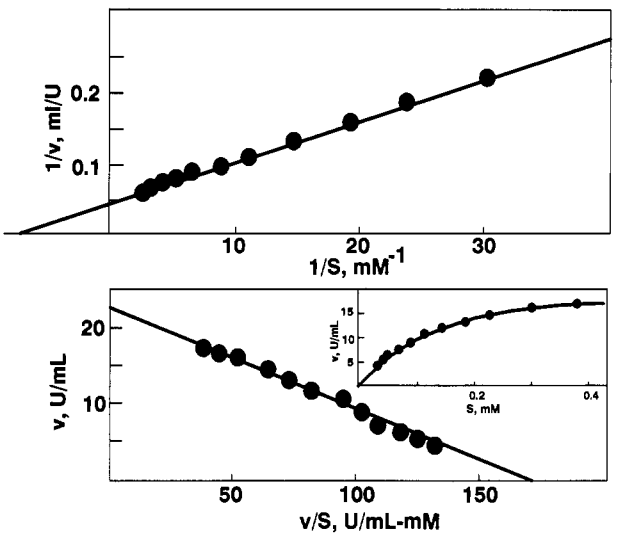

FIGURE 1: Typical output from the kinetic mixing device used to obtain kinetic data. The example shown is the reduction of C_8 by $\pi\pi$-ADH with an initial substrate concentration of 0.85 mM. Initial velocity data are linearized and fit to the Eadie–Hofstee plot and then replotted in Lineweaver–Burk and v vs S forms.

Computer-Graphics Studies. The substrate was fit into the active site with the interactive graphics display program TOM (Cambillau et al., 1984). This program uses FRODO as a base and has a ligand handling routine. The details of the method have been described (Eklund et al., 1987).

Results

Hydration of 4-Hydroxyalkenals. In water, 4-hydroxyalkenals do not form inactive aldehyde hydrates as determined by the method of Deetz et al. (1984). Solutions of 4-hydroxyhexenal (C_6) and 4-hydroxydecenal (C_{10}) were prepared in acetonitrile and water and incubated at 25 °C for 2 h. When diluted to a final concentration of 60 μM in an excess of ADH, there was no difference in the decrease in absorbance at 340 nm between substrates kept in the aqueous and nonaqueous phases.

Reduction of 4-Hydroxyalkenals. Class I and II ADH enzymes readily catalyze the reduction of 4-hydroxyalkenals at pH 7.0. Table I shows the K_m, k_{cat}, and k_{cat}/K_m values for class I, II, and III ADH enzymes with a homologous series of C_5–C_{11} and C_{15} 4-hydroxyalkenals. Most of the kinetic

[1] Abbreviations: ADH, alcohol dehydrogenase; C_5–C_{12}, carbon length of 4-hydroxyalkenals; NAD^+, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; TES, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid.

Table I: Kinetic Parameters for the Reduction of 4-Hydroxyalkenals (5–15 Carbon Atoms) by Various ADH Isozymes[a]

class	isozyme	4-hydroxyalkenal C_5	C_6	C_7	C_8	C_9	C_{11}	C_{15}
				K_m[b] (μM)				
I	$\alpha\beta_1$	ND[d]	2700	1800	1100	250	120	ND
	$\beta_1\beta_1$	ND	2100	1200	820	370	150	ND
	$\beta_1\gamma_1$	ND	ND	ND	ND	520	190	ND
	$\beta_2\beta_2$	ND	ND	ND	ND	1430	330	ND
II	$\pi\pi$	720	310	ND	130	97	97	ND
				k_{cat} (min^{-1})[b]				
I	$\alpha\beta_1$	ND	340	340	570	320	350	ND
	$\beta_1\beta_1$	ND	40	260	520	520	460	ND
	$\beta_1\gamma_1$	ND	ND	ND	ND	1100	700	ND
	$\beta_2\beta_2$	ND	ND	ND	ND	3570	1660	ND
II	$\pi\pi$	2800	3000	ND	3000	2700	3300	ND
				k_{cat}/K_m (μM^{-1} min^{-1})				
I	$\alpha\beta_1$	ND	0.12	0.18	0.51	1.3	3.0	5.7[c]
	$\beta_1\beta_1$	ND	0.12	0.22	0.64	1.4	3.2	5.4[c]
	$\beta_1\gamma_1$	ND	ND	ND	ND	2.0	3.7	3.6[c]
	$\beta_2\beta_2$	ND	ND	ND	ND	2.5	5.0	ND
II	$\pi\pi$	3.9	9.6	ND	23	28	34	ND
III	χ	0.020[c]	ND	ND	ND	0.56[c]	ND	0.36[c]

[a] Reaction conditions: 0.21 mM NADH/50 mM TES, pH 7.0 and 25 °C. [b] K_m and k_{cat} were determined with the kinetic mixing device. [c] Values determined at low substrate concentration (where $v/[E]_{tot} = k_{cat}/K_m[S]$). [d] ND, not determined.

parameters presented in Table I were obtained by use of the kinetic mixing device (Figure 1). The k_{cat} values for the reduction of individual 4-hydroxyalkenals by class I isozymes are all quite similar and comparable to those of the same isozymes toward other aldehydes, e.g., acetaldehyde, pentanal, and octanal (Deetz et al., 1984). The k_{cat} values for $\beta_2\beta_2$ are greater than those for the other class I isozymes in one case by as much as 10-fold. However, the corresponding K_m values are also considerably higher, and consequently the catalytic efficiency expressed as k_{cat}/K_m is in the same range as that for the other class I isozymes. The K_m values for both class I and II ADH enzymes decrease with increasing chain length of the substrate, which parallels their increasing hydrophobicity.

Class II ($\pi\pi$-ADH) reduces 4-hydroxyalkenals with high efficiency. The k_{cat}/K_m values for this enzyme are 10–100-fold higher than those of the class I isozymes, an effect that depends primarily on k_{cat}. In fact, the value of 3300 min^{-1} for C_{11} is the highest k_{cat} yet reported for any $\pi\pi$-ADH substrate.

Class III ($\chi\chi$-ADH) displays the lowest k_{cat}/K_m values due to very high K_m values; that of 4-hydroxynonenal is >6 mM.

Oxidation of 4-Hydroxyalkenals. Three class I ADH isozymes and the class II ADH oxidize 4-hydroxyhexenal and 4-hydroxynonenal at pH 10.0 (Table II). $\alpha\beta_1$ exhibits the highest k_{cat}/K_m toward 4-hydroxyhexenal, primarily due to its low K_m, and was studied in more detail with a homologous series of 4-hydroxyalkenals with chain lengths of 5 to 9 carbon atoms. The kinetic parameters of hydroxyalkenals of chain length 5, 6, and 8 carbon atoms (Table III) are very similar. The K_m, k_{cat}, and k_{cat}/K_m of $\alpha\beta_1$ for these substrates are close, but the C_7 and C_9 hydroxyalkenals are oxidized with much lower catalytic efficiency. This lowered activity of C_7 and C_9, specifically observed with $\alpha\beta_1$, is the result of irreversible inhibition during the assay. Thus, the rate of oxidation decreases during the first 10 min to a constant value of about one-tenth that of the initial velocity. These lower rates were used in determining the kinetic constants for C_7 and C_9. This apparent inhibition requires NAD^+ and does not occur when the enzyme is incubated with 4-hydroxynonenal alone. A 25-fold dilution of the inhibited reaction did not increase the relative activity, which suggests irreversible inhibition, perhaps of a "suicide" nature.

Table II: Kinetic Parameters for Some ADH Isozymes. Oxidation of C_6 and C_9 4-Hydroxyalkenals[a]

class	isozyme	4-hydroxyalkenal C_6	C_9
		K_m (μM)	
I	$\alpha\beta_1$	370	670
	$\beta_1\beta_1$	1800	1000
	$\beta_1\gamma_1$	970	400
II	$\pi\pi$	610	850
		k_{cat} (min^{-1})	
I	$\alpha\beta_1$	208	11
	$\beta_1\beta_1$	10	14
	$\beta_1\gamma_1$	53	57
II	$\pi\pi$	244	223
		k_{cat}/K_m (μM^{-1} min^{-1})	
I	$\alpha\beta_1$	0.56	0.016
	$\beta_1\beta_1$	0.0056	0.014
	$\beta_1\gamma_1$	0.055	0.14
II	$\pi\pi$	0.40	0.26

[a] Conditions: 2.5 mM NAD^+/0.1 M glycine–NaOH, pH 10.0 and 25 °C.

Table III: Kinetic Parameters for $\alpha\beta_1$ ADH Oxidation of 4-Hydroxyalkenals[a]

	4-hydroxyalkenal C_5	C_6	C_7	C_8	C_9
K_m (μM)	300	370	3600	202	670
k_{cat} (min^{-1})	240	208	38	118	11
k_{cat}/K_m (μM^{-1} min^{-1})	0.79	0.56	0.011	0.58	0.016

[a] Reaction conditions: 2.5 mM NAD^+/0.1 M glycine–NaOH, pH 10.0 and 25 °C.

A 4-hydroxynonenal-inhibited enzyme was prepared by mixing $\alpha\beta_1$ (0.026 μM) with 4-hydroxynonenal (~160 μM) at pH 10 in 2.5 mM NAD^+/0.1 M glycine–NaOH and incubating for 10 min. Its oxidizing activity toward ethanol and 4-hydroxyoctenal was only 10% that of native $\alpha\beta_1$. A similar decrease in reducing activity was observed with benzaldehyde as the substrate. The resultant oxidizing catalytic efficiency of the 4-hydroxynonenal-modified $\alpha\beta_1$ is similar to that of native $\beta_1\beta_1$, the latter being 10-fold lower in activity with most substrates (Wagner et al., 1983; Deetz et al., 1984). However, its reducing activity toward C_7–C_{11} 4-hydroxyalkenals was

unchanged. 4-Hydroxyalkenals with 7, 10, 11, or 12 carbon atoms were also found to inhibit $\alpha\beta_1$ selectively in the presence of NAD^+, but hydroxyalkenals with 6 or 8 carbon atoms did not.

Inhibition of Reduction and Oxidation. Both ethanol and 1,10-phenanthroline instantaneously, reversibly, and competitively inhibit 4-hydroxynonenal reduction. Ethanol inhibits 4-hydroxynonenal reduction by $\alpha\beta_1$ with a K_i of 0.64 mM. The K_i values for 1,10-phenanthroline inhibition of 4-hydroxynonenal reduction by $\alpha\beta_1$, $\beta_1\beta_1$, and $\beta_1\gamma_1$ are 0.86, 0.86, and 0.082 mM, respectively. The oxidation of 4-hydroxynonenal by $\beta_1\gamma_1$ is competitively inhibited by 1,10-phenanthroline with a K_i of 0.18 mM.

Catalytic Reaction under Physiological Redox Conditions. A model system that mimics the cytoplasmic redox state in rat liver cytoplasm (Bucher et al., 1972) contained NAD^+ at 0.5 mM and a variable ratio of $[NAD^+]/[NADH]$ of 200–700 (Williamson et al., 1967). The assay system contained 0.13 mM 4-hydroxynonenal and 0.04 μM $\beta_1\beta_1$ or $\alpha\beta_1$ in 50 mM TES, pH 7. With both isozymes, a significant reducing rather than oxidizing activity was observed.

Computer-Graphic Studies. Computer-graphic analyses were undertaken to elucidate the interaction of the α subunit of class I ADH (von Bahr-Lindström et al., 1986) with the inhibitory oxidation products of C_7 and C_9–C_{12} 4-hydroxyalkenals. The basis for this analysis is the known topography of horse ADH (Brändén et al., 1975) and the model for human class I enzyme (Eklund et al., 1987, 1990) derived from it. As in the horse enzyme, two parts of the active site are critically involved in substrate binding to the human enzymes: the long hydrophobic tunnel from the surface of the enzyme to the active-site zinc and a cleft in the vicinity of the metal that is sterically limited by the nicotinamide ring and several protein side chains, particularly residue 93 which is Phe in the β and γ subunits but Ala in the α subunit. The resultant wider cleft in the α subunit accommodates the oxidation products of 4-hydroxyalkenals and allows interaction of the aldehyde group with either Ser-177 or Ser-328, with possible hemiacetal formation. In this wider cleft, the alkyl chains of the long-chain 4-hydroxyalkenals (C_7 and C_9–C_{12}) occupy the same position as the phenyl ring of Phe-93 present in the β_1 and γ_1 subunits.

Discussion

Human alcohol dehydrogenase enzymes are shown here to catalyze the reduction of the aldehyde group of a series of 4-hydroxyalkenals at pH 7 and the oxidation of their secondary hydroxyl group at pH 10.

Reduction. Classes I and II but not class III efficiently catalyze the reduction of 4-hydroxyalkenals (Table I). K_m values decrease with longer chain length, a trend also observed with simple aliphatic alcohols (Wagner et al., 1983). The constancy of k_{cat} values indicates rate-limiting cofactor dissociation. The fact that ethanol and 1,10-phenanthroline, known to compete with other conventional substrates, inhibit 4-hydroxyalkenals competitively suggests that the latter bind at the same site as conventional substrates. Among class I isozymes, it is remarkable that all, and especially $\alpha\beta_1$ and $\beta_1\beta_1$, are characterized by identical kinetic parameters for each substrate. In contrast, with other conventional substrates, the ratios of activity of $\alpha\beta_1$ to $\beta_1\beta_1$ vary; e.g., pentanal is 3.7 and benzaldehyde is 9 (Deetz et al., 1984). Hence, the α subunit probably does not contribute significantly to the reduction of these alkenals (see below).

$\pi\pi$-ADH is particularly effective in catalyzing the reduction of all the 4-hydroxyalkenals examined. The high k_{cat} values, near 3000 min^{-1}, are relatively constant for the different substrates (Table I) which indicates that the rate-limiting step is likely the dissociation of NAD^+.

Oxidation. Oxidation of the 4-hydroxy group of 4-hydroxyalkenals is catalyzed by both class I and II ADHs but at rates far slower than primary alcohols (Wagner et al., 1983; Ditlow et al., 1984). Activity with class III is not detectable. Isozymes containing an α subunit generally exhibit the highest catalytic activity toward any particular substrate (Wagner et al., 1983), and this is also observed with the 4-hydroxyalkenals. The k_{cat} values for 4-hydroxyalkenal oxidation by $\alpha\beta_1$, $\beta_1\beta_1$, and $\beta_1\gamma_1$ are quite similar to those for primary, aromatic, and cyclic alcohols (Wagner et al., 1983); therefore, their lower catalytic efficiencies toward the secondary alcohol group are due to their higher K_m values (Wagner et al., 1983).

Such differences in activity among the class I isozymes have been attributed to a wider substrate cleft in the α subunit due to the change from Phe-93 in β_1 and γ_1 to Ala-93 and from Tyr-319 in β_1 and Phe-319 in γ_1 to Leu-319 in α (Eklund et al., 1987, 1990).

Irreversible Inhibition. The $\alpha\beta_1$-catalyzed oxidation of two of the substrates, 4-hydroxyheptenal (C_7) and 4-hydroxynonenal (C_9), is biphasic and consistent with substrate- or product-induced irreversible inactivation during the assays. This does not occur with 4-hydroxyalkenals of 5, 6, or 8 carbon atoms. That NAD^+ is required for this inactivation suggests the formation of an oxidation product that is irreversibly bound to the enzyme in the manner of suicide inhibition (currently under investigation). Since this effect is not observed with the $\beta_1\beta_1$ or $\beta_1\gamma_1$ isozymes and since the modified $\alpha\beta_1$ isozyme has a finite residual activity which is comparable to that of the $\beta_1\beta_1$ isozyme, it appears that the α subunit is selectively inactivated and that the residual activity is that of the β_1 subunit. The possibility of inhibitory contaminants in the substrate solutions appears unlikely since no corresponding inactivation was obtained with the same preparations used in kinetic studies involving 15 different forms of glutathione transferase (Danielson et al., 1987).

Remarkably, the modification by 4-hydroxynonenal does not change the reducing activity of $\alpha\beta_1$. Two possible explanations of this finding are either that the α subunit is inactive in the reduction reaction to begin with or that the inactivating substrate binds to the α subunit in such a way that it can still bind and reduce certain substrates. Due to the extended structure of the 4-hydroxyalkenal molecules when binding through the aldehyde group, they can be fitted into the long hydrophobic tunnel. The difference in the size of the cleft between the α and β subunits, which appears to be important for the oxidation reaction, does not seem to be important for the reduction reaction.

The modeling studies, performed in an effort to further clarify the nature of this inhibition, indicate that the inactivation of the $\alpha\beta_1$ isozyme by the long-chain 4-hydroxyalkenals could be due to irreversible binding in the limited cleft close to the zinc ion. The results of computer graphics (not shown) can be interpreted to indicate that in binding in a manner that positions the long alkyl group so that it mimics the missing phenyl group of Phe-93, the topography of the active site of the α subunit assumes a structure resembling that of a β_1 or γ_1 subunit.

It has been suggested that lipid peroxidation causes cellular damage through the production of diffusible cytotoxic products such as 4-hydroxyalkenals (Esterbauer, 1982). Under the simulated physiological conditions modeled with respect to the redox environment at pH 7, only a reducing activity of the

4-hydroxyalkenals was observed consistent with the known pH dependence of oxidation (maximal at pH 10) and reduction reactions (maximal near pH 7) catalyzed by alcohol dehydrogenase. Therefore, reduction to diols rather than oxidation of 4-hydroxyalkenals likely is the effective reaction in the cell. The high activity demonstrated by human ADH toward 4-hydroxyalkenals suggests that one or more enzyme forms play a role in the defense system of the cell to remove these products by reduction. On the basis of its high turnover, $\pi\pi$-ADH is the enzyme most likely to contribute the major effect in the conversion of the cytotoxic 4-hydroxyalkenals to the less reactive alcohols. Indeed, $\pi\pi$-ADH is essentially limited to the cytosolic fraction of liver cells where such detoxication has been shown to occur.

While all enzymes of ADH are present in liver, this is not the case in other tissues where it has been shown that certain isozymes are more abundant than others (Beisswenger et al., 1985; Wagner et al., 1984). It remains to establish the relative roles of the enzymes of ADH and of glutathione transferase, respectively, in the protection of individual organs.

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Registry No. ADH, 9031-72-5; 4-hydroxypentenal, 16931-17-2; 4-hydroxyhexenal, 17427-08-6; 4-hydroxyheptenal, 17427-09-7; 4-hydroxyoctenal, 17449-15-9; 4-hydroxynonenal, 29343-52-0; 4-hydroxyundecenal, 29343-58-6; 4-hydroxypentadecenal, 112147-39-4; ethanol, 64-17-5; 1,10-phenanthroline, 66-71-7.

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