β_2 (Oriental) Human Liver Alcohol Dehydrogenases Do Not Exhibit Subunit Interaction: Oxidation of Cyclohexanol by Homo- and Heterodimers†

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ABSTRACT: The steady-state kinetics of isozymes of human liver alcohol dehydrogenase (ADH) containing the β_2 (Oriental) subunit were investigated in order to confirm the supposition [Fong, W. P., & Keung, W. M. (1987) *Biochemistry* (preceding paper in this issue)] that the subunits of such heterodimeric ADHs act independently and noncooperatively. The ADH isozymes $\alpha\beta_2$, $\beta_2\beta_2$, $\beta_2\gamma_1$, and $\beta_2\gamma_2$ as well as $\gamma_1\gamma_1$ were purified by chromatography on DEAE-cellulose, 4-[3-[*N*-(6-aminocaproyl)amino]propyl]pyrazole–Sepharose, and CM-cellulose. Their kinetics were studied at pH 9.0 with cyclohexanol since this substrate permits maximal differentiation between activities of the heterodimeric subunits. Oxidation of cyclohexanol by the homodimers $\beta_2\beta_2$ and $\gamma_1\gamma_1$ follows conventional Michaelis–Menten kinetics. The values of K_m and k_{cat} determined for $\beta_2\beta_2$ and $\gamma_1\gamma_1$ are 0.11 M and 260 min^{-1} and 79 μM and 45 min^{-1}, respectively, indicating that $\beta_2\beta_2$, like the previously studied $\beta_1\beta_1$, has an unusually low binding affinity for cyclohexanol compared to that of the ADH isozymes formed by the combination of α, γ_1, and γ_2 chains. Cyclohexanol oxidation by the heterodimers $\alpha\beta_2$, $\beta_2\gamma_1$, and $\beta_2\gamma_2$ follows biphasic kinetics which can be fully accounted for by the individual subunits, one exhibiting a high and the other a low substrate-binding affinity. Eadie–Hofstee plots resolve the biphasic kinetics into two linear components, each of which yields a set of kinetic parameters. The K_m values for the low-affinity components of the $\alpha\beta_2$ (0.082 M), $\beta_2\gamma_1$ (0.079 M), and $\beta_2\gamma_2$ (0.091 M) heterodimers are similar to that of the $\beta_2\beta_2$ (0.11 M) homodimer, while the K_m values of the high-affinity components fall into the same concentration range (micromolar) as those determined for the α, γ_1, and γ_2 chains [Wagner, F. W., Burger, A. R., & Vallee, B. L. (1983) *Biochemistry 22*, 1857–1863]. Furthermore, the substrate concentration vs. activity profile of $\beta_2\gamma_1$ fits the theoretical curve obtained by averaging the kinetic responses of the parent homodimers, $\beta_2\beta_2$ and $\gamma_1\gamma_1$. Thus, the individual subunits of β_2-containing heterodimeric ADHs do not exhibit subunit interaction. This conclusion is supported by studies of the effects of pH and thiourea on the activities of the high- and low-affinity components of the heterodimers.

Of the more than 20 different isozymes of alcohol dehydrogenase (EC 1.1.1.1) (ADH)[1] that have been found to be present in human liver (Vallee & Bazzone, 1983) only that produced by the ADH_2 gene locus is known to exhibit a marked racial variability. Individuals having the ADH_2^1 allele at the ADH_2 gene locus produce β_1 subunits, while those with the ADH_2^2 allele produce β_2 subunits (Smith et al., 1973). Greater than 85% of livers from Orientals contain the β_2 form, and these can either be homozygous ADH_2 2-2 or heterozygous ADH_2 2-1 phenotypes (Yin et al., 1984a). In contrast, livers from white American and European populations contain predominantly the β_1 form. Yet another variant, β_3 ($\beta_{Indianapolis}$), has been found in about 25% of the Black American population (Bosron et al., 1980).

As part of our ongoing effort to understand the metabolism of ethanol in Orientals we examined the substrate specificity of β_2-containing isozymes of ADH (Fong & Keung, 1987). The $\beta_2\beta_2$ homodimer was found to have a much higher k_{cat} than the $\beta_1\beta_1$ form, confirming previous studies (Yin et al., 1984b). Moreover, in contrast to β_1-containing heterodimers that had previously been found to give linear kinetics (Wagner et al., 1983), β_2-containing heterodimers were found to exhibit biphasic kinetics with secondary alcohols as substrate, suggestive of independent, noninteracting subunits (Fong & Keung, 1987). Two sets of kinetic parameters, corresponding to each of the subunits, were necessary to fit the activity profiles. Among the secondary alcohols studied, cyclohexanol exhibited the maximal difference between the high- and low-affinity activities. We therefore chose this substrate for further detailed investigations of the kinetic behavior of β_2-containing ADH isozymes. The results confirm that in such heterodimers the individual subunits act independently of one another.

Materials and Methods

NAD^+ (grades III and AA-1), Tris (reagent grade), ammonia-free glycine, PMS, MTT, and preswollen microgranular form ion-exchange resins were purchased from Sigma Chemical Co., St. Louis, MO. Thiourea was a product of Eastman Kodak Co., Rochester, NY, and was recrystallized prior to use. Absolute ethanol and cyclohexanol were purchased from BDH Chemical Ltd., England. Cyclohexanol was purified by fractional distillation (bp 161 °C) before use. All other reagents were used without further purification.

Human liver specimens were obtained at post-mortem examination from apparently healthy individuals who had died suddenly. Specimens were stored at −65 °C before use. The phenotype of ADH was determined by the total enzyme ac-

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[1] Abbreviations: ADH, alcohol dehydrogenase; *ADH*, alcohol dehydrogenase locus; Tris, tris(hydroxymethyl)aminomethane; CapGapp, 4-[3-[*N*-(6-aminocaproyl)amino]propyl]pyrazole; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PMS, phenazine methosulfate.

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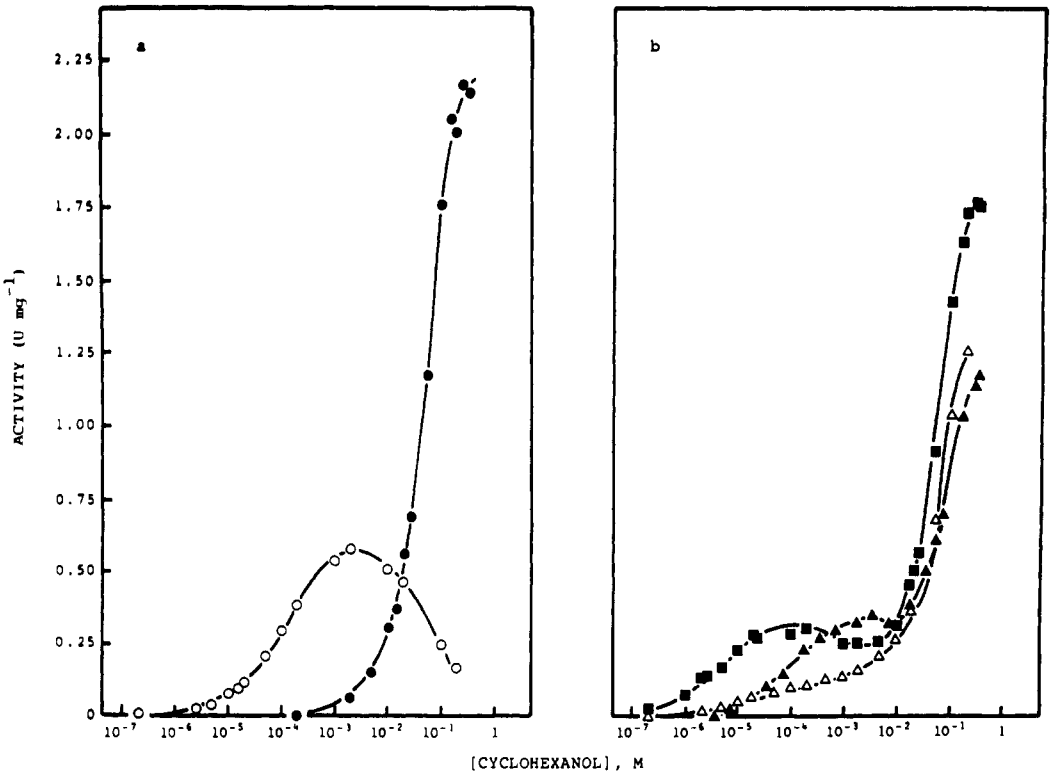

FIGURE 1: Kinetic response of (a) homodimeric and (b) heterodimeric ADH isozymes as a function of cyclohexanol concentration. The purified isozymes $\beta_2\beta_2$ (●), $\gamma_1\gamma_1$ (○), $\alpha\beta_2$ (■), $\beta_2\gamma_1$ (▲), and $\beta_2\gamma_2$ (△) were assayed for cyclohexanol oxidation with 2.4 mM NAD^+ in 0.1 M glycine–NaOH buffer, pH 9.0.

tivity and the pH–rate profile of the extract (von Wartburg et al., 1965).

Class I isozymes $\alpha\beta_2$, $\beta_2\gamma_1$, $\beta_2\gamma_2$, $\beta_2\beta_2$, and $\gamma_1\gamma_1$ were purified by chromatography on DEAE-cellulose, CapGapp–Sepharose, and CM-cellulose according to the procedure of Wagner et al. (1983). The isozymes were identified by starch gel electrophoresis (Bosron et al., 1979) and by determination of the subunit composition of the isozyme on polyacrylamide gels containing 7 M urea (Keung et al., 1985). The activity of ADH was measured spectrophotometrically by following the increase in absorbance at 340 nm in an assay medium containing 0.1 M glycine–NaOH buffer, pH 9.0, and 2.4 mM NAD^+ and the corresponding substrate. All enzyme assays were performed at 25 °C with a Varian Model 210 spectrophotometer. Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

RESULTS

The class I isozymes of ADH from both Oriental and Caucasian livers were isolated by CapGapp–Sepharose affinity chromatography. The resultant mixture of Oriental class I isozymes has a specific activity of about 5 units/(mg of protein) when assayed at pH 9.0 with 40 mM ethanol and 2.4 mM NAD^+, about 5 times higher than that of the corresponding Caucasian liver enzymes assayed at pH 10.0 (Wagner et al., 1983). The pH optimum for ethanol oxidation by the Oriental enzyme[2] ranges from 8.5 to 10.0, depending on the ethanol concentration used in the assay. Thus, at 5 M ethanol, the maximal rate occurs near pH 10.0, but as the ethanol concentration decreases, the pH optimum also decreases, so that at 0.5 M ethanol the pH optimum is 8.7. A pH of 9.0 was selected for the present study, since at that pH the β_2-containing isozymes have the highest k_{cat}/K_m value for ethanol oxidation. For purposes of comparison, isozymes not containing the β_2 subunit were therefore also studied at pH 9.0.

Table I: Kinetic Parameters[a] of ADH Isozymes toward Cyclohexanol

isozyme	high-affinity activity		low-affinity activity	
	K_m (μM)	k_{cat} (min^{-1})	K_m (μM)	k_{cat} (min^{-1})
$\gamma_1\gamma_1$	79	45		
$\alpha\beta_2$	4.2	31	82 000	180
$\beta_2\gamma_1$	74	27	79 000	140
$\beta_2\gamma_2$	17	9.6	91 000	150
$\beta_2\beta_2$			110 000	260

[a] Enzyme reaction rates were measured in 0.1 M glycine–NaOH buffer, pH 9.0, and 2.4 mM NAD^+. Kinetic parameters of the heterodimers were estimated from Eadie–Hofstee plots.

The class I isozymes from the livers of individual Oriental subjects can be resolved by CM-cellulose chromatography using the same procedures as for the Caucasian enzymes (Wagner et al., 1983). Usually, three or four major isozymes ($\alpha\beta_2$, $\beta_2\beta_2$, $\beta_2\gamma_1$, and $\beta_2\gamma_2$) are obtained from each of the Oriental livers studied. They constitute over 90% of the total ADH activity of these livers. Since very little, if any, $\gamma_1\gamma_1$ isozyme can be isolated from Oriental livers, the $\gamma_1\gamma_1$ isozyme used for the present study was isolated from a Caucasian liver by similar procedures.

When the concentration of NAD^+ is fixed at 2.4 mM, the rate of cyclohexanol oxidation by $\gamma_1\gamma_1$ increases progressively as the substrate concentration increases from 1 μM to 2 mM (Figure 1a). Although substrate inhibition becomes evident at higher cyclohexanol concentrations, the activity–substrate curve for the lower concentration range fits a simple Michaelis–Menten scheme. The values of K_m and k_{cat} determined by Lineweaver–Burk analysis are 79 μM and 45 min^{-1}, re-

[2] What are now referred to as the Oriental and Caucasian enzymes were first called "atypical" and "normal" by von Wartburg et al. (1965). The Caucasian enzyme is also known as the "usual" or "typical" enzyme.

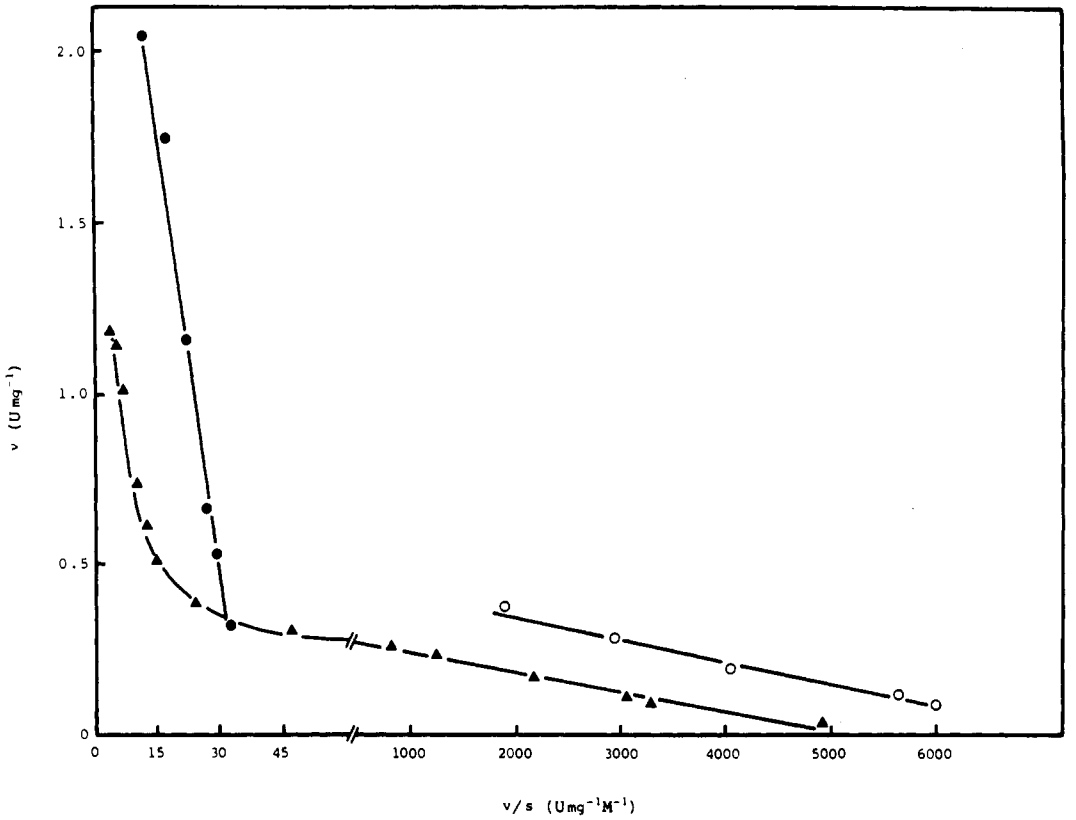

FIGURE 2: Eadie–Hofstee plots of the kinetic response of $\beta_2\beta_2$ (●), $\gamma_1\gamma_1$ (○), and $\beta_2\gamma_1$ (▲) to cyclohexanol oxidation. The kinetic constants derived from these plots are listed in Table I.

spectively (Table I). The activity–substrate concentration curve of $\beta_2\beta_2$ is also shown in Figure 1a. Below 300 μM cyclohexanol, activity is hardly detected, but it increases dramatically above about 10 mM, indicating that, while cyclohexanol has a low binding affinity toward $\beta_2\beta_2$, its maximal rate of oxidation is quite high. The K_m and k_{cat} values determined for $\beta_2\beta_2$ are 0.11 M and 260 min^{-1}, respectively (Table I).

In contrast to the simple Michaelis–Menten kinetics of the homodimers, the activity–substrate concentration curves measured for the heterodimers $\alpha\beta_2$, $\beta_2\gamma_1$, and $\beta_2\gamma_2$ are biphasic (Figure 1b). The first plateau or activity maximum is reached at low or below millimolar cyclohexanol concentrations (100 μM, 3 mM, and around 100 μM for $\alpha\beta_2$, $\beta_2\gamma_1$, and $\beta_2\gamma_2$, respectively). Increasing the cyclohexanol concentration leads to a second phase of activity, which in all cases approaches a maximum at about 0.2 M. Eadie–Hofstee plots (Figure 2) indicate that each of the heterodimers has a high- and a low-affinity component associated with catalysis of cyclohexanol oxidation. The K_m and k_{cat} values for each of these components are also listed in Table I.

The effect of pH on the rate of cyclohexanol oxidation by the homo- and heterodimers was studied to further characterize the high- and low-affinity components. Figure 3a shows the pH–activity profiles of $\gamma_1\gamma_1$ and $\beta_2\beta_2$ each measured at two different cyclohexanol concentrations, 100 μM and 200 mM. At the lower substrate concentration, $\beta_2\beta_2$ does not exhibit appreciable activity throughout the pH range studied, whereas $\gamma_1\gamma_1$ has maximal activity at pH 10.2. At the higher cyclohexanol concentration, the pH optimum of $\gamma_1\gamma_1$ shifts to pH 10.7. More significantly, the activity of $\beta_2\beta_2$ becomes substantial under these conditions and differs from that of $\gamma_1\gamma_1$ by having a pH optimum around 9.2. The pH–activity profiles of the heterodimer $\beta_2\gamma_1$ measured by using low and high cyclohexanol concentrations are shown in Figure 3b. With 100 μM cyclohexanol, the profile of $\beta_2\gamma_1$ is virtually identical with that of $\gamma_1\gamma_1$ with an optimum at 10.2, while with 200 mM cyclohexanol it resembles that of $\beta_2\beta_2$. Comparable results were also obtained for the other two β_2-containing heterodimers (not shown).

Thiourea has been shown to have different effects on the activities of Oriental and Caucasian liver ADH: it inhibits the Oriental and activates the Caucasian enzyme (von Wartburg et al., 1965).[2] We therefore examined the effects of thiourea on cyclohexanol oxidation by the individual homo- and heterodimers in order to help establish the identity of the high- and low-affinity components (Figure 4). Since $\beta_2\beta_2$ has no appreciable activity with 100 μM cyclohexanol, the effect of thiourea was only examined at 200 mM. Under these conditions, 0.8 M thiourea inhibits $\beta_2\beta_2$ almost completely. In contrast, thiourea activates $\gamma_1\gamma_1$ at either cyclohexanol concentration. Activation is moderate with 100 μM cyclohexanol, but with 200 mM cyclohexanol it is almost 300%. When thiourea is tested with the heterodimer $\beta_2\gamma_1$, two opposite effects are observed. The high-affinity component, which is assayed with 100 μM cyclohexanol, is activated, whereas the low-affinity component, assayed with 200 mM cyclohexanol, is inhibited. Similar results were also obtained for $\alpha\beta_2$ and $\beta_2\gamma_2$ (not shown). Thus it would appear that the high-affinity activity of the heterodimer is contributed by the γ (or α) component, while the low-affinity activity is due to the β_2 component.

Discussion

The ADH activities found in most Oriental livers are readily differentiated from those of Caucasian livers both by their higher turnover number and by their lower pH optimum. A similar differentiation had been recognized earlier among the so-called "normal" and "atypical" segments of the Swiss population (von Wartburg et al., 1965). These differences in

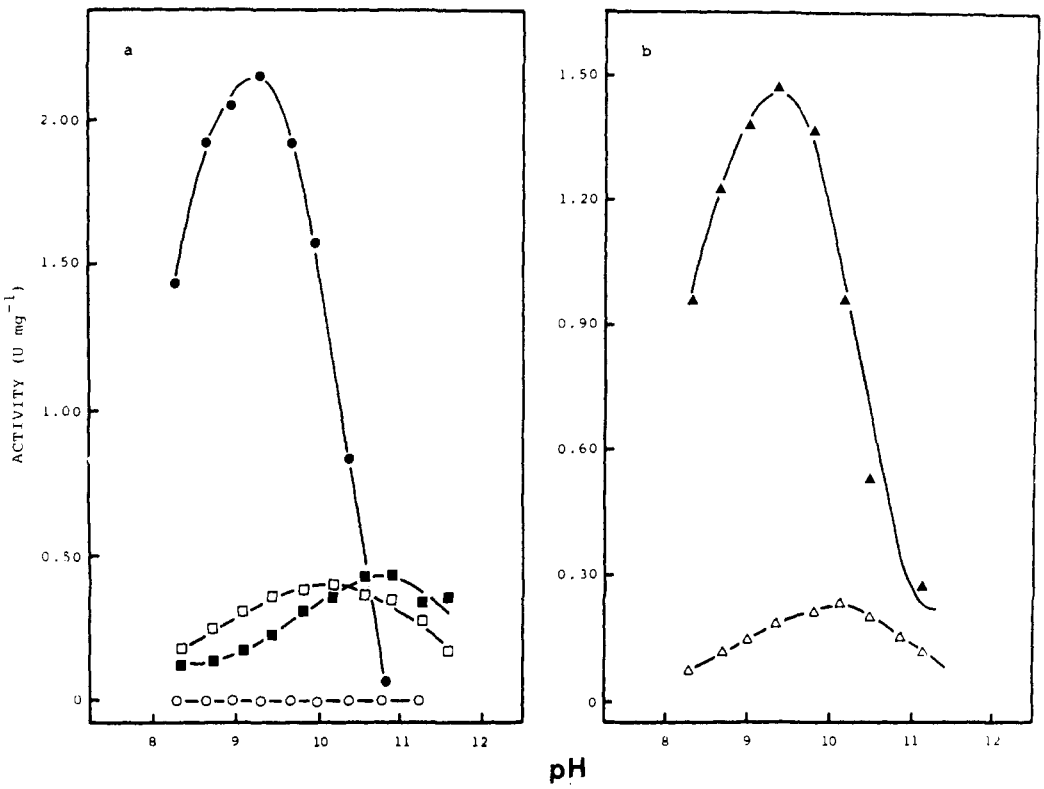

FIGURE 3: pH–activity profiles of (a) $\beta_2\beta_2$ (circles), $\gamma_1\gamma_1$ (squares), and (b) $\beta_2\gamma_1$ (triangles) measured at 100 μM (open symbols) and 0.2 M cyclohexanol (closed symbols).

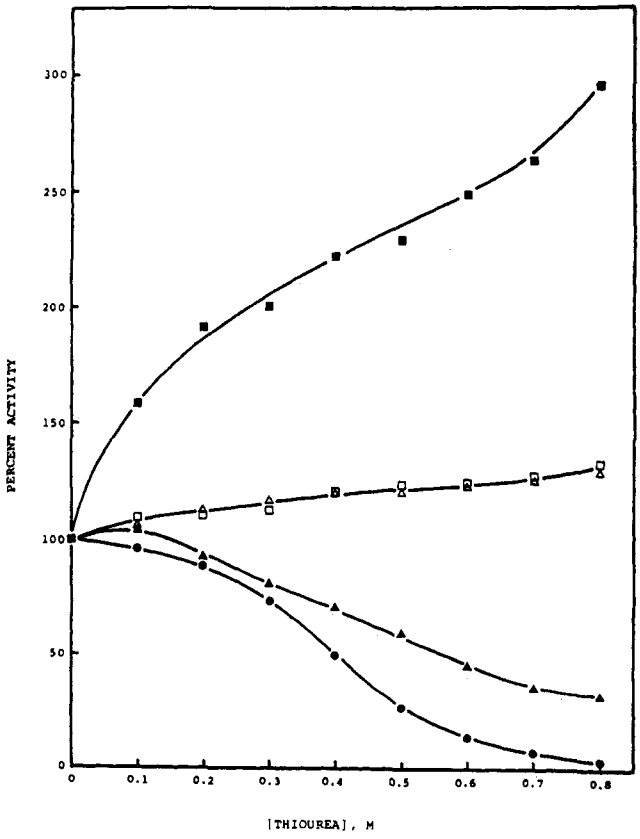

FIGURE 4: Effect of thiourea on the rate of cyclohexanol oxidation by $\beta_2\beta_2$ (circles), $\gamma_1\gamma_1$ (squares), and $\beta_2\gamma_1$ (triangles) measured at 100 μM (open symbols) and 0.2 M cyclohexanol (closed symbols).

catalytic properties are primarily due to the replacement of the Caucasian β_1 subunit with the β_2 subunit, a change brought about by mutation at the ADH_2 locus (Smith et al., 1973; Stamatoyannopoulos et al., 1975; Harada et al., 1980). Recently, Jörnvall et al. (1984) reported that the functional differences between these two homodimeric phenotypes arise from the substitution of Arg-47 of $\beta_1\beta_1$ by a histidine residue in $\beta_2\beta_2$. This single replacement results in a significant change in turnover number and pH optimum, but its effect on the values of K_m toward various alcohols is much less. Thus, the values of K_m for $\beta_2\beta_2$ toward the alcohols listed in Table II are 3–10-fold higher than those measured for $\beta_1\beta_1$ at pH 10.0 (Wagner et al., 1983). In general, these K_m values reveal that both $\beta_1\beta_1$ and $\beta_2\beta_2$ prefer primary alcohols with bulky substituents. Both of them also have a very high K_m for cyclohexanol compared to those of the isozymes containing α, γ_1, and γ_2 chains. This seems to be one of the few features of specificity shared by the two β-type isozymes.

Table II: K_m Values (μM) for $\beta_1\beta_1$ and $\beta_2\beta_2$ toward Various Alcohols

alcohol	isozyme $\beta_1\beta_1$[a]	isozyme $\beta_2\beta_2$
methanol	6000	39 000[b]
ethanol	1200	3 200[b]
benzyl alcohol	120	1 700[b]
cyclohexanol	23000	110 000[c]

[a] Data taken from Wagner et al. (1983a). Measured at pH 10.0. [b] Data taken from Fong and Keung (1987). [c] Data obtained in the present study. Measured at pH 9.0.

While cyclohexanol oxidation by the homodimers $\gamma_1\gamma_1$ and $\beta_2\beta_2$ follows simple Michaelis–Menten kinetics, the heterodimer $\beta_2\gamma_1$ exhibits biphasic kinetics. The two sets of kinetic parameters estimated from the kinetic curves largely reflect the kinetic properties of the parent homodimers (Table I). Thus, the two K_m values obtained for the high- and low-affinity components of $\beta_2\gamma_1$ (74 μM and 79 mM) agree quite well with those obtained for $\gamma_1\gamma_1$ (79 μM) and $\beta_2\beta_2$ (110 mM), respectively. Similarly, the two k_{cat} values determined for $\beta_2\gamma_1$ (27 and 140 min^{-1}) are about half of those for $\gamma_1\gamma_1$ (45 min^{-1}) and $\beta_2\beta_2$ (260 min^{-1}), respectively. The simplest interpretation

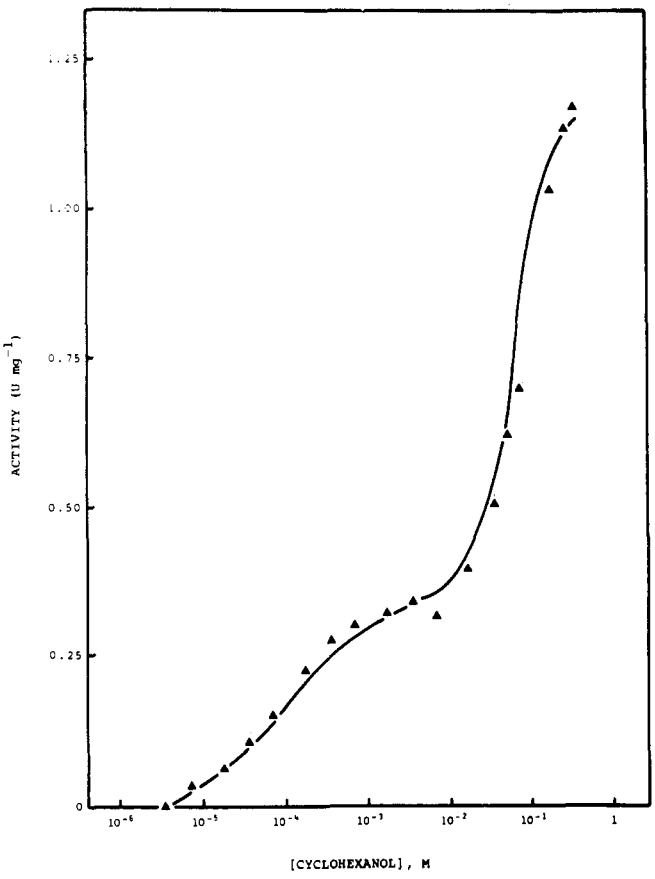

FIGURE 5: Kinetic response of $\beta_2\gamma_1$ to cyclohexanol concentration. Enzyme activity was determined as described in the legend of Figure 1. The data points (▲) represent the observed response of $\beta_2\gamma_1$, and the solid line is calculated from half the activities of $\beta_2\beta_2$ plus $\gamma_1\gamma_1$ taken from Figure 1a.

for these findings is that the individual subunits in the heterodimers act in an independent, noncooperative manner. Thus, the high-affinity activities measured for the heterodimers $\alpha\beta_2$, $\beta_2\gamma_1$, and $\beta_2\gamma_2$ are contributed mainly by the α, γ_1, and γ_2 subunits, respectively, whereas the low-affinity activities are contributed mainly by the β_2 subunit. The close agreement between the activity–concentration curve observed for $\beta_2\gamma_1$ and that calculated by averaging those for $\gamma_1\gamma_1$ and $\beta_2\beta_2$ (Figure 5) supports this proposal.

Further substantiation for this hypothesis regarding the origin of the biphasic kinetics is provided by the effects of pH and thiourea on the low- and high-affinity activities of the heterodimers. The Oriental isozyme $\beta_2\beta_2$ has three distinct characteristics not shared by human class I isozymes that lack this subunit: it is (1) more active; (2) optimally active around pH 8.5–9.0 instead of near pH 10.5; and (3) inhibited rather than activated by thiourea. Thus, if biphasic kinetics indeed reflect independent contributions by the constituent subunits of heterodimers, thiourea should activate the high-affinity activities, i.e., those contributed by the α, γ_1, or γ_2 subunits, but inhibit the low-affinity activity, i.e., that contributed mainly by the β_2 subunit. As demonstrated in Figure 4, thiourea does indeed activate the high-affinity activity of $\beta_2\gamma_1$ measured with 100 μM cyclohexanol. Moreover, the percent activation agrees closely with that of the homodimer $\gamma_1\gamma_1$ studied at the same cyclohexanol concentration. Further, and again consistent with the hypothesis, thiourea inhibits the low-affinity activity of $\beta_2\gamma_1$ measured with 200 mM cyclohexanol. Actually, at low thiourea concentration where the inactivation of the β_2 subunit is minimal, there is a slight activation, but at higher concentrations the effect is inhibitory and similar, though less profound than that with $\beta_2\beta_2$. These observations can be explained readily if the β_2 chain contributes the low-affinity activity.

The contribution of the α, γ_1, and γ_2 subunits to the low-affinity activities of $\alpha\beta_2$, $\beta_2\gamma_1$, and $\beta_2\gamma_2$, respectively, is usually very small because their k_{cat} values are so much lower than that of the β_2 subunit, and they are subject to substrate inhibition at high cyclohexanol concentrations. However, in the presence of thiourea their contribution to the low-affinity activities could become significant because they are activated while the β_2 subunit is inhibited. The present study also shows that the activation effect of thiourea on $\gamma_1\gamma_1$ is more pronounced when assayed at high cyclohexanol concentrations (Figure 4). The mechanism by which thiourea exerts its effects on ADH has not been established.

The pH–activity profile of the high-affinity component of $\beta_2\gamma_1$ has an optimum near pH 10.2, similar to that of $\gamma_1\gamma_1$, while the low-affinity component has an optimum at pH 9.2, similar to that of $\beta_2\beta_2$ (Figure 3). This again is consistent with the interpretation that the two subunits of $\beta_2\gamma_1$ act independently.

Previous studies had not detected unequivocal biphasic kinetics for heterodimeric ADH isozymes (Wagner et al., 1983; Bosron et al., 1980, 1983; Yin et al., 1984b). Most likely this was due to the fact that α, γ_1, γ_2, β_1, and β_2 all have similar affinities (K_m values) for ethanol and for most of the primary alcohol substrates studied.[3] However, because the isozymes containing α, γ_1, and γ_2 chains have K_m values for secondary alcohols, and especially cyclohexanol, that differ so greatly ($\sim$1000-fold) from those of $\beta_2\beta_2$, the use of such substrates clearly reveals that the subunits of dimeric ADH isozymes can act independently. There is no reason to surmise that this independence is a unique property observed only when cyclohexanol is the substrate. Yin et al. (1984b) showed that the specific activities of the heterodimers $\alpha\beta_2$ and $\beta_2\gamma_1$ for ethanol oxidation agree well with the mean specific activities calculated from their respective homodimer values and interpreted this to mean that the subunits of β_2-containing heterodimers act independently. However, the kinetic data obtained with heterodimers formed by the combination of β_1 with α, γ_1, and γ_2 were interpreted to indicate an interaction between subunits (Wagner et al., 1983; Deetz et al., 1984). Thus it would seem that the type of β subunit (β_1 or β_2) present in these heterodimers will determine whether or not subunit interaction will be observed.

Structural information regarding the subunits of all human ADHs has begun to accumulate (Hempel et al., 1985), and their primary structures are sufficiently diverse to expect large differences in kinetic properties. In fact, under appropriate conditions and with the use of certain substrates or inhibitors, some subunit specific differences have been observed. Thus at pH 7.5, negative cooperativity was observed for $\gamma_1\gamma_1$ and $\gamma_2\gamma_2$ but not for $\alpha\alpha$ and $\beta_1\beta_1$ (Bosron et al., 1983).

More recently, Mårdh et al. (1986) have shown that testosterone selectively binds to and inhibits the γ-containing homo- and heterodimeric isozymes of class I human ADH but not those composed only of α and/or β subunits. Thus, the results of the present study and those just mentioned will clearly be essential to the understanding of the action and regulatory mechanisms of ADH. The definition of the structural properties of all the subunits should soon permit generalization and integration of these independent observations.

[3] In addition to the particular substrate employed, other variables such as pH, temperature, ionic strength, etc. may play a role in determining whether or not subunit interaction will be observed.

Registry No. ADH, 9031-72-5; cyclohexanol, 108-93-0.

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Conformational Aspects and Rotational Dynamics of Synthetic Adrenocorticotropin-(1-24) and Glucagon in Reverse Micelles†

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ABSTRACT: The tryptophan (Trp) rotational dynamics and the secondary structure of the peptide hormones adrenocorticotropin-(1-24) [ACTH(1-24)]—the fully active N-terminal fragment of adrenocorticotropin-(1-39)—and glucagon were studied in aqueous solutions and in reverse micelles of sodium bis(2-ethylhexyl) sulfosuccinate (AOT)/water/isooctane, a system selected to mimick the membrane–water interface. In aqueous solutions, the total fluorescence intensity decays of their single Trp residue [Trp-9 and Trp-25 for ACTH(1-24) and glucagon, respectively] are multiexponential. This is also the case for ACTH(5-10), a fragment of the adrenocorticotropin "message" region. Time-resolved fluorescence anisotropy data evidence a high degree of rotational freedom of the single Trp residue. Transfer of these peptides from water to the aqueous core of reverse micelles induces severe restrictions of the Trp internal motion and of its local environment. The results indicate that the Trp-9 residue in ACTH(1-24) is maintained in the close neighborhood of the water–AOT molecular interface where the water molecules are strongly immobilized. By contrast, the Trp residues in ACTH(5-10) and glucagon are likely to be located closer to the center of the micellar aqueous core where the water molecules are in a more mobile state. Furthermore, the above location of Trp can be extended to the peptide chains themselves as evidenced by the overall correlation time values of the peptide-containing micelles. Nevertheless, in all peptides, the indole ring remains susceptible to oxidation by *N*-bromosuccinimide. Circular dichroism measurements evidence the induction in glucagon of α-helices remaining unaffected by the micellar water content. Conversely, β-sheet structures are favored in ACTH(1-24) at low water-to-surfactant molar ratios (w_0) but are disrupted by subsequent additions of water. These results are discussed in terms of the possible role of the micellar interfaces in selecting the preferred peptide dynamical conformation(s).

The conformational adaptability of peptide hormones has been investigated in a variety of homogeneous solvents (Gratzer et al., 1968; Greff et al., 1976; Nabedryk-Viala et al., 1978; Toma et al., 1981, 1985). Further, the use of surfactant micelles and of lipid vesicles in water has provided a more accurate description of the peptide–hormone interactions with a membrane-like interface, since in these systems the anisotropic and amphipathic nature of biological membranes is preserved to a certain extent (Bornet & Edelhoch, 1971; Schneider & Edelhoch, 1972; Braun et al., 1983; Gremlich et al., 1983; Kaiser & Kézdy, 1983; Epand & Surewicz, 1984;

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