YEAST ALCOHOL DEHYDROGENASE

I. THE EFFECT OF PYRIDINE DERIVATIVES ON THE REACTION*

JAN VAN EYS AND NATHAN O. KAPLAN

The McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Md. (U.S.A.)

The reaction catalyzed by yeast ADH** is identical with that catalyzed by horse liver ADH. The binding of DPNH by the two enzymes appears to be different, since a spectral shift of the 340 mμ band of DPNH can be observed when the enzyme-coenzyme complex is formed with the mammalian enzyme[2] and not with the yeast dehydrogenase. A binding of DPN and DPNH by the yeast enzyme can, however, be observed by the ultracentrifugal separation technique of HAYES AND VELICK[3]. Furthermore, a detailed study of the kinetics of the reaction catalyzed by the enzyme gave results compatible with a reaction scheme in which the enzyme-coenzyme complexes are intermediates[4,5].

From direct measurement and determination of the Michaelis-Menten constants for DPN and DPNH, it can be inferred that the binding of DPNH is stronger than that of DPN with yeast ADH[3]. A direct competition between the two forms of the coenzyme, can, however be demonstrated[3]; this indicates that the same site may be involved in the binding of both DPN and DPNH.

We have observed that pyridine derivatives are inhibitors of the reaction catalyzed by yeast ADH. The inhibiting pyridine derivatives studied fall into three categories: (a) free pyridine bases; (b) N^1-methylpyridinium salts; (c) analogues of DPN. This inhibition of pyridine nucleotide dependent dehydrogenases has been observed in isolated instances: on lactic dehydrogenase[6], glucose dehydrogenase[6,7], and malic dehydrogenase[8].

The pyridine derivatives do not act on yeast ADH as simple competitive type inhibitors for DPN, but they can, in some instances, influence the oxidation of alcohol to a different extent than they can the reduction of acetaldehyde, and also at low concentrations they can increase the rate of reaction.

These results and their significance in interpreting the mechanism of action and the nature of coenzyme binding by yeast ADH will be described in this paper.

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** Abbreviations used in this paper are: ADH: alcohol dehydrogenase; DPN and DPNH: oxidized and reduced diphosphopyridine nucleotide; beta-picoline DPN, pyridine DPN, and ethyl nicotinate DPN: oxidized analogue of DPN of beta-picoline, pyridine, and ethyl nicotinate, respectively; APDPN and APDPNH: oxidized and reduced analogues of DPN of acetyl pyridine; Py-3AlDPN and Py-3AlDPNH: oxidized and reduced analogue of DPN of pyridine-3-aldehyde respectively; TRIS: tris-(hydroxymethyl)aminomethane.

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MATERIALS AND METHODS

Yeast ADH was a commercial preparation* used without recrystallization. A fresh dilution was made just prior to use. Rates were determined by following the change in absorption at 340 mμ in the Beckman spectrophotometer. For DPN reduction ethanol was used at a final concentration of 0.5 *M*; for DPNH oxidation acetaldehyde was used at a final concentration of 0.07 *M*. These compounds represented in all cases minimally a 1000 fold excess of substrate over coenzyme.

The enzyme concentration was adjusted so that the reaction remained approximately zero order for the first 90 seconds. Rates were defined as the change in optical density at 340 mμ in the 15 to 30 second interval after starting the reaction with enzyme.

The substituted pyridines were commercial preparations. Acetyl pyridine was redistilled prior to use; all others were used without prior purification.

The N^1-methylpyridine derivatives were prepared from methyl iodide and the corresponding pyridine compounds in the usual manner**. When the inhibition of the methylpyridinium iodide compounds was tested, an equivalent amount of potassium iodide was included in the control reaction mixture. No significant inhibition by potassium iodide at the concentrations employed was ever observed.

The analogues of DPN were prepared from DPN, using the appropriate pyridine compound, by the action of pig brain DPNase, as described for the *iso*nicotinic acid hydrazide and acetyl pyridine analogues of DPN[9,10]. The only significant contaminant in the analogue preparations was the alpha isomer of DPN[11]. The pure alpha isomer, however, up to a 50 fold excess of that present in the most heavily contaminated analogue preparations had no effect on the reactions catalyzed by yeast ADH.

DPNH, APDPNH and Py-3AlDPNH were prepared enzymically as described previously[12].

The values of the p*Ka* of the pyridine derivatives used were taken from the data of JAFFÉ AND DOAK[13]. Values not reported by JAFFÉ AND DOAK were estimated from the sigma value[14] of the substituent. The sigma values were those compiled by JAFFÉ[15].

RESULTS

Free pyridine bases

The inhibition of free pyridine bases was discovered with β-picoline as an inhibitor. But it was soon found that the inhibition was not peculiar to this compound, but rather, that all free pyridine bases tested exhibited this property. The degree of inhibition was markedly dependent upon the pH of the reaction mixture. As an illustration; at a given concentration in pyrophosphate buffer beta-picoline gave the following inhibition at pH 9.6: 13.4%; at pH 8.6: 29.1%; at pH 8.3: 56.4%.

This same pH dependence was shown by other pyridine derivatives, though the relation between pH and inhibitory action was different for the different compounds. For this reason a series of different pyridine derivatives were tested in their effect on the reduction of DPN and compared on the basis of their basicity. It was obvious that, with the exception of pyridine-3-sulfonic acid, the inhibition was proportional to the p*Ka* of the ring nitrogen. This is illustrated in Fig. 1. This startling relationship at once pointed toward the importance of the pyridine ring nitrogen for the inhibition.

The inhibition of pyridine derivatives, at least for the more strongly basic ones, appeared to be truly competitive, as determined in a conventional LINEWEAVER-BURK plot[16].

It could be observed that the inhibition was dependent on the coenzyme used. When DPN was used as a coenzyme, the inhibition by beta-picoline was much less than when APDPN was used as a coenzyme. 10^{-3} *M* beta-picoline inhibited coenzyme reduction 20% in the case of DPN and 45% in the case of APDPN at the concentration of coenzyme of $1.5 \cdot 10^{-4}$ *M*. Although the picoline inhibition is much different, the Michaelis-Menten constants for the two nucleotides, determined in the usual

* Worthington Biochemical Corporation.

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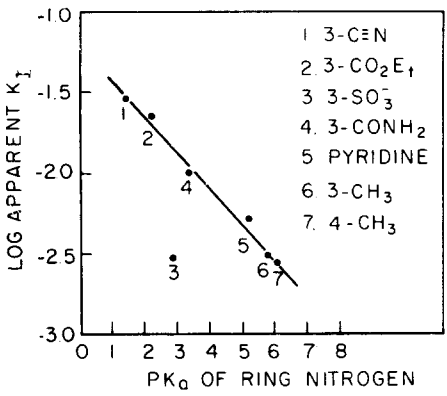

Fig. 1. The relationship between the pKa of the ring nitrogen and the inhibitory power of pyridine bases. The word apparent K_I is used since only at relatively high concentrations is a true competitive inhibition observed. The K_I values were determined in the usual manner from a LINEWEAVER BURK plot[16]:

$$1/v = \left(1 + \frac{1}{K_I}\right) \cdot K_m/V_{max} \cdot 1/\text{DPN} + 1/V_{max}.$$

manner, are quite close: for DPN the K_m was determined to be $4 \cdot 10^{-4}$ moles/liter, for APDPN the K_m was found to be $2 \cdot 10^{-4}$ moles/liter.

N'-methylpyridine derivatives

Since the ring nitrogen of pyridine compounds appeared to be of importance in inhibiting yeast ADH the corresponding N^1-methyl derivatives were tested for their inhibitory capacity. Here it became clear that the degree of inhibition was related to the substituent on the ring. The inhibition, however, was not in the order of the free pyridine bases but approximately in the reverse order. This order gave a series in which the inhibitory action was greater, the stronger the electronegativity of the side chain.

The inhibition was, at least for some members, competitive with DPN. The order of inhibition of the series of N^1-methylpyridinium ions was the same as that of the pyridine bases when the concentration of the bases was expressed as the amount of pyridinium ion present§. This made it appear likely that the inhibiting species was the

TABLE I

COMPARISON OF INHIBITION BY PYRIDINE DERIVATIVES

The figures are based on a reaction mixture consisting of DPN $1.3 \cdot 10^{-4}$ M, ethanol 0.5 M, Tris buffer 0.05 M, pH 9.3

Substituent	*pK_a pyridine base*	*Concentration required for 50% inhibition*		
		Pyridine derivatives		*N^1-methyl-pyridinium derivatives (molar)*
		Total base (molar)	*pyridinium ion (molar)**	
4-CH_3	6.11	0.020	$1.3 \cdot 10^{-5}$	**
3-CH_3	5.82	0.040	$1.3 \cdot 10^{-5}$	$5.8 \cdot 10^{-3}$
none	5.27	0.070	$6.6 \cdot 10^{-6}$	$5.5 \cdot 10^{-3}$
3-$CONH_2$	3.40	0.23	$2.9 \cdot 10^{-7}$	$5.0 \cdot 10^{-3}$
3-$COCH_3$	3.39	***	***	$4.0 \cdot 10^{-3}$
3-CHO	3.37	***	***	$4.2 \cdot 10^{-3}$
3-$CO_2C_2H_5$	2.24	0.30	$2.6 \cdot 10^{-8}$	$3.6 \cdot 10^{-3}$
3-CN	1.45	0.60	$8.5 \cdot 10^{-9}$	$3.2 \cdot 10^{-3}$
3-SO_3^-	2.9	0.013	$5.1 \cdot 10^{-9}$	**

* These figures are calculated from the previous column as indicated below§.

** Compound not available.

*** The absorption at 340 mμ of solutions of these compounds was so high that no reliable values could be obtained. For the same reason the figures for 3-amino-pyridine and 3-hydroxypyridine and the corresponding N^1-methyl derivatives were not included in the table. However, the values were in the range expected from their pKa.

§ Calculated from the Henderson-Hasselbach equation, assuming the amount of unionized pyridine compound equal to the total amount present. At pH 9.3 this approximation introduced an error of less than 1 part in 10^5 maximally.

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pyridinium ion rather than the unionized bases. As a consequence the N^1-methyl derivatives were chemically the stronger inhibitors, but the pyridinium ions were far more potent than the corresponding N^1-methylpyridinium ions (see Table I). This can be illustrated by comparing the 3-cyanopyridine derivatives: the amount of inhibition given by N^1-methyl-3-cyanopyridinium iodide in the concentration of $5 \cdot 10^{-3}$ *M* required about 0.1 *M* 3-cyanopyridine, which at pH 9.3 corresponded to a concentration of 3-cyanopyridinium ion of 10^{-9} *M*.

DPN analogues

A similar series could not be derived for the DPN analogues since the only analogue inhibiting DPN reduction was the beta-picoline analogue of DPN. The pyridine analogue and the ethyl nicotinate analogue of DPN had little or no effect on the rate, while APDPN and Py-3AlDPN will actually substitute for DPN in the reaction of yeast ADH[10,17]. The inhibition by the beta-picoline analogue was again competitive with DPN.

The effect of pyridine derivatives on DPNH oxidation

When the various compounds were tested for their inhibition of the reverse reaction, *i.e.*, DPNH oxidation by acetaldehyde, an unexpected result was obtained. It was found that some of the inhibitors affected the reverse rate much less than the forward reaction, even though the concentration of pyridinium ions was far greater. This was especially true for the stronger bases in the free pyridine series: gamma- and beta-picoline. In contrast the pyridine analogue of DPN, which has little effect on the oxidation of ethanol, was a strong inhibitor of the reduction of acetaldehyde (see Fig. 2). This pointed to a qualitative difference in the binding of DPN and DPNH apart from the known quantitative difference[3].

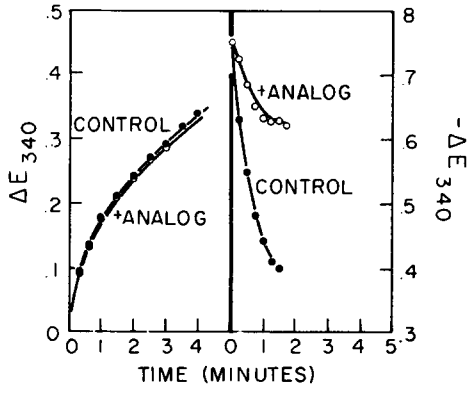

Fig. 2. The effect of pyridine DPN on the forward and backward reaction of yeast ADH. DPNH concentration $0.15 \cdot 10^{-3}$ *M*, analogue concentration in the forward reaction $5 \cdot 10^{-3}$ *M*, in the backward reaction $2 \cdot 10^{-4}$ *M*. Forward reaction TRIS buffer pH 9.3, backward reaction phosphate buffer pH 7.8.

Activation by pyridine derivatives

The effect of those pyridine derivatives which inhibited relatively strongly the reduction of acetaldehyde, such as nicotinic acid and nicotinamide, could activate DPN reduction, when the concentrations of the compounds were lowered (Fig. 3). This activation was dependent on the DPN concentration used (Fig. 4).

The above cited observations make necessary the following conclusions with regard to the coenzyme binding and the reaction sequence: (a) The binding of DPN and DPNH is not only quantitatively but also qualitatively different. (b) The binding of the coenzymes involves a negatively charged grouping since pyridinium ions are strongly inhibitory. This binding site is less important for DPNH binding, since strong

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bases are relatively poor inhibitors of DPNH oxidation. (c) A potentially positive site is involved in the coenzyme binding since the inhibitors with a strong electronegative side chain are more effective than the ones with weak electronegative side chains. This site is relatively more important for DPNH binding than for DPN binding since it takes a strongly negative grouping on pyridine derivatives for them to be inhibitors for the reduction of acetaldehyde.

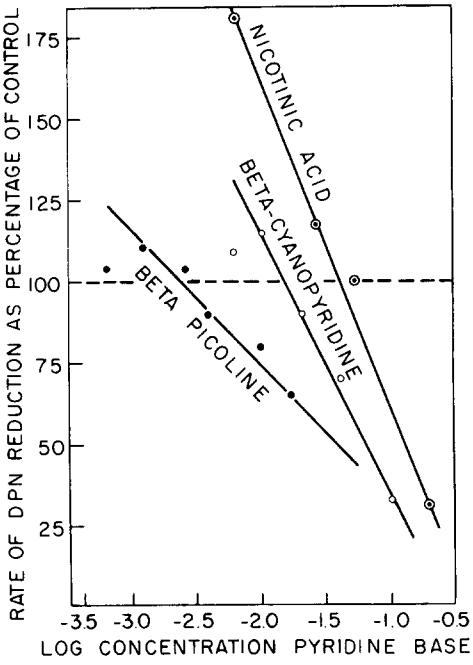

Fig. 3. The effect of pyridine derivatives on the relative rate of yeast ADH in the presence and absence of inhibitor. TRIS buffer pH 9.3. DPN concentration $0.15 \cdot 10^{-3}$ *M*. The concentration of the pyridine compounds is expressed as total amount added.

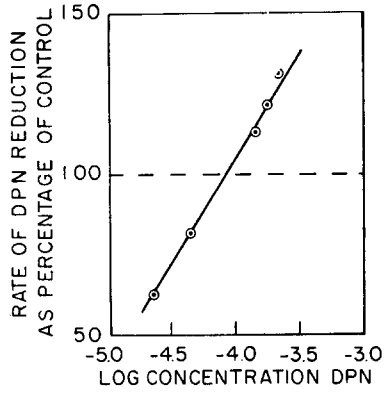

Fig. 4. The effect of increasing amounts of DPN on the relative rate of DPN reduction in the absence and presence of nicotinic acid. Concentration of sodium nicotinate 0.04 *M*. To the control was added 0.04 *M* NaCl. TRIS buffer pH 9.3.

THEORETICAL

To explain the increase in the rate of reaction promoted by some of the pyridine derivatives, the following reaction sequence has been considered.

$$E\text{-DPN} + \text{alcohol} \rightleftharpoons E\text{-DPNH} + \text{acetaldehyde} + H^+ \quad (1)$$

$$E\text{-DPNH} + \text{DPN} \rightleftharpoons E\text{-DPN} + \text{DPNH} \quad (2)$$

where step (2) is the rate-limiting step*.

This second reaction does not involve transhydrogenation[18], but rather a direct competition between DPN and DPNH for the enzyme.

In the presence of a pyridinium ion the sequence of events would be:

$$E\text{-DPN} + \text{alcohol} \rightleftharpoons E\text{-DPNH} + \text{acetaldehyde} + H^+ \quad (3)$$

$$E\text{-DPNH} + \text{pyridine} \rightleftharpoons E\text{-pyridine} + \text{DPNH} \quad (4)$$

$$E\text{-pyridine} + \text{DPN} \rightleftharpoons E\text{-DPN} + \text{pyridine} \quad (5)$$

If we take the normal reaction, assuming all n sites on the enzyme to be equal and independent, we can write the equilibrium:

$$(E\text{-DPN}) = K_c[(E_t) - (E\text{-DPN})][(\text{DPN})/(\text{DPNH})] \quad (6)$$

* In the circumstance that ethanol is not present in very large excess, this is not the case, and one of the reactions in step (1) becomes the actual rate-limiting step.

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where E-DPN stands for the concentration of sites on the enzyme occupied by DPN and E_t stands for the total number of sites on the enzyme. In the steady state the rate will be proportional to the left side of equation (6) since the rate is directly proportional to E-DPN. When a pyridine ion (Py) is present, we have two competing equilibria:

$$K' = (E\text{-Py})(\text{DPNH})/(E\text{-DPNH})\,(\text{Py}) \tag{7}$$

and

$$K'' = (E\text{-DPN})(\text{Py})/(E\text{-Py})(\text{DPN}) \tag{8}$$

where E-Py, E-DPNH and E-DPN stand for the concentration of the sites on the enzyme occupied by the pyridinium ion, by DPNH and by DPN, respectively.

Solving equation (7) for Py and substituting the solution in (8) we obtain

$$(E\text{-DPN}) = K'K''\,(E\text{-DPNH})\,[(\text{DPN})/(\text{DPNH})] \tag{9}$$

which can be rewritten by substituting E-DPNH as:

$$(E\text{-DPN}) = K'K''\,[(E_t) - (E\text{-DPN})]\,[(\text{DPN})/(\text{DPNH})] - K'K''(E\text{-Py})\cdot[(\text{DPN})/(\text{DPNH})] \tag{10}$$

The relative rate in the presence and absence of the pyridinium ion, then, will be the result of dividing equations (10) and (6). This results in equation (11):

$$V_r = (K'K''/K_c)\left[1 - \frac{(E\text{-Py})}{(E_t) - (E\text{-DPN})}\right] \tag{11}$$

Since the DPNH concentration is relatively negligible in the period of zero order kinetics, which is under investigation, we may substitute for E-Py the appropriate solution of equation (8), yielding equation (12). In this solution, it must be considered that at a given enzyme concentration, during zero order kinetics E-DPN and E_t are constants:

$$V_r = K'K''/K_c\,[1 - C(\text{Py})/(\text{DPN})] \tag{12}$$

The constant C has then the value

$$C = (E\text{-DPN})/[(E_t) - (E\text{-DPN})]K''$$

(DPN) stands for the initial DPN concentration.

It is obvious that when $K'K''/K_c > 1$ the relative rate can be greater than one. The relative rate will be inversely proportional to the concentration of pyridinium ion and directly proportional to the concentration of DPN. This is in agreement with the data shown in Figs. 3 and 4. The proportionality is, however, not directly to Py but to the logarithm of Py. The significance of this discrepancy will be discussed in the following paper[19].

DISCUSSION

It has been assumed in the derivation above that all sites on the enzyme act completely autonomously so that kinetically the enzyme concentration is equivalent to the concentration of active sites. That the sites are, at least with respect to coenzyme binding, independent, has been shown by the work of HAYES AND VELICK[3]. For a general picture of the enzyme action, however, the number of sites is not important, and it is only necessary to assume that there exists a direct competition between DPN and DPNH, and that the rate of regeneration of the enzyme-DPN complex is the rate-limiting step.

For the explanation of the action of the pyridinium compounds, a specialized picture of the *mode* of binding by the enzyme is required, a picture in which DPN and DPNH are bound by different mechanisms. The work of BARRON[20], when recalculated for the now accepted molecular weight of the enzyme[3], shows that two sulfhydryl groups are taken up per mole of DPN bound. Four moles of DPN are bound per mole of enzyme. Furthermore, the work of VALLEE AND HOCH[21] has established the presence of four moles of zinc per mole of enzyme.

On the basis of the above information the binding of DPN can be pictured as in

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Fig. 5. One sulfhydryl group is linked through the amino group of DPN. Corroborating evidence is found in the diminished activity of deamino DPN as compared to DPN[12], and in the decreased stability of the enzyme in the presence of deamino DPN*. The zinc is pictured as linked through the pyrophosphate bond. The second sulfhydryl is attached to the pyridinium nitrogen. The greater positive charge of DPN as compared to DPNH lowers the affinity of the pyrophosphate grouping of DPN for the zinc. Furthermore, it appears from Pauling-Corey models of nicotinamide mononucleotide that a strong hydrogen bonding exists between the carboxamide grouping of the nicotinamide moiety and the phosphate**. Such an interaction in the DPN would again materially weaken the pyrophosphate bond. This hydrogen bonding is absent in the reduced nucleotide since the angle of the plane of the nicotinamide ring to the ribose ring is altered in converting the nitrogen atom from a quaternary pyridinium nitrogen to a tertiary nitrogen.

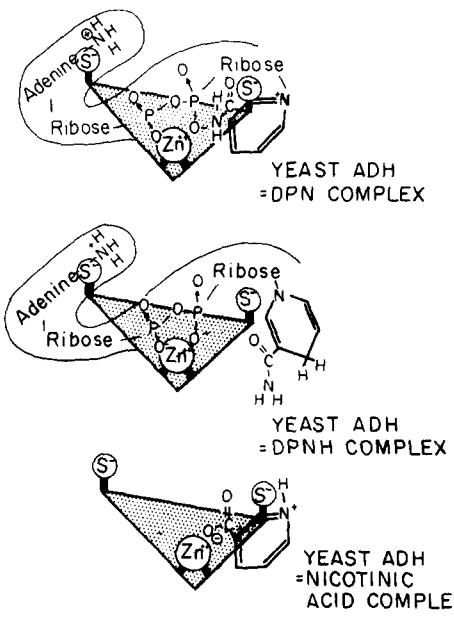

Fig. 5. The binding of DPN, DPNH, and nicotinic acid by yeast ADH. See text for details.

Thus DPN is relatively weakly bound through the pyrophosphate, but strongly bound through the pyridinium grouping. DPNH is strongly bound through the pyrophosphate, and little, if at all, through the nitrogen of the reduced pyridine ring.

The net effect is that DPNH is bound more strongly to yeast ADH than is DPN, especially because of the high affinity of the pyrophosphate in DPNH for the zinc.

Nicotinic acid, which will compete for both the zinc and the sulfhydryl grouping may easily displace the pyrophosphate grouping of DPN. On the other hand, the pyridinium bond of DPN should be broken only with difficulty. In the case of DPNH, the sulfhydryl grouping is easily competed with, while the pyrophosphate group should be quite resistant to action of nicotinic acid.

The free pyridine type compounds compete apparently solely for the sulfhydryl grouping, and hence have a greater effect on the oxidized form of DPN. The pyridine analogue of DPN has no hydrogen bonding and will attack the zinc more strongly than will DPN; however, the sulfhydryl grouping will be attacked with the same activity of DPN. DPN competes with the pyridine analogue at both sites. On the other hand, DPNH has effective competition only at the pyrophosphate site, and would therefore be more inhibited by the analogue.

Finally, the stronger the interaction is between a side chain of a given DPN analogue and the first phosphate the weaker is the zinc-pyrophosphate linkage. This appears to be the explanation for the greater inhibition of beta-picoline on APDPN, than on DPN since APDPN has an apparent stronger hydrogen bond.

* J. VAN EYS, M. M. CIOTTI AND N. O. KAPLAN, to be published.
** N. O. KAPLAN AND L. GROSSMAN, unpublished observations.

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SUMMARY

The effect of pyridine derivatives on yeast alcohol dehydrogenase has been described. These compounds have been found to compete with DPN or DPNH for the active site on the enzyme. Three classes of compounds have been studied: pyridine bases, N^1-methylpyridinium salts, and analogues of DPN.

A number of pyridine compounds have been found to show inhibitory powers different for the DPN reduction than for the DPNH oxidation. The inhibition of pyridine bases appears to be due to their ionized forms.

Although pyridine bases inhibit the yeast alcohol dehydrogenase reaction, some of these compounds could actually, at low concentrations, stimulate the rate of reaction.

A partial reaction sequence and an enzyme-coenzyme model are proposed to explain the experimental data.

REFERENCES

1 J. VAN EYS, *Federation Proc.*, 15 (1956) 374.
2 H. THEORELL AND R. K. BONNICHSEN, *Acta Chem. Scand.*, 5 (1951) 1105.
3 J. E. HAYES, JR., AND S. F. VELICK, *J. Biol. Chem.*, 207 (1954) 225.
4 A. P. NYGAARD AND H. THEORELL, *Acta Chem. Scand.*, 9 (1955) 1300.
5 A. P. NYGAARD AND H. THEORELL, *Acta Chem. Scand.*, 9 (1955) 1551.
6 H. VON EULER, *Ber. deut. Chem. Ges.*, 75 (1942) 1876.
7 N. G. BRINK, *Acta Chem. Scand.*, 7 (1953) 1090.
8 P. FEIGELSON, J. N. WILLIAMS, JR. AND C. A. ELVEHJEM, *J. Biol. Chem.*, 198 (1951) 361.
9 L. J. ZATMAN, N. O. KAPLAN, S. P. COLOWICK AND M. M. CIOTTI, *J. Biol. Chem.*, 209 (1954) 453.
10 N. O. KAPLAN AND M. M. CIOTTI, *J. Biol. Chem.*, 221 (1956) 823.
11 N. O. KAPLAN, M. M. CIOTTI, F. E. STOLZENBACH AND N. R. BACHUR, *J. Am. Chem. Soc.*, 77 (1955) 815.
12 M. E. PULLMAN, S. P. COLOWICK AND N. O. KAPLAN, *J. Biol. Chem.*, 194 (1952) 593.
13 H. H. JAFFÉ AND G. O. DOAK, *J. Am. Chem. Soc.*, 77 (1955) 4441.
14 L. P. HAMMETT, *Physical Organic Chemistry*, 1940, McGraw-Hill Book Co., New York, Chapter VII.
15 H. H. JAFFÉ, *Chem. Revs.*, 53 (1953) 191.
16 H. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.
17 N. O. KAPLAN, M. M. CIOTTI AND F. E. STOLZENBACH, *J. Biol. Chem.*, 221 (1956) 833.
18 N. O. KAPLAN, S. P. COLOWICK, L. J. ZATMAN AND M. M. CIOTTI, *J. Biol. Chem.*, 205 (1953) 31.
19 J. VAN EYS, M. M. CIOTTI AND N. O. KAPLAN, *Biochim. Biophys. Acta*, 23 (1957) 581.
20 E. S. GUZMAN BARRON AND S. LEVINE, *Arch. Biochem. Biophys.*, 41 (1952) 175.
21 B. L. VALLEE AND F. L. HOCH, *J. Am. Chem. Soc.*, 77 (1955) 821.

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YEAST ALCOHOL DEHYDROGENASE*

II. PROPERTIES OF THE CATALYTICALLY ACTIVE SITE*

JAN VAN EYS, MARGARET M. CIOTTI AND NATHAN O. KAPLAN

The McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Md. (U.S.A.)

In the previous paper a reaction scheme and a model of the enzyme-coenzyme complex of yeast alcohol dehydrogenase** were proposed[1]. In the development of the theoretical rate in the presence and absence of a pyridinium ion, it was assumed that

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** Abbreviations used in this paper are: ADH: alcohol dehydrogenase; DPN and DPNH: oxidized and reduced diphosphopyridine nucleotide, respectively; Py-3AlDPN and Py-3AlDPNH: oxidized and reduced analogs of DPN of pyridine-3-aldehyde, respectively; pCMB: *para*-chloromercuribenzoate.

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