A HYDROPHOBIC REGION AT THE ACTIVE SITE OF YEAST ALCOHOL DEHYDROGENASE[1]

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Many compounds structurally analogous to nicotinamide adenine dinucleotide (NAD^+) have been demonstrated to be competitive inhibitors of NAD^+ in reactions catalyzed by yeast alcohol dehydrogenase (Van Eys and Kaplan, 1957; Wallenfels et al., 1957; Anderson and Kaplan, 1958; Anderson et al., 1963). Inhibition by a series of pyridine bases in these reactions was attributed to the protonated forms of these compounds as evidenced by an observed linear relationship between the logarithm of inhibitor constants and the pK_a' for the various ring nitrogens involved (Van Eys and Kaplan, 1957). The corresponding N^1-methylpyridinium derivatives were likewise shown to be inhibitors of yeast alcohol dehydrogenase and in several cases competitive inhibition with respect to NAD^+ was observed. In other studies (Hoch et al., 1960), inhibition of yeast alcohol dehydrogenase by commercially obtained N^1-methylnicotinamide chloride was reported as an inhibition which was non-competitive with respect to NAD^+ and ethanol. In this case, the inhibition was shown to be dependent upon enzyme concentration and was attributed to the presence of silver ions in the N^1-methylnicotinamide chloride preparation.

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The results of the present investigation indicate that N^1-alkylnicotinamide chlorides can competitively inhibit reactions of yeast alcohol dehydrogenase and the effectiveness of these inhibitors increases with increasing chain length of the alkyl substituent.

N^1-alkylnicotinamide chlorides containing as alkyl substituents the methyl, propyl, amyl, heptyl, octyl, decyl, and lauryl groups were prepared according to Karrer and Stare (1937). These compounds were each recrystallized five times from appropriate solvents. Carbon, hydrogen and nitrogen analyses of these compounds compared extremely well with theoretical values. The functioning of these compounds as inhibitors of yeast alcohol dehydrogenase was studied at 25° in 3 ml reaction mixtures containing 0.01M sodium pyrophosphate buffer, pH 7.85, 0.1M ethanol, NAD^+ in concentrations ranging from 1.6×10^{-5}M to 1.6×10^{-4}M, and 1.3 μg of twice crystallized enzyme (Worthington Biochemical Corporation). The reactions were initiated by addition of enzyme, and initial velocities were obtained by measuring the change in optical density at 340 mμ. Each N^1-alkylnicotinamide chloride was studied at two different concentrations and velocities were obtained at six concentrations of NAD^+ with and without inhibitor present. Actual concentrations of inhibitors used varied due to the variation in effectiveness of the different alkyl derivatives. The data obtained, when plotted according to Lineweaver and Burk (1934), demonstrated that the N^1-methyl, propyl, amyl and heptyl nicotinamide chlorides are competitive inhibitors with respect to NAD^+ in these reactions. A mixed inhibition was observed in studies of the octyl, decyl and lauryl derivatives. Inhibitor constants (K_I) determined from these plots varied from 6.51×10^{-2}M for the N^1-methyl derivative to 3.65×10^{-4}M for the N^1-lauryl derivative. A linear relationship between the logarithm of the reciprocal of the K_I and the number of carbons of the alkyl substituent was observed in the region of five to twelve carbons (Figure 1). The change in free energy for the binding of these inhib-

itors to the enzyme was calculated as a function of chain length and a value of 0.39 kcal/mole per methylene group was obtained. This value is consistent with an enhancement of binding through dispersion forces (Webb, 1963). The competitive nature of this relationship would indicate the presence of a hydrophobic region at the active site of yeast alcohol dehydrogenase which could greatly influence the binding of pyridine nucleotides. The divergent values of the N^1-methyl and N^1-propyl derivatives indicate the need for four or more carbons in the alkyl group for the non-polar effect to be exhibited. It appears that the hydrophobic influence of the smaller methyl and propyl groups is obviated by the close proximity of the positively charged ring nitrogen.

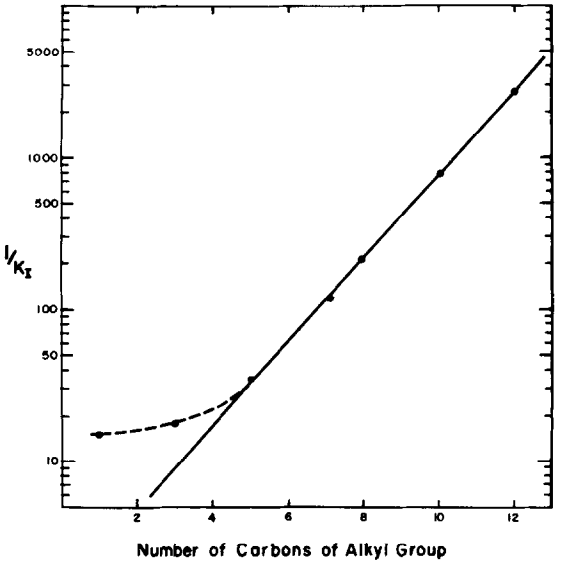

Fig. 1. The relationship of inhibition to the increase in non-polarity of the inhibitor. $1/K_I$ is expressed as molarity^{-1}

The inhibition of yeast alcohol dehydrogenase by the N^1-propyl, N^1-amyl, N^1-lauryl and N^1-decyl derivatives was studied as a function of ethanol concentration ranging from 8×10^{-3}M to 5×10^{-2}M. The reaction mixtures were prepared as described above with the exception that the

NAD^+ concentration was held constant at 1.3×10^{-3}M. The results of these studies indicate these inhibitors to be non-competitive with respect to ethanol.

Thionicotinamide adenine dinucleotide ($TNAD^+$) functions as a hydrogen acceptor in yeast alcohol dehydrogenase reactions, however, the maximum velocity for the reduction of this analogue is 1/200 the maximum velocity for the reduction of NAD^+ under the same conditions (Anderson et al., 1963). Inhibition of $TNAD^+$ reduction by N^1-lauryl nicotinamide chloride was studied under conditions described above for the NAD^+ reactions. It was necessary to use 200-times the enzyme concentration used in the NAD^+ studies in order that comparable initial velocities be obtained. As in the NAD^+ studies two different N^1-lauryl nicotinamide chloride concentrations were used (1.67×10^{-4}M and 3.33×10^{-4}M) and six concentrations of $TNAD^+$ ranging from 2.7×10^{-5}M to 1.35×10^{-4}M were studied. The reactions were followed at 398 mμ, the absorption maximum of reduced $TNAD^+$ (Stein et al., 1963). The inhibition of $TNAD^+$ reduction by the N^1-lauryl derivative in these reactions was demonstrated to be competitive with respect to $TNAD^+$ and the K_I was calculated to be 3.21×10^{-4}M. This value compares favorably to the K_I value (3.65×10^{-4}M) obtained for this inhibitor in the studies of NAD^+ reduction. It is of interest that very little change in K_I was observed although a 200-fold difference in enzyme concentration was employed.

The more effective longer chain N^1-alkyl derivatives were studied as possible hydrogen acceptors in the yeast alcohol dehydrogenase reactions. No reduction of the N^1-octyl, decyl and lauryl derivatives was observed in a two hour period using high concentrations of the enzyme.

The N^1-alkylnicotinamide chlorides are presently being studied as inhibitors of other pyridine nucleotide-requiring enzymes to investigate the possibility that dispersion forces can be of general significance in the enzyme binding of pyridine nucleotides.

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