Characterization and Kinetics of Native and Chemically Activated Human Liver Alcohol Dehydrogenases*

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Acetimidylation of the amino groups of alcohol dehydrogenase from human and horse liver yields several modified enzyme forms, which differ in electrophoretic mobility and can be separated by ion exchange chromatography, but which are similar in kinetic characteristics. The acetimidylated, as well as the methylated, enzymes from human livers of the normal phenotype have increased activity and larger Michaelis and inhibition constants. These results suggest that the human enzyme has amino groups at the active sites, as was shown previously for the enzyme. The variant subunit occurring in the enzyme isolated from atypical human livers does not seem to be activated by acetimidylation, which may indicate that substitution of proline for Ala-230 or modification of Lys-228 is sufficient to fully activate the enzyme. Results of product inhibition studies of native and modified human enzymes are consistent with an Ordered Bi Bi mechanism. However, the major isoenzyme of native human liver alcohol dehydrogenase exhibits nonlinear kinetics over a wide range of ethanol concentrations. This result may indicate that subunits with different kinetic characteristics are present or that there is negative cooperativity between subunits. After chemical modification, the kinetic patterns become linear, suggesting that the mechanism is altered.

When ε-amino groups of lysine residues at the active sites of horse liver alcohol dehydrogenase (EC 1.1.1.1) are alkylated or amidinated with substituents that retain the positive charge, the maximum velocities of the enzymatic reactions are increased up to 10-fold (1–3). The modification of lysine residue 298 is responsible for the activation (4, 5). The kinetic mechanisms of the modified enzymes appear to be the same as that of native enzyme, that is, Ordered Bi Bi (1, 2, 6). The increased activity of the modified enzymes is due to increased rates of dissociation of the enzyme–coenzyme complexes, the rate-limiting steps in the reaction catalyzed by the native enzyme (2, 7–9). With the horse liver enzyme, small, positively charged substituents, such as methyl or acetimidyl, increase turnover numbers and Michaelis constants for ethanol, but do not greatly change the Michaelis constants for NAD⁺ (3); such modified enzymes could be more active in vivo (10).

The main isoenzymes of horse and human liver alcohol dehydrogenases have been shown to be highly homologous (11, 12). On the other hand, the horse and human enzymes differ somewhat in catalytic properties, such as turnover number, pH optimum, and substrate specificity. In order to compare their reaction mechanisms and the effects of modifications of amino groups, we have studied the kinetics of native and chemically modified human enzymes. We were also interested in determining whether the genetically determined "atypical" variant of the enzyme, which has higher specific activity and a lower pH optimum than the normal form (13, 14), would be activated by modification of amino groups. The atypical form has been shown to have a proline substituted for alanine at residue number 230, 2 residues away from lysine residue 228 at the active site (12).

EXPERIMENTAL PROCEDURES

Purification of Liver Alcohol Dehydrogenase—Human livers from adults of both sexes were obtained from legal medical autopsies and frozen at -20°C within 10 to 20 h after death. The enzyme was purified by fractionation with ammonium sulfate and chromatography on DEAE-cellulose and CM-cellulose by procedures adapted from the literature (15, 16). The main isozyme BB from normal or atypical livers was isolated according to the method of Berger et al. (12). Step 6 of their procedure was replaced by gel filtration on a Sephadex G-50 column (3.0 x 50 cm) equilibrated with 100 mM Tris/HCl buffer, pH 8.0. Horse liver alcohol dehydrogenase isozyme AA was prepared as described by Lutstorf et al. (17).

Enzyme Assay—Assays in 33 mM NaH₂PO₄/HCl buffer (pH 8.8) with 1.6 mM NAD⁺ and 16 mM ethanol at 25°C (17) were used for comparison of the activity and the relative activation by chemical modification of the human enzyme to that of the horse enzyme; assays were also conducted in 0.32 M ethanol and 1.75 mM NAD⁺ at pH 9 and 25°C (1). The pH-rate profiles were measured as described by Berger et al. (12).

Protein Concentration—This was estimated by the method of Lowry et al. (18) or by determination of the absorbance at 280 nm using an extinction coefficient of 0.607 per cm for a 1 mg/ml solution of the human enzyme (12).

Chemical Modification of Alcohol Dehydrogenase—For acetimidylation, a solution of enzyme in 0.5 M triethanolamine/HCl buffer, pH 8.0, at 25°C was made 0.1 M in ethyl acetimidate (freshly dissolved and neutralized). After 1 h, freshly prepared ethyl acetimidate was added

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RESULTS AND DISCUSSION

Acetimidylation of Horse Liver Alcohol Dehydrogenase—In previous studies, the horse enzyme was repetitively acetimidylated until the activity was maximally increased, and the kinetics of the modified protein were determined without a detailed chemical characterization of the products (3). Recently, it has been suggested that acetimidylation at pH 8 may produce a variety of products (20). In order to determine if the heterogeneity of the products would be reflected in the kinetic characteristics of the modified horse enzyme, and presumably also of the human enzyme, we first separated the products by ion exchange chromatography. As shown in Fig. 1, five different peaks can be separated. The mixture of products can also be resolved electroforetically; four discrete bands were observed after starch gel electrophoresis (Fig. 2, Band B). Various fractions isolated by chromatography were also subjected to electrophoresis. As compared to native enzyme (Fig. 2, Band A), and as judged from the correlation between the electrophoretic mobility (17) and the amino acid sequences (21) of the isozymes of the horse liver enzyme, the various acetimidylated forms apparently differ progressively, by 1 unit of charge per dimeric molecular weight (Figs. 2, Bands C to F). Since acetimidylation per se does not change the charge of an ε-amino group at pH 8.8, the more anodic mobility of the acetimidylated enzyme forms could be due to reactions of intermediate N-alkyl imidates (20), such as cross-linking of 2 lysine residues by the monofunctional reagent or to formation of acetylated lysines, which occur in model systems (22) and result in the loss of positive charge. However, it has been reported that all of the lysine residues in enzyme acetimidylated at pH 8 can be accounted for as acetimidylated or free lysine (20). Another possible explanation for the origin of the forms is that acetimidylation produces conformational isomers which differ in the exposure of ionic groups.

Although the acetimidylated forms differ structurally, they have similarly enhanced activities as compared to native enzyme (Table I). Both the $K_m$ values toward ethanol and the turnover numbers are increased. The major acetimidylated product (Band 2) shows the highest activation. Eadie-Hofstee plots of the kinetic data were linear over the range of ethanol concentrations from 0.1 to 10 mM, but showed substrate inhibition above 20 mM.

Acetimidylation of Human Liver Alcohol Dehydrogenases—Isoenzyme BB of normal human phenotype, with the subunit composition $B_B$, was acetimidylated and chromatographed on CM-Sephadex (Fig. 3). In contrast to the horse enzyme, discrete peaks were not resolved, although electrophoresis showed that forms with a slightly different mobility were present. Most of the material had electrophoretic mobilities the same (Pool II) or slightly slower (Pool I) than native enzyme. The Michaelis constants toward ethanol of the enzymes in Pools I and II were found to be similar, but the highest maximum velocity was associated with the predominant form (Pool II, Table II). As shown in Fig. 4, the native and modified forms exhibited substrate inhibition with concentrations of ethanol above about 0.1 M (pH 8.8, 25°, 1.67 mM NAD+). The different shapes of the curves will be discussed later. The predominant forms were maximally active at pH 10, whereas the native enzyme had a pH optimum at pH 10.3 (Fig. 5A).

The native isoenzyme BB of atypical phenotype is a mixture of

![Fig. 1](left). Chromatography of acetimidylated horse liver alcohol dehydrogenase (isoenzyme AA) on CM-Sephadex. About 50 mg of enzyme was applied to a column (1.8 x 20 cm) of CM-Sephadex C-50 equilibrated with 5 mM Tris/HCl buffer, pH 8.0, and eluted with a linear gradient prepared from 500 ml each of 5 and 50 mM Tris/HCl buffers at 4°. Fractions of about 8 ml were collected. Enzyme activity is given in international units per ml.

![Fig. 2](right). Starch gel electrophoresis of native and acetimidylated horse liver alcohol dehydrogenase. The gel was stained for enzymatic activity (17). A, native enzyme; B, mixture of modified enzymes; C to F, forms of modified enzyme separated chromatographically as shown in Fig. 1; C, Band 4, Fraction 11; D, Band 3, Fraction 21; E, Band 2, Fraction 30; F, Band 1, Fraction 40.

**Table I**

| Specific activity | $K_m$, ethanol | Turnover number |
|-------------------|----------------|----------------|
| units/mg | mM | s⁻¹ |
| Native | 5.7 | 0.89 | 4.0 |
| Acetimidylated | | | |
| Band 1 | 16 | 2.2 | 13 |
| Fraction 40 | | | |
| Band 2 | 23 | 3.7 | 18 |
| Fraction 30 | | | |
| Band 3 | 16 | 3.4 | 13 |
| Fraction 21 | | | |
Kinetics of Human Liver Alcohol Dehydrogenases

**Figure 3 (left).** Chromatography of acetimidylated human liver alcohol dehydrogenase (normal phenotype, isoenzyme B,B,) on CM-Sephadex. About 60 mg of enzyme was applied to a column (1.8 x 20 cm) of CM-Sephadex C-50 equilibrated with 5 mM Tris/HCl buffer, pH 8.0, and eluted with a linear gradient prepared from 150 ml each of 10 mM and 100 mM Tris/HCl buffers at 4°C. Fractions of 6 ml were collected. Enzyme activity is given in international units per ml.

**Figure 4 (right).** Oxidation of ethanol by native and acetimidylated human liver alcohol dehydrogenase of the normal phenotype. The acetimidylated enzyme forms were separated as shown in Fig. 3 (pool numbers given). The activities were determined with 1.67 mM NAD+ at pH 8.8 and the indicated concentrations of ethanol. The activities were adjusted for the different amounts of protein used, and the activity of the native enzyme in 16.7 mM ethanol was set equal to 1.

**Table II**

Kinetic characteristics of acetimidylated forms of human liver alcohol dehydrogenases

| Specific activity | $K_{m}$, ethanol | $V_{max}$ |
|------------------|------------------|----------|
| **Normal**       |                  |          |
| Native           | 1.4              | 0.19, 5.5b | 110     |
| Pool II          | 5.1              | 12       | 640     |
| Pool I           | 3.5              | 12       | 400     |
| **Atypical**     |                  |          |
| Native           | 1.6              | 3.1      | 105     |
| Modified mixture | 2.1              | 8.3      | 210     |

* The maximum velocities are expressed relative to activities of the native enzymes determined with 16.7 mM ethanol.

* As shown in Fig. 9, native normal enzyme exhibits nonlinear kinetics; the limiting $K_{m}$ values were determined by fitting the data to the "2/1" equation given in Table IV and then calculating $K_{1}$ and $K_{2}$ as described in Table V. Acetimidylated normal phenotype and the atypical enzymes gave linear Eadie-Hofstee plots over the concentration ranges used.

of the normal subunit B, and the mutant subunit Bz and is characterized by a pH-rate profile with an optimum at pH 8.8 due to the more active Bz subunit and a shoulder above pH 10 due to the subunit B1 (Fig. 5B and Refs. 12 and 14). In contrast to the normal BzBz enzyme, the acetimidylated BzBz enzyme was prone to precipitation, and it lost about 50% of its activity per day. This instability prevented the isolation and characterization of the different bands revealed by electrophoresis of the freshly modified atypical enzyme. The electrophoretic pattern was similar to that of modified normal enzyme. Therefore, freshly modified, but unfractonated, enzyme was characterized. The pH-rate profile of the soluble, acetimidylated atypical enzyme was very similar to that of the modified normal enzyme (Fig. 5B). Acetimidylation increased the maximum activity by only about 2 fold (Table II). This might indicate that the substitution of proline in subunit Bz for Ala-230 in subunit B, alters the local structure of the enzyme in a manner similar to that obtained by the acetimidylation of Lys-228 in subunit B1. The net effect is that alteration of residues 230 or 228 increases activity, but alteration of both residues does not...
activate additively. On the other hand, the acetimidylated B₂ subunit is apparently less stable than the acetimidylated B₁ subunit, so it is possible that the 2-fold activation observed is lower than it should be simply because the acetimidylated B₂ subunit was denatured and only the activity of the acetimidylated B₁ subunit remains to be expressed.

Steady State Kinetic Studies - Product inhibition studies on the predominant form of enzyme in normal livers were carried out in order to determine the mechanism of the enzyme and the magnitudes of the kinetic constants. As shown in Fig. 6, A and B, the coenzymes appear to be competitive against one another. Fig. 6, C and D show that ethanol and acetaldehyde were essentially competitive against one another (p > 5% for the pair of intercepts differing most). For the Ordered Bi Bi mechanism, one would expect these patterns to be noncompetitive (24, 25). In order to further elucidate the mechanism, the experiments shown in Fig. 6, E and F, were performed.

Ethanol is certainly a noncompetitive, if not uncompetitive, inhibitor against NADH (Fig. 6E) since the slopes are almost the same (p > 5%). This result eliminates the simple Rapid Equilibrium Random Bi Bi mechanism and also suggests that the Theorell-Chance mechanism does not obtain (24, 25). The results in Fig. 6E are not as conclusive as those in Fig. 6F, but again fit the Ordered mechanism. (We recognize, of course, that the results are consistent with more complicated mechanisms, such as Random Bi Bi.)

The product inhibition studies for the acetimidylated enzyme were carried out as with native enzyme except that somewhat higher (e.g. 2- to 10-fold) concentrations of substrates and products were used in order to accommodate the higher kinetic constants of the acetimidylated enzyme. The inhibition patterns obtained were very similar to those shown in Fig. 6. In particular, NAD⁺ and NADH were linear competitive inhibitors against one another. Acetaldehyde appeared to be a linear competitive inhibitor against ethanol; that is, significant differences in intercepts (p > 1%) could not be demonstrated. Ethanol gave weakly noncompetitive inhibition (for closest intercepts, p = 1 to 5%) against acetaldehyde (Fig. 7A). Acetaldehyde gave noncompetitive inhibition against NAD⁺ (Fig. 7B). Thus, the results with acetimidylated enzyme also appear to fit the Ordered Bi Bi mechanism.

The substrate concentrations used for the product inhibition studies for methylated enzyme were similar to those used for acetimidylated enzyme. Again, NAD⁺ and NADH gave linear, competitive inhibition against one another, as did acetaldehyde against ethanol as substrate. However, in contrast to the results with native and acetimidylated enzyme, ethanol was certainly a noncompetitive inhibitor against acetaldehyde (Fig. 7C), and acetaldehyde appeared to be competitive against NAD⁺ (Fig. 7D). Less extensive product inhibition studies on the methylated enzyme were also performed at pH
The values and their standard errors were computed by fitting all of the data for a product inhibition experiment (see Fig. 6, for instance) to the equation describing the type of inhibition (1, 23). The values were corrected for subsaturating concentrations of nonvaried substrates on the assumption of an Ordered Bi Bi mechanism. \( K \) is a Michaelis constant; \( K_i \), an inhibition constant; \( a, b, p, \) and \( q \) represent NAD\(^+\), ethanol, acetaldehyde, and NADH, respectively. \( V_{\max}/E_i \) is the turnover number for the reaction of NAD\(^+\) and ethanol, and \( V_{\max}/E_i \) for NADH and acetaldehyde. The buffers used were 0.1 \( \mu \) \( \text{mol} \) of sodium phosphate at the pH indicated at 25°C.

| Group                        | Native | Acetimidyl | Methyl | Methyl | 4-Hydroxybutyrimidyl |
|------------------------------|--------|------------|--------|--------|----------------------|
| \( K_{\text{m}}, \mu\text{M} \) | 17 ± 3 | 40 ± 3     | 40 ± 5 | 16 ± 1 | 600 ± 40             |
| \( K_{\text{m}}, \text{mM} \)   | 1.8 ± 0.3a | 33 ± 4 | 5.8 ± 0.5 | 22 ± 2 | 90 ± 10               |
| \( K_{\text{m}}, \mu\text{M} \) | 15.5 ± 0.2a | 6.8 ± 0.5 | 15 ± 2 | 13 ± 2 | 16 ± 2               |
| \( K_{\text{m}}, \text{mM} \)   | 10 ± 1  | 61 ± 5     | 120 ± 20| 100 ± 10| 190 ± 20             |
| \( K_{\text{m}}, \mu\text{M} \) | 49 ± 5  | 150 ± 10   | 580 ± 60| 560 ± 30| 1100 ± 100           |
| \( K_{\text{m}}, \text{mM} \)   | 92-703a | 300 ± 60   | 140 ± 20| -c     | -c                   |
| \( K_{\text{m}}, \mu\text{M} \) | 1-160a | 5.4 ± 0.5  | 6 ± 2  | -c     | -c                   |
| \( K_{\text{m}}, \text{mM} \)   | 2.1 ± 0.3 | 6.2 ± 0.4 | 6.3 ± 0.6| 5.2 ± 0.3| 34 ± 2               |
| \( K_{\text{m}}, \mu\text{M} \) | 9 ± 2   | 76 ± 9     | 140 ± 40| 150 ± 30| 70 ± 10              |
| \( K_{\text{m}}, \text{mM} \)   | 0.23 ± 0.06 | 1.7 ± 0.4 | 0.58 ± 0.05| 0.20 ± 0.02| 3.5 ± 0.4           |
| \( V_{\max}/E_i, \text{s}^{-1} \) | 1.4 ± 0.1 | 4.7 ± 0.8 | 7.4 ± 0.6| 5.7 ± 0.5| 22 ± 2               |
| \( V_{\max}/E_i, \text{s}^{-1} \) | 34 ± 2  | 110 ± 6    | 220 ± 20| 240 ± 20| 350 ± 20             |
| pH of kinetics               | 8.0     | 8.0        | 8.0    | 7.0    | 8.0                  |

| Units/mg, pH 9d | Native | Acetimidyl | Methyl | Methyl | 4-Hydroxybutyrimidyl |
|-----------------|--------|------------|--------|--------|----------------------|
| Units/mg, pH 8.8e | 3.0    | 22         | 28     | 28     | 42                    |

\[ a \] These are apparent values, obtained with high concentrations of substrates.

\[ b \] The large range of values is due to large corrections for subsaturating concentrations of nonvaried substrates.

\[ c \] These values were not obtained because the product inhibition patterns appeared to be competitive, probably because the intercept inhibition constants are much larger than slope inhibition constants.

7.0, in order to determine the effect of pH on the kinetic constants. The four patterns corresponding to those shown in Fig. 6, A to D, all appeared to be competitive.

A derivative of the human enzyme has also been prepared with 4-hydroxybutyrimidyl substituents on the amino groups (26). This modified enzyme is even more active than the acetimidylated and methylated enzymes and has even larger kinetic constants.

Previous studies with the horse liver enzyme have shown that larger, positively charged substituents give enzymes with larger kinetic constants, also. The product inhibition patterns analogous to those shown in Fig. 6, A to D (obtained with 2- to 10-fold higher concentrations of substrates and products), all appeared to be competitive. These results would be consistent with several mechanisms, but because the native human and horse enzymes and several of their activated forms have mechanisms consistent with Ordered Bi Bi (1, 3, 6) we have assumed that the 4-hydroxybutyrimidylated (and methylated) enzymes also have Ordered mechanisms.

The kinetic constants in the equation describing the velocity of the reaction as a function of substrate and product concentrations (25) were computed from the results of the product inhibition studies and are presented in Table III. The Michaelis and inhibition constants for the native human enzyme are larger, up to 5-fold, than the constants for the horse enzyme at the same pH (3, 7). These kinetic constants are increased by acetimidylation and methylation, and interestingly, the relative increases are about the same using either the horse or human enzymes as references for their modified enzymes. This observation strongly suggests that a lysine residue homologous to number 228 in the horse enzyme is also present in human enzyme, which has been previously suggested on the basis of sequence work (12). Furthermore, it suggests that the mechanisms of the two enzymes and the role of the amino group in the active site are similar.

It also appears that human liver enzyme modified with small, positively charged substituents might be useful in vivo for accelerating ethanol metabolism, as was suggested previously on the basis of studies with the horse enzyme (10). The basis for this suggestion was that the modified enzymes are more active (as indicated by the 3- to 10-fold larger turnover numbers), the Michaelis constants for NAD\(^+\) are nearly the same as native enzyme so that the modified enzyme could compete for the NAD\(^+\) available in the cell, and the Michaelis constants for ethanol are increased so that the modified enzyme would become saturated at more highly intoxicating concentrations of ethanol than is native enzyme (29, 30). This suggestion is quite hypothetical and speculative, of course, but does indicate the potential value of these results for developing methods to accelerate ethanol metabolism. It may also be noted that the 4-hydroxybutyrimidylated enzyme has such large kinetic constants that it may not be more active under in vivo conditions.

**Nonlinear Kinetics of Native Human Enzyme**—Although the B, B, native enzyme has a mechanism consistent with Ordered Bi Bi when results obtained over a limited range of concentrations are considered, use of much wider ranges of ethanol as substrate reveals nonlinear kinetics. The Line-weaver-Burk plots show downward curvature with concentrations of ethanol up to 0.1 \( \text{mM} \); at higher concentrations substrate inhibition becomes apparent (cf. Fig. 4). A product inhibition pattern plotted according to Eadie and Hofstee for the best representation of nonlinear kinetics, is given in Fig. 8. It is apparent that acetaldehyde gives competitive inhibition against varied concentrations of ethanol. It is also noteworthy that when limited ranges of ethanol are considered (0.2 to 1.0 \( \text{mM} \), 4 to 20 \( \text{mM} \), or 20 to 100 \( \text{mM} \)), each set of data fitted a
that the preparation contains two forms of enzyme that differ in kinetic characteristics. Although the preparation is chromatographically and electrophoretically homo- 
geneous (19, 14), microheterogeneity, analogous to the valine/alanine ex-
change at position 43 in the horse enzyme (11) cannot be excluded. Substitution of an amino acid residue at a critical location at the active site could greatly affect activity, as is found with the substitution of proline for alanine at position 230. If it is assumed that there are only two different kinds of sites and that these act independently, the activities observed should simply be the sum of the individual activities (31). The equation for the sum is a 2/1 function and can be solved for the kinetic constants pertaining to each form of enzyme. The constants given in Table V suggest that the postulated forms differ greatly in magnitudes of Michaelis constants for ethanol, but that they have approximately equal maximum velocities. This might suggest that there are equal amounts of the two forms. The constants calculated in Table V can also be used to evaluate the effect of acetaldehyde as an inhibiting product on activity. For each form, acetaldehyde gave linear inhibition (apparent K/V plotted against acetaldehyde concentra-
tion), with an apparent constant (K/KV) of about 0.22 mM. That these inhibition constants and the turnover numbers have about the same magnitudes, whereas the Michaelis constants for ethanol are very different, may be interpreted to indicate that the two forms of enzyme would differ primarily because the rates of binding of ethanol to the enzyme· NAD+ complexes (k) are different. (K/KV/KV) = k (k + K)/k. 

A product inhibition study similar to that shown in Fig. 6D, except that the concentrations of acetaldehyde were varied from 0.5 to 10 mM also suggested that the kinetic behavior was nonlinear, with apparent activation by substrate. Attempts to fit these data to the 2/1 function failed, however, as indicated by 

negative parameters or large standard errors. This failure precluded further attempts to differentiate the kinetic properties of the postulated enzyme forms.

Another explanation for the nonlinear kinetics could be that the dimeric enzyme exhibits negative cooperativity, in that

| Table V |

| Kinetic constants for oxidation of ethanol in presence of acetaldehyde calculated on assumption that two forms of human enzymes are present in normal phenotype |

For a mixture of isozymes, it may be assumed that the observed velocity is the sum of the activities contributed independently by the two forms:

\[ v = \frac{V_1S}{K_1 + S} + \frac{V_2S}{K_2 + S} = \frac{(V_1 + V_2)S^2 + (V_1K_1 + V_2K_2)S}{S^2 + (K_1 + K_2)S + K_1K_2} \]

By comparison to the equation fitted in Table IV, therefore, the kinetic constants can be calculated as follows:

\[ K_1 = 0.5B \pm 0.5(B^* - 4C)^{1/2}, \frac{V_1}{V_2} - \frac{V_1}{V_2} - \frac{V_2}{V_2} - \frac{V_1}{V_1} \]

Kinetic constants can also be calculated from the other data at 0.1, 0.4, and 0.8 mM, a more consistent value of 0.3 mM can be calculated.

**Table IV**

| Kinetic constant | Concentration of acetaldehyde |
|------------------|-----------------------------|
| B, mM | 24 ± 5 | 16 ± 2 | 32 ± 9 | 51 ± 10 |
| C, mM² | 12 ± 4 | 10 ± 2 | 51 ± 24 | 130 ± 30 |
| D, mM | 11 ± 3 | 6.6 ± 1.1 | 14 ± 5 | 23 ± 5 |
| K, mM | 4.0 ± 0.5 | 5.3 ± 0.2 | 9 ± 1 | 14 ± 1 |
| V, units/ml | 1.68 ± 0.03 | 1.07 ± 0.01 | 1.08 ± 0.04 | 1.13 ± 0.04 |

* This value appears to be erroneously high. By extrapolation from the other data at 0.1, 0.4, and 0.8 mM, a more consistent value of 0.3 mM can be calculated.
the binding of the 1st molecule of ethanol causes decreased affinity for the 2nd molecule of ethanol. A more extreme form of negative cooperativity, called "half-of-the-sites reactivity" has been proposed previously for the action of horse liver alcohol dehydrogenase on NADH and aromatic aldehydes (33). However, the correctness of the basic observation has been questioned recently (34). If the native human enzyme does exhibit some negative cooperativity, it is interesting that the acetimidylated enzyme does not, at least over the range of ethanol concentrations from 0.1 mM to 0.1 M. This result could indicate that modification of amino groups disrupts cooperative interactions between subunits and also that acetimidyla-

tion activates by altering the rates of conformation isomerizations, which may occur during the binding of the coenzymes (1, 6). Because of the potential significance of the nonlinear kinetics of the native human enzyme, it is clear that more extensive studies on this problem are required.

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