ON THE TEMPERATURE DEPENDENCE AND MECHANISM OF ACTION OF ALCOHOL DEHYDROGENASE

by

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INTRODUCTION

Enzymes are catalytically active proteins; they exert forces on substrates with which they are in contact to facilitate the reaction between them. For an understanding of the catalytical activity, it is therefore necessary to distinguish between two aspects —the binding of the substrates and their activation once they are attached to the enzyme. In the following chapters the kinetics of alcohol dehydrogenase (ADH) from yeast is studied from this point of view.

The rate of a reaction often follows a law of the Michaelis-Menten type:

$$v = v_0 \frac{1}{1 + \frac{K}{c}} \qquad (1)$$

where c is the concentration of the substrate, and K the Michaelis constant. If the reaction of the substrates at the enzyme surface is rate limiting, and not for instance the dissociation of the products, v_0 is this limiting rate for the enzyme saturated with substrate. In many instances this is not the case (ADH from animal tissues[1], succinic acid dehydrogenase[2]). However, in ADH from yeast the dissociation constants between enzyme and the substrates were measured directly by HAYES AND VELICK[3] with the ultracentrifugal method, and were found to agree fairly well with the Michaelis constants from the reaction kinetics; thus we may consider both identical and take v_0 for the turnover number. Studies on competitive inhibition and competitive reaction with two kinds of alcohol have been made and were found to support this assumption. The kinetics of ADH from yeast give K and v_0 values which describe the attachment between enzyme and substrate, and the activation of the substrate once it is attached.

These quantities are in themselves of little theoretical significance unless reduced to physical terms, the energy and entropy of activation and association. In these terms one obtains:

$$v_0 = n \cdot \frac{kT}{h} \cdot e^{\frac{s}{k} - \frac{q}{kT}} \qquad (2a) \qquad K = K_0 e^{\frac{S}{k} - \frac{Q}{kT}} \qquad (2b)$$

(k = Boltzmann's constant, T = absolute temperature, h = Planck constant, and s and S entropies, q and Q energies of activation and association). v_0 is the number of substrate molecules catalyzed per enzyme molecule and second for the enzyme saturated with substrates. n is the number of active

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References p. 121.

sites per enzyme molecule which is 4 for ADH[3]. kT/h is the frequency factor according to the theory of EYRING[4] and is of the order of molecular vibrations ($0.6 \cdot 10^{13}$ sec^{-1}). The activation entropy thus refers to the activation of a single substrate molecule and accounts for the change in randomness of molecular structure and positions in course of the activation. For K_0, we choose the concentration of the substrate in the pure condensed state. Standard entropies may be obtained by adding $k \ln K_0$ to this association entropy (Table I). The sign of S and Q is defined to make Q positive; Q is thus the energy liberated on association.

To get the energies and entropies separately, one has to use the temperature dependence of the v_0 and K. Their logarithms, plotted against the reciprocal of absolute temperature, should give straight lines with the slope q/k or Q/k and the corresponding entropies can then be calculated with the help of eq. (2). The reaction catalyzed by ADH is given, within the pH range of these experiments, by the equation:

$$CH_3CH_2OH + DPN^+ \rightleftharpoons CH_3CHO + DPNH + H^+ \qquad (3)$$

In the following experiments, the activation and association energies for the reaction in both directions and with all substrates are measured. The values for K are obtained by plotting the reciprocal of the rate $1/v$ against $1/c$ of the particular substrate with constant concentration of the other substrate:

$$\frac{1}{v} = \frac{1}{v_0}\left(1 + \frac{K_1}{c_1}\right)\left(1 + \frac{K_2}{c_2}\right) \qquad (4)$$

v_0 is obtained by the extrapolation to high substrate concentrations. The rates v are initial rates, obtained by extrapolating to the beginning of the reaction where no inhibiting effects by the products and inactivating effects occur. The complicating effects of the H^+ ion in eq. (3) will be considered below.

EXPERIMENTAL PROCEDURE

The rate of the reaction is followed with a Beckman spectrophotometer. This is easily done since DPNH has an absorption band at 340 mμ where DPN has none.

The temperature is regulated by circulating water from a water bath through coils on both sides of the cell compartment in the Beckman and through a mixing apparatus[5] with two syringes, one of which contains the enzyme and the other the substrates. At the moment of mixing, the temperature is therefore already adjusted so that the extrapolation to initial rate is easily possible. The enzyme is used in its pure form (Worthington products). It is diluted to get a stock solution from which further dilutions are made immediately before each series of measurements. There is a decrease in activity during each series of measurements, which is checked by measuring a test solution of substrates with the same enzyme solution before and after the series; corrections are applied to take this decrease into account. These corrected rates, extrapolated to initial velocity, are used to determine the turnover numbers and Michaelis constants.

The experiments were carried out in 0.05 M phosphate buffer. 1% glycine was added to the enzyme solutions to prevent denaturation by heavy metal ions. The water used was double distilled.

The dehydrogenation of the alcohol is strongly inhibited by the aldehyde that is produced in the reaction. To prevent this inhibition, 0.02 M semicarbazide is added to the reaction mixture of this reaction so that the aldehyde is removed.

EXPERIMENTAL RESULTS

To measure the temperature dependence of the turnover numbers and Michaelis constants, for each substrate three or four solutions with different concentrations of these substrates were measured. Fig. 1 shows as an example the results for DPN^+. The reciprocal of the rate is plotted against the reciprocal of the concentration; the rate is given in relative values so that it is 1 for $1/c = 0$. According to eq. (4), the slope of the curve is the Michaelis constant. It is seen to increase with increasing temperature. In Fig. 2 this constant is plotted logarithmically against the reciprocal of temperature. The slope is proportional to the energy of association according to eq. (2b). Fig. 3 represents a

References p. 121.

measurement for alcohol. With the help of these Michaelis constants, the turnover numbers v_0 are extrapolated (eq. 4). Fig. 4 shows their logarithm, plotted against the reciprocal of temperature. The slope is proportional to the energy of activation. Fig. 5 gives the temperature dependence of the Michaelis constant for butyl alcohol. The entropies of association and activation are then calculated with eq. (2) on the basis of a molecular weight of 150,000 and four active sites for each enzyme molecule.

The results thus obtained are collected in Table I. In the first column is indicated the substrate, the concentration of which is varied. The data of association refer to this particular substrate. Most of the measurements have been carried out at pH 7.8. To check for possible variations with pH, data are given for pH around 6.3, mostly based on measurements at two temperatures (3 and 20° C). At temperatures above 30° C the instability of the enzyme made measurements rather uncertain.

The experimental errors with regard to the energies of association and activation are estimated, in view of the several extrapolations that are necessary for each value, to be about 1 kcal/mol, the errors in the entropies 4 cal/mol degree. Further, the interpretation of K and v_0 as the true dissociation constants and turnover numbers may be subjected to some corrections. Thus, the energies themselves may be dependent on the temperature, the enzyme may show some degree of self-association at low temperatures, the pH influence may contribute to the temperature dependence, the correspondence between dissociation and Michaelis constants may not be quantitative so that other steps in the reaction contribute to a smaller degree to v_0, and the buffer solution may affect the reaction.

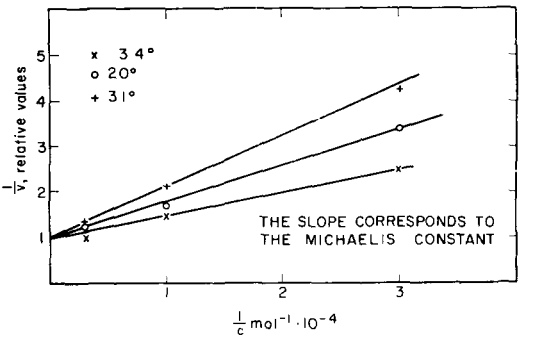

Fig. 1. Dependence of the Michaelis constant of DPN$^+$ on temperature. $1/v$ is plotted in relative values ($1/v = 1$ for $1/c = 0$) against $1/c$. The slope is the Michaelis constant.

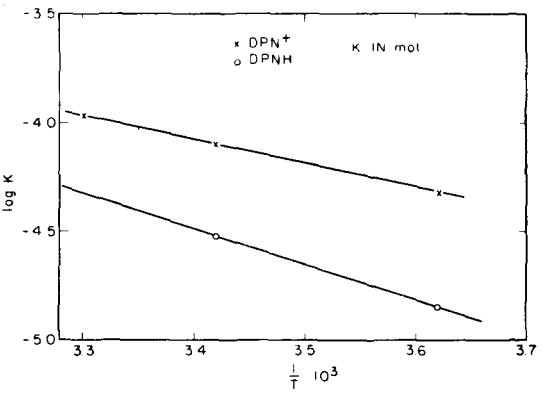

Fig. 2. Michaelis constants of DPN$^+$ and DPNH plotted logarithmically against $1/T$. The slope is proportional to the association energy (eq. 2). Association energies; DPN$^+$: 5 kcal/mol; DPNH: 7 kcal/mol.

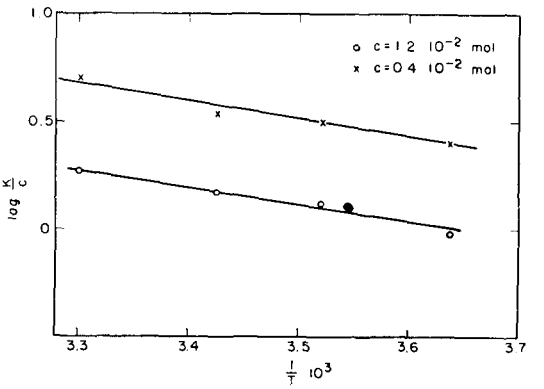

Fig. 3. Michaelis constant for alcohol at two different concentrations, plotted logarithmically against $1/T$. The slope is proportional to the association energy. Association energy; 3.8 kcal/mol.

References p. 121.

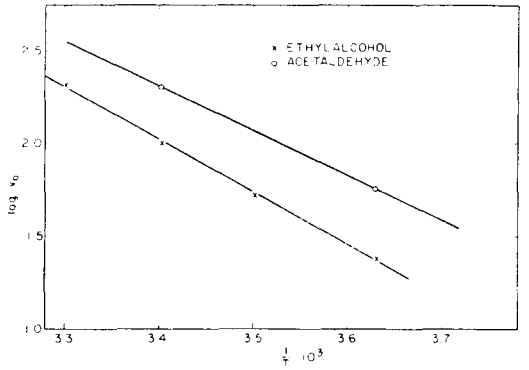

Fig. 4. Turnover number v_0, per second and enzyme site, of the dehydrogenation of alcohol and the hydrogenation of aldehyde, plotted logarithmically against $1/T$. The slopes are proportional to the energies of activation. Activation energies; Ethylalcohol: 14 kcal/mol; Acetaldehyde: 12.5 kcal/mol.

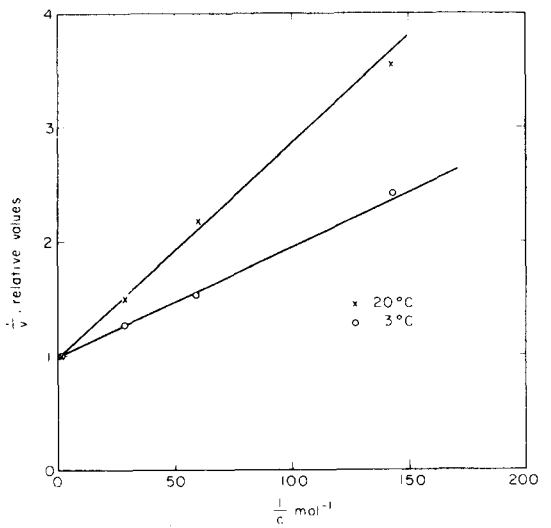

Fig. 5. Michaelis constant for butyl alcohol at two temperatures (see Fig. 1).

The pH influence on the energies and entropies is not found to be large. A theory for this influence (see the discussion) indicates that the true turnover numbers and association energies are obtained at high pH for the alcohol, at low pH for the aldehyde reaction. The other corrections may somewhat effect the quantities in the table, but will probably not be large enough to affect the general results.

To check the results for ADH, the temperature dependence of the equilibrium constant was measured. It corresponds to an energy of reaction of 6 kcal/mol. The equilibrium constant is given by

$$\frac{c_{CH_3CHO}\, c_{DPNH}}{c_{CH_3CH_2OH}\, c_{DPN^+}} = \frac{v_0 \cdot K_{CH_3CHO} \cdot K_{DPNH}}{v_0' \cdot K_{CH_3CH_2OH} \cdot K_{DPN^+}} \qquad (5)$$

Therefore the energy of reaction should be

$$-Q = q' - q + (Q_{CH_3CH_2OH} - Q_{CH_3CHO}) + (Q_{DPN^+} - Q_{DPNH}) \qquad (6)$$

With the values from Table I, one obtained 4 ± 3 kcal/mol. The correspondence is satisfactory in view of the seven independently measured quantities involved, but indicates again that all values are uncertain within about a kcal. These general results from Table I may be summarized as follows:

1. The energies of activation are around 14 kcal/mol.

2. The entropies of activation are very small, their contribution to the free energy of activation, $T \cdot s$, is smaller than 1 kcal/mol.

3. The energies of association are between 3 and 8 kcal/mol; that is, within the range of intermolecular forces.

4. The entropies of association are small in most cases ($T \cdot s < 1$ cal/mol) if they are related to the concentration of the substrate in the pure condensed state as standard concentration (eq. 2b).

For comparison, some results for lactic acid dehydrogenase are included in the table. For LDH, no measurements of the dissociation constants are available until now, so that their correspondence with the Michaelis constants is not established. Both the variation of the rates with different substrates[6] and the pH dependence[6] resembles, however, the action of ADH from yeast rather than that from animal tissues, and it

References p. 121.

TABLE I*

Reaction	*pH*	*Enzyme*	*Michaelis constant* 20° C	*Assoc. energy* kcal mol	*Assoc. entropy* cal mol°	*Turnover number* 20° C; sec⁻¹	*Activation energy* kcal mol	*Activation entropy* cal mol°
DPN^+ (+ Ethanol)	7.8	ADH	$0.8 \cdot 10^{-4}$	5	— 2.3	$4 \cdot 10^2$	14	— 2
Ethanol (+ DPN^+)	7.8	ADH	0.016	4	— 0.4			
DPNH (+ Acetaldehyde)	7.8	ADH	$3 \cdot 10^{-5}$	7	+ 2.3	$8 \cdot 10^2$	12.5	— 6
Acetaldehyde (+ DPNH)	7.8	ADH	$3 \cdot 10^{-4}$	5	— 5.0			
DPN^+ (+ Ethanol)	6.3	ADH	10^{-4}	5	— 1.8	$1.5 \cdot 10^2$	14.5	— 2
Ethanol (+ DPN^+)	6.3	ADH	0.038	4	+ 1.4			
DPNH (+ Acetaldehyde)	6.3	ADH	$3 \cdot 10^{-5}$	7	+ 2.3	$9 \cdot 10^2$	13.5	— 2
Acetaldehyde (+ DPNH)	6.3	ADH	10^{-4}	5	— 7.5			
Butanol (+ DPN^+)	7.7	ADH	0.018	5	+ 4.0	40	14.5	— 5
Amylalcohol (+ DPN^+)	7.7	ADH	0.04	6	+ 9.0	15	13.5	— 9
Pyruvate (+ DPNH)	7.7	LDH	$5 \cdot 10^{-4}$	10	+ 14.0	$5 \cdot 10^2$	14	+ 2
Pyruvate (+ DPNH)	6.3	LDH	$1 \cdot 10^{-4}$	7	— 1.0	$5 \cdot 10^2$	14	+ 2

* For definition of energies and entropies see eq. (2).

is probable therefore that also in LDH v_0 is the true turnover number. Indeed, the results of the measurements on the temperature dependence show the same pattern as for ADH, particularly the low entropies of activation, and it is not unlikely that also their interpretation should be similar as in the case of ADH (see discussion).

In Table II, the variation of turnover numbers and Michaelis constants for the reaction of ADH with pH are collected. It is seen that v_0 and K_{CH_3CHO} increases, while $K_{CH_3CH_2OH}$ decreases with increasing pH. The variation of K_{DPN^+} and K_{DPNH} seems not to be great. It is significant that the turnover number of the reverse reaction v_0' varies only slightly in this pH range.

TABLE II

pH-DEPENDENCE OF ALCOHOL DEHYDROGENASE AT 20° C

	Reaction with alcohol:	
pH = 7.65	v_0 = (1)	$K_{CH_3CH_2OH}$ = $1.5 \cdot 10^{-2}$ mol
6.9	0.56	$3.3 \cdot 10^{-2}$ mol
6.0	0.31	$4.0 \cdot 10^{-2}$ mol
	Reaction with aldehyde	
pH = 5.9	v_0' = (1)	K_{CH_3CHO} = $1 \cdot 10^{-4}$ mol
6.9	0.9	$2 \cdot 10^{-4}$ mol
7.9	0.9	$3 \cdot 10^{-4}$ mol
8.4	0.9	—

In Table III, the rate for different substrates of ADH is listed. All values are for high substrate concentrations, so that they may be considered as turnover numbers. There is a strong decrease of the rate with increasing chain length of higher homologs of alcohols, which has been observed before[7]. Very low rates are found for diols. Studies of competitive reaction between a substrate of low rate and ethyl alcohol have been carried out. If a substrate with low rate is added to a solution of ethyl alcohol, one would expect a decrease of the rate when compared with the rate of the pure alcohol solution. The effect has been found to exist in the expected order of magnitude for the higher

References p. 121.

homologs of alcohol. (Very high concentrations inhibit even stronger, as one would expect from their Michaelis constants; the nature of this effect is not clear.) Diols are, however, found to be weak inhibitors. The active sites of the enzymes are therefore even at high diol concentrations only incompletely covered by the substrates. Whether this indicates that the four active sites of ADH are different is not clear.

TABLE III

REACTIONS WITH HOMOLOGUES

Initial rates at 20° C, pH 7.7

Alcohol	*Molarity*	*Relative rate*
1,4-Butanediol	0.8	8
1,3-Butanediol	0.7	90
1,3-Propanediol	0.06	8
1,2-Propyleneglycoll	0.70	6
1,4-Butanediol	0.047	6
1,3-Butanediol	0.047	45
tert.-Amylalcohol	0.20	0
*iso*Butylalcohol	0.28	8
*iso*Propylalcohol	0.55	130
Heptylalcohol	0.012	100
Amylalcohol	0.08	120
n-Butylalcohol	0.32	260

One may conclude from the measurements of the variation with the substrates that homologs of alcohol have similar Michaelis constants as ethyl alcohol, but the turnover number decreases strongly with increasing length of the aliphatic chain. The measurements with diols indicate that a second hydroxyl group in this chain lowers either the association or the activation of the substrate.

DISCUSSION

Enzyme catalysis is most probably brought about by a combination of two effects:

(a) Binding of the substrates to the enzyme in relative positions that are favorable for the reaction and

(b) Activation of the shift of atoms that constitutes the reaction. The binding effect (a) cannot be the only effect of the enzyme. At concentrations of alcohol and DPN^+ which are high enough so that about 10^{-8} mol must always be in a proper state of attachment by reason of statistical collisions, no measurable reaction occurs within days, while 10^{-8} mol of enzyme catalyze a rapid reaction. The enzyme must therefore exert, aside from the binding effect (a) an activating effect (b). The mostly intermolecular forces that bind the substrate to the protein can either cause a straining of bonds within the substrate that facilitates the reaction[8], or lower the energy barrier by electric forces.

A. *Activation and association of the substrates*

The results of the temperature studies have shown that the entropies of activation and association for both directions of the ADH reaction are very low. To draw the conclusions with regard to enzyme mechanism, it is useful to separate mechanisms into two groups. In one of them the enzyme molecule is assumed to undergo major structural

References p. 121.

changes in the course of the reaction, whereas in the other the enzyme remains rather rigid. The first assumption has recently been made by EYRING AND LUMRY[9] on the basis of an analogy between enzyme action and reversible denaturation of proteins caused by small organic molecules. Such "conformation changes" are accompanied by large entropy changes; in many enzyme reactions, however, the entropies are much smaller. In the particular case of ADH the very small entropy of activation is not compatible with major structural changes of the enzyme molecule. Since the entropies of activation are small in both directions of the reaction and for the association of both the substrates and the products, there is no possibility in any step of the process for a major entropy term to occur. It seems very unlikely that within one step (association or activation) large entropy changes cancel each other. The low entropy changes therefore indicate strongly that the enzyme remains rather rigid in course of the reaction. Eventually some side chains of the protein to which the substrates are attached change their configuration, but the protein as a whole will not change its structure.

The next question arises with regard to the nature of the activating step. Probably the shift of atoms from a substrate molecule is influenced by groups of the enzymic protein with which the substrates are in direct contact. For such an activation the substrate must be attached with several of its groups to the protein in positions determined within fractions of an A. In this way, one arrives at the hypothesis that the main reaction is preceded by a rigid, crystal-like attachment of the substrates to the enzyme surface and consists only of a simple shift of the transferred atoms (for instance hydrogen) without major rearrangements of the positions of the rest of the molecule. Thus, not only the structure of the protein, but also the mode of attachment of the substrates should not undergo major structural changes. If the activation follows such a simple pattern without intermediate steps, the entropies of activation should be very small and this has been found to be the case for ADH.

With regard to the association, the problem is more difficult. The association of a substrate is a complicated replacement reaction of water molecules from the surfaces of both the enzyme and the substrates, and a crystal-like attachment does not necessarily imply that the entropies of association are low, particularly if ionized groups are involved. The low entropies of association are thus to be regarded as a rule rather than a general law. There is, however, a semi-empirical approach to the problem on the basis of the analogy to crystallization which is applied here to alcohol and may prove useful for other enzymic substrates. An alcohol crystal binds an alcohol molecule rather rigidly and specifically, and will have a similar surface structure of the binding site as that of the enzyme. In aqueous solution the terms for the replaced water molecules should therefore be approximately the same, and one would expect a similar entropy of association to the enzyme and entropy of crystallization out of an aqueous solution of alcohol to a (hypothetical) alcohol crystal. The latter term can be calculated on the basis of thermodynamic data available in the literature and is found to be fairly small (-3 cal/degree) and in agreement with the low entropy of association to the enzyme. This supports the hypothesis of crystal-like attachment of the substrates to the enzyme.

B. *The nature of the activating step*

The last section provided evidence that the activation of the dehydrogenation occurs in an enzyme substrate complex that undergoes no major structural changes. It is therefore presumably due to the effect of forces between the enzyme and the substrates

References p. 121.

in the rather small contacting area. To investigate the nature of these effects the variation of quantities other than the temperature must be studied, such as the pH value and the substrate.

(a) *Variation of pH.* Much progress with regard to the mechanism of hydrogen transfer has recently been made by the investigations of WESTHEIMER *et al.*[10] with the use of deuterium. In the reaction

$$CH_3CH_2OH + DPN^+ \longrightarrow CH_3CHO + DPNH + H^+ \quad (3)$$

the hydrogen from the α carbon atom of the alcohol was shown to be transferred directly to the DPN^+. It is interesting to follow the path of the other hydrogen form of the hydroxyl group, which finally ends up in the solution as H^+ ion. It cannot be followed by the isotopic method since it exchanges rapidly with the solution. The kinetics with regard to the H^+ ion concentration should give information on its path. The problem is complicated by the fact that the H^+ ion is not only a product of the reaction (and a substrate in the reverse reaction), but may also influence other groups of the enzyme that affect its activity. It is assumed that such effects do not change the general pattern of the theory based on the treatment of the H^+ ion as a product of the reaction, but may well affect somewhat the quantitive aspects of such a theory.

If the proton should go from the OH group of the alcohol into the solution directly, it would come directly out of the solution in the reverse reaction. The turnover number of the reverse reaction v_0' should therefore be proportional to the H^+ ion concentration, while all Michaelis constants and v_0 should be independent of pH. As shown in Table II, in the pH range 6–8 exactly the opposite is the case: while v_0' varies very little in this range, the Michaelis constants of alcohol and aldehyde and v_0 are strongly affected. (Little variation is observed for DPN and DPNH.) Thus, the proton does not go into the solution directly, but is accepted, and, in the reverse reaction, donated by the enzyme-substrates complex.

Since the Michaelis constants of DPN and DPNH are not strongly dependent on pH, the H^+ ion must be accepted by a site of the enzyme rather than the coenzymes. Evidently the association of alcohol or aldehyde to this site of the enzyme will be strongly dependent on whether the H^+ ion is dissociated from it or not. This accounts for the pH dependence of the respective Michaelis constants. Therefore, the H^+ ion cannot be dealt with by introducing for it a Michaelis constant as a product of the reverse reaction. The treatment is more involved and will be presented elsewhere[11]. We obtain from the slopes of the v and K the dissociation constant of the enzyme and this H^+ ion; it is about 10^{-7} mol. At this concentration the H^+ ion is available at half of the enzyme molecules, whereas by mere statistical collisions derived from the solution it would be available at this site only in about the 10^{-8} part of all enzyme molecules. This site thus donates the proton to increase the rate by a factor up to 10^{8*}; accordingly, in the dehydrogenation the proton is extracted by this site of the enzyme to increase the rate by this factor compared with water molecules as acceptors. Thus the enzyme provides in a proper position for the reaction a polar group that acts in a way analogous to general acid base catalysis[12]. It constitutes probably the strongest accelerating effect of the enzyme and makes it quite obvious why the reaction cannot go without the enzyme even at very high substrate concentrations.

* The factor is somewhat reduced by indirect influences of the polar group on the v_0 and K. The experience from acid base catalysis would be in favour of a factor between 10^4 and 10^8 [11].

References p. 121.

(b) *Variation of the substrates.* The specificity of the reaction shows that the catalytic effect depends not only on the H–O–C–H group. In particular, there is a strong decrease in the rate for the higher homologs of ethyl alcohol; in addition methyl alcohol shows a rather low rate. Other catalysts such as the sulphate[13] ion and heterogenous metal oxide catalysis[14] do not show this strong effect, which is therefore to be attributed to a special effect on the aliphatic chain of the enzymic protein. It has been suggested that this aliphatic chain, by covering active sites of the protein exerts a sort of self inhibition. Also, it replaces water molecules from the protein surface and thus gives rise to forces that influence the reaction.

Whether or not these influences are strong, there is an additional effect that will contribute to the specific activation. It may have a more general significance since it is an example of activation by the straining of chemical bonds that has been suggested many times for enzyme mechanisms[8].

In the course of the dehydrogenation of ethyl alcohol the carbon atom that is dehydrogenated changes the bond configuration from the tetrahedral to the planar one[15]. The relative positions of the O, C, and CH_3 groups are thus changed. WESTHEIMER[10] has shown that the hydrogen atom that is transferred to DPN^+ is transferred in a stereospecific way. It follows that the alcohol is bound with at least three points to the enzyme, presumably the groups with O, C, and CH_3. These groups, and in particular the weakly bound CH_3 group, therefore have to change their position relative to the enzyme surface. The change depends on the direction of hydrogen transfer, that is, on the site of the DPN^+, and will be a fraction of an A. The activation will, of course, be dependent on the potential energy of this CH_3 group during the shift according to the interaction with the enzyme surface, and the latter should be so adapted to facilitate the shift by "proper straining" of the CH_3 group with a minimum of potential energy in some configuration between the tetrahedal and the planar one. The low rate of methyl alcohol would be the rate without this effect, which may thus be estimated to accelerate by a factor up to 10^2. Higher chain length in homologs would have a hindrance effect since the strained $-CH_2$-group is fixed by the rest of the chain. The complicated effects of a second hydroxyl group in diols on the association and activation requires further studies and will be discussed elsewhere.

C. *Conclusions on the mechanism of ADH action*

The previous analysis of the activation and association of the substrates of ADH together with the studies of WESTHEIMER on hydrogen transfer and of VELICK on association, make the following description of the enzyme mechanism the most probable:

The enzyme undergoes no major structural changes in its catalytical action. To its surface, the substrates (*e.g.* ethylalcohol and DPN^+) are attached in a rather rigid, crystal-like manner, probably by intermolecular forces. The enzyme then activates the shift of the two hydrogen atoms. The polar hydrogen atom of the alcohol is shifted in the form of a hydrogen ion over a hydrogen bridge to a basic group of the enzyme. The provision of this basic group accelerates the rate by a factor of the order of 10^4–10^8 when compared with water molecules as acceptors. The other hydrogen goes over directly to the DPN^+ while it is attached to the enzyme. In course of this shift, the carbon atom changes its bond configuration, which causes a shift of the position of the methyl group in the alcohol with respect to the enzyme surface. It is probable that this shift is supported by forces from the enzyme that strain the $C–CH_3$ bond.

References p. 121.

It seems likely that proper binding, bond straining and proton extraction (or donation) constitute the main catalytic effect of the enzyme. Its surface provides a specific combination of relatively simple effects that have often been proposed for the explanation of catalytic activity. Thus, ADH provides probably a more simple example of enzyme mechanisms than those enzymes that change their structure during the reaction.

SUMMARY

The temperature dependence of reactions catalyzed by alcohol dehydrogenase is measured. The activation energies are about 14 kcal/mol. The entropies of activation and association of both the substrates and the products are small. It is concluded that the enzyme does not undergo major structural changes in the course of the reaction, and that the substrates are attached in a rather rigid, crystal-like manner to the enzyme surface that activates the hydrogen transfer.

The dependence of the kinetical data on pH suggests that this activation is partially brought about by hydrogen transfer over a hydrogen bridge between the alcohol and a basic group of the enzyme. This shift accelerates the reaction in analogy to general acid base catalysis by a factor 10^4–10^8. The dependence of the rate on the structure of the substrate indicates that the enzyme affects the methyl group of the alcohol. It is suggested that the enzyme activates, by a straining of the $C-CH_3$ bond, the shift of the position of the methyl group that accompanies the change in the bond configuration in the alcohol molecule during the reaction.

It seems that the influences of binding, bond straining and proton shifting by groups of the enzyme surface constitute the main catalytic effects of the enzyme.

RÉSUMÉ

L'influence de la température sur les réactions catalysées par la déhydrogenase alcoolique a été mesurée. Les énergies d'activation sont à peu près 14 kcal/mole. Les entropies d'activation et d'association des substrata et des produits de la réaction sont petites. On en conclu que l'enzyme ne subit pas de large changements structuraux, et que les substrata sont attachés à la surface de l'enzyme, qui active le transport de l'hydrogène, d'une façon plutôt rigide, à la maniere d'un cristal.

La manière dont la cinétique de la réaction dépend du pH, suggère que cette activation est en partie due à un transport d'hydrogène, par un pont d'hydrogène entre l'alcool et un groupe basique de l'enzyme. Le transport accélère la réaction par un facteur de 10^4–10^8 d'une manière analogue à la catalyse acido-basique en général. La vitesse de la réaction depend de la structure du substratum, indiquant que l'enzyme a une action sur les groupes méthyliques de l'alcool. On suggère que, par une tension de la liaison $C-CH_3$, l'enzyme cause un changement de position des groupes méthyliques, qui accompagne le changement de configuration de la molécule d'alcool pendant la réaction.

Il semble que l'attachement du substratum, la tension ou la déformation de la liaison chimique, et le transport d'un proton par des groupes de la surface enzymatique, constituent les effets essentiels de l'action catalytique de l'enzyme.

ZUSAMMENFASSUNG

Die Temperaturabhängigkeit der enzymatischen Dehydrogenierung von Alkohol mit ADH wird untersucht. Die Aktivierungsenergien sind etwa 14 kcal/mol, während die Aktivierungs und Assoziationsentropien der Substrate und Produkte der Reaktion sehr klein sind. Daraus ist zu folgern, dass das Enzym seine Struktur im Verlaufe der Reaktion nicht stark ändert, und dass die Substrate, ähnlich wie bei der Kristallisation, ziemlich fest an das Enzym gebunden sind, wenn dieses die Wasserstoffübertragung aktiviert.

Die Abhängigkeit der kinetischen Grössen vom pH Wert weist darauf hin, dass die Aktivierung teilweise auf der Übertragung eines Wasserstoffions über eine Wasserstoffbrücke zwischen dem Alkohol und Enzymmolekül beruht. Dies beschleunigt die Reaktion nach der Art der allgemeinen Säure-Base-Katalyse um einen Faktor von etwa 10^4–10^8. Die Substratabhängigkeit der Reaktionsgeschwindigkeit zeigt eine Beeinflussung der Methylgruppe des Alkohols durch das Enzym. Vermutlich biegt das Enzym die $C-CH_3$ Gruppe, sodass die Änderung der Konfiguration des Alkohols im Verlauf der Reaktion aktiviert wird.

Es scheint, dass die Bindung der Substrate, das Biegen oder Strecken von Bindungen und die Übertragung vonProtonen den Hauptteil der katalytischen Wirkung des Enzyms ausmachen.

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