Kinetics and Reaction Mechanism of Yeast Alcohol Dehydrogenase with Long-Chain Primary Alcohols

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Kinetic studies of yeast alcohol dehydrogenase with NAD^+ and ethanol, hexanol or decanol as substrates invariably result in non-linear Lineweaver–Burk plots if the alcohol is the variable substrate. The kinetic coefficients determined from secondary plots are consistent with an ‘equilibrium random-order’ mechanism for extremely low alcohol concentrations and for all alcohols, the transformation of the ternary complexes being the rate-limiting step of the reaction. This mechanism also applies to long-chain substrates at high concentrations, whereas the rate of the ethanol– NAD^+ reaction at high ethanol concentrations is determined by the dissociation of the enzyme– NADH complex. The dissociation constants for the enzyme– NAD^+ complex and for the enzyme–alcohol complexes obtained from the kinetic quotients satisfactorily correspond to the dissociation constants obtained by use of other techniques. It is suggested that the non-linear curves may be attributed to a structural change in the enzyme itself, caused by the alcohol.

Yeast alcohol dehydrogenase (EC 1.1.1.1) catalyses the reversible oxidation of many primary and secondary alcohols (Sund & Theorell, 1963). For a considerable time, detailed studies of the kinetics and reaction mechanism were available only for ethanol oxidation and acetaldehyde reduction (Wratten & Cleland, 1963; Silverstein & Boyer, 1964). Dickinson & Monger (1973) were the first to investigate the kinetics by using other substrates and obtained the kinetic parameters for the oxidation of ethanol, propan-1-ol, propan-2-ol and butan-1-ol, and for the reduction of acetaldehyde and butyraldehyde.

We showed previously (Schöpp & Aurich, 1973) that the enzyme also reacts with substrates with much longer chains of carbon atoms (up to 16 carbon atoms) within the regular pattern of the hydrophobic protein–ligand interaction. The pK_m values for the substrates with between 4 and 10 carbon atoms show a linear increase with the chain length, whereas the maximal reaction velocities decrease with increasing chain lengths but reach a second shallow maximum at 8 carbon atoms. A kinetic examination of the reaction with substrates that are within this systematic range should give results leading to conclusions of general interest.

The present paper aims at a thorough investigation of the kinetics of the reactions of yeast alcohol dehydrogenase with long-chain alcohols to obtain more information about the reaction mechanism of the enzyme.

Materials and Methods

Crystalline alcohol dehydrogenase was prepared from dried baker's yeast (Racker, 1950; Dickinson,

1970); a freeze-dried enzyme was supplied by Boehringer (Mannheim, West Germany). The specific activity (measured at 25°C in 0.1 M-sodium pyrophosphate buffer, pH 8.7, and with 0.5 M-ethanol and 0.8 mM- NAD^+) was normally between 380 and 430 $\mu\text{mol}/\text{min}$ for the three-times-recrystallized fresh preparation, and between 200 and 250 $\mu\text{mol}/\text{min}$ for the commercial enzyme (per mg of protein).

NAD^+ and GSH* were supplied by Boehringer; ethanol and pentanol by VEB Laborchemie (Apolda, German Democratic Republic); hexanol and decanol by Fluka A.G. (Buchs, Switzerland).

Initial rates were determined fluorimetrically with a Beckman DK 2 A spectrophotometer with fluorescence attachment or with an arrangement used in an earlier study (Schöpp & Aurich, 1973). The original fluorescence attachment of the DK 2 A spectrophotometer was converted to accommodate a cuvette holder for a 3 ml cuvette. The light-source for both arrangements was a mercury-vapour lamp (HQE 40, Osram, West Berlin). The changes in fluorescence were registered by recorders. All measurements were taken at 25°C in 0.067 M-sodium phosphate buffer, pH 8.0, which contained 0.5 mM-GSH. The stock solutions of the enzyme were prepared in 0.067 M-sodium phosphate buffer (pH 7.5) which also contained 0.5 mM-GSH. In these solutions the enzyme was stable for at least 2 h, depending on the extent of dilution. In each case the total test volume was 2 ml. The reaction was started with 50 μl each of enzyme solution. The enzyme concentration was determined by spectrophotometry at 280 nm by using an extinc-

*Abbreviation: GSH, reduced glutathione.

tion coefficient (e) of $189 \text{ litre} \cdot \text{mmol}^{-1} \cdot \text{cm}^{-1}$ (Hayes & Velick, 1954).

The kinetic coefficients in the equation:

$$\frac{e}{v_0} = \phi_0 + \frac{\phi_1}{[S_1]} + \frac{\phi_2}{[S_2]} + \frac{\phi_{12}}{[S_1][S_2]}$$

were determined graphically from primary and secondary plots (Dalziel, 1957). In the equation, e is the enzyme concentration measured and multiplied by a factor of 2.7. This value was obtained by Dickinson (1970) for the number of NAD^+ binding sites on the enzyme. S_1 is NAD^+ , and S_2 is alcohol.

The kinetic parameters for alcohol dehydrogenase with ethanol, hexanol and decanol given in the present paper were determined simultaneously by using two different instruments. In each case two or three measurements were done. A measurement was repeated after the rates of reaction for five other substrate concentrations had been determined. The procedure adopted was not to keep $[S_1]$ constant first and vary $[S_2]$ but to vary $[S_1]$ and $[S_2]$ alternately over the entire range of concentration, according to a pre-set random sequence. This greatly decreased the possibility of systematic errors resulting from changes in the activity of the enzyme. The enzyme preparations used did not catalyse the reduction of NAD^+ in the absence of alcohol. The tests were made over a period of 1 week by using the same enzyme preparation, but diluted enzyme solutions prepared for the kinetic tests were used only for about 60 min. The rates of reaction measured are generally reproducible to within 5% and the kinetic coefficients to within 25%.

Results

Stability of the diluted enzyme

A major problem in examining the oxidation of long-chain alcohols was that albumin could not be added to assays. This is normally required to stabilize the enzyme. Albumin binds long-chain alcohols (Reynolds *et al.*, 1968), thus decreasing the free concentration of the substrate in the test solution. As a result kinetic values obtained with added albumin are probably not reliable. We showed earlier (Schöpp, 1973; Schöpp & Aurich, 1974a) that the presence of foreign protein drastically decreases the rate of enzymic reactions, especially with hydrophobic substrates. This can give a sigmoid character to the kinetics.

In our first kinetic experiments with long-chain alcohols and aldehydes (Schöpp & Aurich, 1973) we did not add albumin, and prepared the enzyme stock solutions in 0.067M-sodium phosphate buffer that contained 0.1% sucrose. Although the activity of the enzyme in these solutions was constant over several hours, we found it rather difficult at times to reproduce our results. This depended particularly on the enzyme preparation.

Fig. 1. Effects of albumin and GSH on the activity of yeast alcohol dehydrogenase at high dilution

The test solution contained 25mM-ethanol and 2nM-alcohol dehydrogenase (mol.wt. 150000) in 0.067M-sodium phosphate buffer, pH7.5 (total volume 20ml). Samples (1.9ml) were taken, and the reaction was initiated by adding 0.1 ml of NAD^+ solution (final concn. 0.82mM). ●, 0.05% bovine serum albumin; ○, 0.5mM-GSH; ○, without albumin and GSH.

Initially, therefore, we examined enzyme activity behaviour under test conditions. Fig. 1 shows that a considerable decrease in activity occurs, the extent of which also depends on the concentration of the substrate. In stabilization studies of several alcohol dehydrogenases, Takemori *et al.* (1967) observed a constant activity of the enzymes in the presence of 1mM-GSH. Under our conditions, also, GSH had a remarkable stabilizing effect that was greatest at a concentration of 0.5mM. There was virtually no difference between the stabilizing effect of GSH on enzyme activity and that of albumin (see Fig. 1).

Kinetics of alcohol- NAD^+ reactions

The results of the initial-rate measurements for hexanol are shown as primary plots in Fig. 2. When we used very low hexanol concentrations we observed deviations from the linear pattern (Schöpp & Aurich, 1974b). The same observation was made for decanol. On the other hand, we invariably found a linear pattern when we plotted NAD^+ in the range of concentrations studied (Fig. 3). The secondary plots for hexanol are shown in Fig. 4. For better differentiation, the coefficients obtained at high alcohol concentrations were marked ϕ^{max} , and those for low concentrations were marked ϕ^{min} .

For better comparison of the resulting parameters with those for ethanol we examined the kinetics of the enzyme with ethanol and with long-chain alcohols under identical conditions, except for pH. With ethanol, too, we observed deviations from the linear pattern for very low alcohol concentrations. The resulting secondary plots are shown in Fig. 5. In a control experiment we examined the kinetics with

Fig. 2. Primary plots of alcohol dehydrogenase reaction: variation of the specific initial rate (e/v_0) at pH 8 and 25°C with the reciprocal of hexanol concentration for several constant NAD^+ concentrations

○, 820; ●, 410; ○, 308; ●, 205; ●, 140 μM - NAD^+ .

ethanol at pH 8. We found that the variations in the individual coefficients and their quotients were negligible in comparison with the coefficients of the long-chain substrates. In addition, we made measurements under the conditions described by Dickinson & Monger (1973), with ethanol in the presence of albumin, and obtained coefficients similar to those of these authors. With regard to ethanol, it can therefore be said that the coefficients vary only negligibly when albumin is present, at least in the range 0.01–1 M-ethanol. We have not performed a complete study of the kinetics with aldehyde substrates, but variation of hexanoic aldehyde concentration (0.016–15 mM) with constant NADH concentrations (100 μM) also showed non-linear plots.

All coefficients obtained from secondary plots are summarized in Table 1. The absolute values of the kinetic parameters are subject to substantial error owing to the marked instability of yeast alcohol dehydrogenase in dilute solutions. However, this seems to be negligible in a comparison of the coefficients or their quotients depending on the chain-length of the substrate, which is the main purpose of the present paper. We obtained the same curves and

similar kinetic quotients for fresh enzyme preparations, commercial preparations having only 40–70% of the activity of the fresh preparation, and for preparations with as little as 10% activity and a thiol-group content of only 11 mol/mol of protein.

To obtain additional information, we studied the simultaneous oxidation of two alcohols, namely hexanol and ethanol, catalysed by alcohol dehydrogenase (Fig. 6). In the presence of hexanol plus ethanol the measured rate is significantly higher than the calculated one if both alcohols are oxidized at the same site and are mutually exclusive. The same behaviour can be observed with other long-chain alcohols and ethanol. At present we cannot provide a complete theoretical basis for the interpretation of this complex situation.

Discussion

The results of our kinetic experiments might suggest the occurrence of a non-equilibrium random-order mechanism (Scheme 1). Such a mechanism would be compatible with the isotope-exchange measurements of Silverstein & Boyer (1964).

Fig. 3. Primary plots of alcohol dehydrogenase reaction: variation of the specific initial rate (e/v_0) at pH 8 and 25°C with the reciprocal of NAD^+ concentration for several constant hexanol concentrations

●, 8.0; ○, 4.3; ▲, 3.2; ◐, 2.1; □, 1.6; ▼, 0.8; ◑, 0.48; ■, 0.32; ▽, 0.22; △, 0.16 mM-hexanol.

Dickinson & Monger (1973) also suggested this mechanism, with some restrictions, for ethanol, propanol and butanol.

The function for all non-equilibrium mechanisms with a ternary complex approaches a straight line asymptotically only if the substrate concentration is low enough, in which case an accurate value cannot be forecast when the velocity constants are not known (Pettersson, 1972). This straight line cannot be experimentally differentiated from the bilinear Dalziel (1957) equation. This in any case requires the restrictive conditions $k_{+3}[S_2] \ll k_{-1}$ and $k_{+4}[S_1] \ll k_{-2}$ in order to obtain linear relationships (Dalziel, 1958).

Assuming that the non-linearity of our curves were to be interpreted as approaching such an asymptote, a smooth and gradual transition between the limiting linear sections would be expected. In our case, an approximation to an asymptote is unlikely, because the transition is relatively abrupt. On the other hand, the inflexion point in the Lineweaver-Burk plots could mean that different routes may lead to the formation of the ternary complex, depending on the

concentration of the alcohol. If so, the kinetic parameters obtained from the two linear ranges of the curves should fulfil the conditions of an effectively ordered mechanism (Pettersson, 1972), where either NAD^+ (for low alcohol concentrations), or the alcohol itself (for high alcohol concentrations), is the first substrate. This would mean that $\phi_1\phi_2/\phi_{12}$ must be less than or equal to ϕ'_0 (Dalziel, 1957). However, this is not the case. Table 2, which contains only ϕ_0 values (and not ϕ'_0), shows that $\phi_1\phi_2/\phi_{12}$ is always greater than ϕ'_0 , because ϕ'_0 is always less than ϕ_0 for all reactions studied (the factor is approx. 5 for pH 8). This applies to the $\phi'^{\max}$ and $\phi'^{\min}$ as well as to the $\phi^{\min}$ and $\phi^{\max}$ values (W. Schöpp, unpublished work). ϕ'_0 and $\phi_1\phi_2/\phi_{12}$ are identical only for high ethanol concentrations (Dickinson & Monger, 1973).

On the other hand, the quotient $\phi_1\phi_2/\phi_{12}$ agrees well with ϕ_0 in all other cases. This identity is obtained for an equilibrium random-order mechanism. Because this mechanism predicts strictly linear Lineweaver-Burk plots, it seems that our non-linear curves may be attributed to a structural change, caused by the alcohol, in the enzyme itself.

Fig. 4. Secondary plots of alcohol dehydrogenase reaction: variation of slopes (a) and intercepts (b) of the primary plots (Figs. 2 and 3) with the reciprocals of the hexanol concentration (●) and NAD^+ concentration (○) respectively.

In (b) the common intercept of (1) and (2) is ϕ_0^{max} and of (3) and (4) ϕ_0^{min} . The slopes of (1) and (3) are ϕ_2^{max} and ϕ_2^{min} and those of (2) and (4) ϕ_1^{max} and ϕ_1^{min} . In (a), the common slope of (1) and (2) is ϕ_{12}^{max} and of (3) and (4) ϕ_{12}^{min} .

Table 3 summarizes the relationships between the rate constants and the Dalziel coefficients (Dalziel, 1957), allowing for the relationships established by Petterson (1972). These apply to all mechanisms possible in terms of Scheme 1. For an equilibrium random-order mechanism, the terms of second degree (in ϕ_1 and ϕ_2) vanish. $(A+B)$ and $(A+R)$ are equal to 1 (Dalziel, 1958).

The kinetically determined values for $K_1(\phi_{12}/\phi_2)$ and $K_4(\phi_1/\phi_0)$ with hexanol and decanol as substrates are between 130 and 200 μM , which are considerably less than the dissociation constants for the enzyme- NAD^+ complex determined by Hayes & Velick (1954) and Dickinson (1972) by a non-kinetic method, which

Fig. 5. Secondary plots of alcohol dehydrogenase reaction: variation of slopes (a) and intercepts (b) of the primary plots with the reciprocals of the ethanol concentration (●) (0.82–51 mM) and NAD^+ concentration (○) (82–820 μM) respectively at pH 7 and 25°C

resulted in values of approximately 300 μM . Further studies (Yamada & Yamoto, 1973) have shown that the NAD^+ -binding sites of the enzyme interact negatively with each other, so that two different dissociation constants may be determined. The values measured were 120 and 310 μM . Although the second value is satisfactorily compatible with our dissociation constant resulting from the ethanol-oxidation reaction, the values found for the oxidation of hexanol and decanol clearly approach the lower dissociation constant. This would suggest that the long-chain alcohols, even at lower concentrations, affect the conformation of the enzyme so that a tighter binding of NAD^+ is achieved.

If we compare the quotients ϕ_{12}/ϕ_1 and ϕ_2/ϕ_0 (Table 2) it is noteworthy that both values are identical. However, marked differences can be observed here when the quotients are determined at

Table 1. Kinetic coefficients of yeast alcohol dehydrogenase for the oxidation of several primary alcohols

	$\phi_0^{\max.}$ (s)	$\phi_0^{\min.}$ (s)	$\phi_1^{\max.}$ ($\mu\text{M}\cdot\text{s}$)	$\phi_1^{\min.}$ ($\mu\text{M}\cdot\text{s}$)	$\phi_2^{\max.}$ ($\mu\text{M}\cdot\text{s}$)	$\phi_2^{\min.}$ ($\mu\text{M}\cdot\text{s}$)	$\phi_{12}^{\max.}$ ($\mu\text{M}^2\cdot\text{s}$)	$\phi_{12}^{\min.}$ ($\mu\text{M}^2\cdot\text{s}$)
Decanol (pH8)	0.07	0.16	14	22	6.3	3.7	1200	650
Hexanol (pH8)	0.065	0.13	11	20	210	100	32000	12700
Butanol (pH7)	0.054*		13.5*		1730*		270000*	
(with albumin)								
Ethanol (pH7)	0.0024		0.20		43		14000	
(with albumin)	0.0031*		0.32*		65*		21000*	
Ethanol (pH7)	0.0028	0.0063	0.27	1.8	67	49	17000	12000

* These data are from Dickinson & Monger (1973); they were originally calculated on the basis of two active sites per molecule (F. M. Dickinson, personal communication); we have converted these data by using the factor 2.7 to compare those with our results.

Fig. 6. Reduction of NAD^+ ($820\mu\text{M}$) by mixtures of ethanol and hexanol in the presence of alcohol dehydrogenase

●, Without hexanol; ○, +7.5mm-hexanol; ----, theoretical curve for the rate of oxidation of mixtures of ethanol and hexanol is given for the case where both alcohols are oxidized at the same site and are mutually exclusive.

high or low alcohol concentrations. If we assume an equilibrium random-order mechanism for the oxidation of long-chain alcohols, both of the quotients are identical with the dissociation constants of the enzyme-alcohol complexes. The identity of K_m and K_s would accord with the result that the maximum velocity of long-chain aldehyde reduction is much greater than for long-chain oxidation (Dickinson & Monger, 1973; Schöpp & Aurich, 1973). If $\log(\phi_2^{\max.}/\phi_0^{\max.})$ or $\log(\phi_{12}^{\max.}/\phi_1^{\max.})$ are plotted against the chain length of the alcohol (Fig. 7), the same linear relationship in the range between 5 and 10 carbon atoms is seen, as we have previously described (Schöpp & Aurich, 1973). This is normally observed for the protein-ligand interaction in a homologous series (Franke, 1970). The fact that the values for $\log(\phi_2^{\min.}/\phi_0^{\min.})$ or $\log(\phi_{12}^{\min.}/\phi_1^{\min.})$ are nearly perfectly in parallel suggests interaction of the same substances at the same binding site. It is a general phenomenon observed in the interaction of proteins with hydrophobic ligands that here the stability of the enzyme-alcohol complex is much greater. For instance, long-chain alcohols bind more

Scheme 1. Common mechanism for the yeast alcohol dehydrogenase reaction with two ternary enzyme substrate complexes

E, Alcohol dehydrogenase; S_1 , NAD^+ ; S_2 , alcohol; S'_1 , NADH ; S'_2 , aldehyde.

Table 2. Relationships between kinetic coefficients for the oxidation of ethanol, hexanol and decanol catalysed by yeast alcohol dehydrogenase

The tests were done at pH8, for decanol and hexanol, but at pH7 for ethanol. $\phi^{\min.}$ = values found at low alcohol concentrations; $\phi^{\max.}$ = values found at high alcohol concentrations.

	$\phi^{\min.} (\mu M)$		$\phi^{\max.} (\mu M)$		$\phi^{\min.} (\mu M)$		$\phi^{\max.} (\mu M)$		$\phi^{\min.} (s)$		$\phi^{\max.} (s)$	
	ϕ_1/ϕ_0	ϕ_{12}/ϕ_2	ϕ_1/ϕ_0	ϕ_{12}/ϕ_2	ϕ_2/ϕ_0	ϕ_{12}/ϕ_1	ϕ_2/ϕ_0	ϕ_{12}/ϕ_1	$\phi_1\phi_2/\phi_{12}$	ϕ_0	$\phi_1\phi_2/\phi_{12}$	ϕ_0
Decanol	137	175	200	206	23	32	90	90	0.125	0.16	0.067	0.07
Hexanol	142	127	177	152	714	640	3400	3000	0.15	0.14	0.072	0.065
Ethanol	285	244	96	253	7800	6700	23900	25000	0.0073	0.0063	0.011	0.003

Table 3. Relationships between rate constants and Dalziel coefficients for all cases inherent in the ternary-complex mechanism shown in Scheme 1 (Pettersen, 1972; Dalziel, 1958)

$$\phi_0 = \frac{(A+B)}{k}$$

$$\phi_{12} = \frac{(A+R) K_1 K_3}{k} = \frac{(A+R) K_2 K_4}{k}$$

$$\phi_1 = \frac{(A+R) K_4}{k} + \frac{(k_{-3}R)^2}{k_{+1}}$$

$$\phi_2 = \frac{(A+R) K_3}{k} + \frac{(k_{-4}R)^2}{k_{+2}}$$

where

$$A = 1 + \frac{k'}{(k'_{-3} + k'_{-4})}; R = \frac{1}{(k_{-3} + k_{-4})};$$

$$B = \frac{k(k'_{-1}k'_{-2} + k'_{-2}k'_{-3} + k'_{-1}k'_{-4})}{k'_{-1}k'_{-2}(k'_{-3} + k'_{-4})}$$

K_1 , K_2 , K_3 and K_4 are the dissociation constants of the binary (ES_1 , ES_2) and of the ternary (ES_1S_2) complexes (see Scheme 1).

Fig. 7. Dependence of some kinetic coefficients on the chain length of the substrates for the oxidation of primary alcohols by yeast alcohol dehydrogenase

○, $\log(\phi^{\max.}/\phi_0^{\max.})$; $\log(\phi_{12}^{\max.}/\phi_1^{\max.})$ (see Table 2); ●, $\log(\phi_2^{\min.}/\phi_0^{\min.})$; $\log(\phi_{12}^{\min.}/\phi_1^{\min.})$ (see Table 2).

Table 4. Rate constants of the conversion of different ternary enzyme-substrate complexes of yeast alcohol dehydrogenase, calculated from the kinetic coefficients.

$k^{\max.}$, constants calculated from $\phi^{\max.}$ values; $k^{\min.}$, constants calculated from $\phi^{\min.}$ values.

	$k^{\max.} (s^{-1})$	$k^{\min.} (s^{-1})$
Decanol (pH8)	14	6
Hexanol (pH8)	15	8
Butanol (pH7)	25*	
Ethanol (pH7)	> 420*	160

*These data are from Dickinson & Monger (1973).

strongly to bovine serum albumin at low alcohol concentrations than at high concentrations (Reynolds *et al.*, 1968).

All kinetic findings therefore suggest that the formation of the ternary complex between alcohol dehydrogenase and long-chain primary alcohols follows an equilibrium random-order mechanism where k limits the overall rate of reaction (see Scheme 1). This does not apply to ethanol at high concentrations as shown previously by Silverstein & Boyer (1964), Wratten & Cleland (1963) and Dickinson & Monger (1973). Our values indicated the same result when we used high ethanol concentrations. It is only at extremely low concentrations that quotients are obtained here that fulfil the conditions of an equilibrium random-order mechanism. We believe, therefore, that all substrates of yeast alcohol dehydrogenase are oxidized by this mechanism. This is not contrary to the findings of Silverstein & Boyer (1964) for

ethanol, because it is possible that structural changes of the enzyme occur under the influence of high ethanol concentrations, yielding changes in kinetic properties, especially k .

Table 4 contains the rate constants determined from ϕ_0 for the rate-limiting step of the reaction. It is obvious that k changes as a function of the alcohol concentration. The ternary complexes are much more slowly transformed than the enzyme-NAD⁺-ethanol complex if a longer-chain alcohol is involved. This means that although the reaction mechanism is the same, there are differences in the mechanism of binding between long-chain alcohols and ethanol. This is also clearly indicated by the ϕ_2 values, which increase dramatically as the chain length decreases, an indication that hydrophobic interaction is decreased. By contrast, the value for ethanol is quite inconsistent with this sequence. The value $k = 160\text{s}^{-1}$ (Table 4) for low ethanol concentrations is fairly close to the value found by Brooks & Shore (1971) for the rate of hydride transfer in liver alcohol dehydrogenase ($k = 140\text{s}^{-1}$). As the ethanol concentration rises, k must increase considerably, so that the rate of reaction is now limited by the dissociation of NADH from the enzyme ($k'_{-1} = 420\text{s}^{-1}$) (Dickinson & Monger, 1973) and the reaction ceases to follow an equilibrium random-order mechanism.

Besides a different binding mechanism for ethanol (compared with that for long-chain alcohols, primarily reflected in the ϕ_2 values), allowance should also be made for structural changes in the enzyme resulting from interactions with the hydrocarbon chain of the alcohols. It is possible that our results demonstrated in Fig. 6 are an expression of such a behaviour. Differences in the reaction with the enzyme are, however, also noticeable in the range of the long-chain alcohols at high concentrations. Their extent depends on the chain length, and they may naturally have an additional effect on the kinetic parameters. In our studies of enzyme reactions in emulsions and suspensions of longer-chain alcohols, we found an inhibiting effect on the enzyme for hexanol already in true solution, and for decanol in emulsion, but virtually no inhibition for tetradecanol suspensions (Schöpp & Rothe, 1975). It should be possible therefore to limit undesirable side-effects to a minimum, solely through investigation at low substrate concentrations. This should also represent more realistically the effect of the enzyme *in vivo*. One factor not to be overlooked here is probably that the enzyme concentration is also significant in the interpretation of the results. Since the specific activity of the enzyme with respect to ethanol is much higher than that with respect to the long-chain alcohols, the enzyme concentration is always considerably lower in kinetic studies of ethanol than in studies of long-chain alcohols. In view of the fact that the activity of the enzyme is greatly decreased at high dilution, differences between the reaction mechanisms of ethanol and the long-chain alcohols may be attributable to this. The

stability-enhancing effect of the addition of albumin or a peptide, such as glutathione, on yeast alcohol dehydrogenase may also be explained in terms of the inhibition of dissociation or conformation changes in the enzyme when highly diluted. As a result, all values obtained at relatively high enzyme concentrations seem to be more realistic. This applies to all experiments with alcohols with more than 5 carbon atoms. This leads to the conclusion that yeast alcohol dehydrogenase is capable of oxidizing all primary alcohols with chain-lengths between 2 and 10 carbon atoms according to a rapid-equilibrium random-order mechanism, as suggested by Nygaard & Theorell (1955) and Mahler & Douglas (1957).

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References

- Brooks, R. L. & Shore, J. D. (1971) *Biochemistry* **10**, 3855-3858
- Dalziel, K. (1957) *Acta Chem. Scand.* **11**, 1706-1723
- Dalziel, K. (1958) *Trans. Faraday Soc.* **54**, 1247-1253
- Dickinson, F. M. (1970) *Biochem. J.* **120**, 821-830
- Dickinson, F. M. (1972) *Biochem. J.* **126**, 133-138
- Dickinson, F. M. & Monger, G. P. (1973) *Biochem. J.* **131**, 261-270
- Franke, R. (1970) *Acta Biol. Med. Germ.* **25**, 757-788
- Hayes, J. E. & Velick, S. F. (1954) *J. Biol. Chem.* **207**, 225-244
- Mahler, H. R. & Douglas, J. (1957) *J. Am. Chem. Soc.* **79**, 1159-1166
- Nygaard, A. P. & Theorell, H. (1955) *Acta Chem. Scand.* **9**, 1300-1305
- Pettersson, G. (1972) *Biochim. Biophys. Acta* **276**, 1-11
- Racker, E. (1950) *J. Biol. Chem.* **184**, 313-320
- Reynolds, J., Herbert, S. & Steinhardt, J. (1968) *Biochemistry* **7**, 1357-1361
- Schöpp, W. (1973) *Acta Biol. Med. Germ.* **31**, 29-38
- Schöpp, W. & Aurich, H. (1973) *Acta Biol. Med. Germ.* **31**, 19-28
- Schöpp, W. & Aurich, H. (1974a) *Acta Biol. Med. Germ.* **32**, 585-591
- Schöpp, W. & Aurich, H. (1974b) *Abstr. FEBS Meet. 9th, Budapest*, p. 41
- Schöpp, W. & Rothe, U. (1975) *Acta Biol. Med. Germ.* **34**, 197-201
- Silverstein, E. & Boyer, P. D. (1964) *J. Biol. Chem.* **239**, 3908-3914
- Sund, H. & Theorell, H. (1963) *Enzymes*, 2nd edn., **7**, 25-83
- Takemori, S., Furuya, E., Suzuki, H. & Katagiri, M. (1967) *Nature (London)* **215**, 417-418
- Wratten, C. C. & Cleland, W. W. (1963) *Biochemistry* **2**, 935-941
- Yamada, T. & Yamoto, M. (1973) *J. Biochem. (Tokyo)* **74**, 971-984