ROLE OF α-NADH FOR MICROSOMAL ETHANOL OXIDATION

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SUMMARY

α-NADH* was found to serve as electron donor for microsomal ethanol oxidation of rat liver. Almost no ethanol oxidation was observed with β-NADH. The α-NADH-dependent ethanol oxidation was almost completely inhibited by 0.1 mM cyanide or azide and strongly abolished in the presence of formate. α-NADH-dependent ethanol oxidation was increased by 1 mM SKF-525A, an inhibitor of microsomal mixed-function oxidase, to about 200%.

These results suggested that hydrogen peroxide generated from α-NADH and molecular oxygen in microsomes might be a prerequisite step in the over-all reaction, eventually leading to the peroxidatic ethanol oxidation by catalase to acetaldehyde.

Since the discovery by Orme-Johnson and Ziegler (3) of a microsomal alcohol oxidation, considerable work has been carried out to elucidate the nature of alcohol-oxidizing system in microsomes. However, the existence of a unique microsomal alcohol-oxidizing system has recently become a subject of controversy. Isselbacher and Carter (4) and Thurman et al. (5) report that in addition to the peroxidatic reaction of catalase, there is no evidence for the existence of a separate and distinct alcohol-oxidizing system in liver microsomes. Recently, evidence has been presented by us for the existence of microsomal enzyme system(s) catalyzing the oxidation of α-NADH (1,2).

In this communication, we wish to report a possible partici-

* Reduced form of α-isomer of nicotinamide adenine dinucleotide (1,2).

pation of α-NADH in the ethanol oxidation of rat liver microsomes.

MATERIALS and METHODS

Male Wistar rats weighing from 180g to 220g, which had been maintained on commercial complete diet, were used. After homogenization of liver in KCl (0.15 M), microsomes were obtained by centrifugation according to the previous method (6), except that the 9,000 x g centrifugation lasted 30 min for removing the contamination of lysosomes and microbodies to microsomal fraction (7). α-NADH was prepared as previously described (1,2) and also purchased from Sigma. β-NADH and NADPH were products of Sigma. SKF-525A (2-Diethylaminoethyl-2,2-Diphenylvalerate, HCl) was purchased from Smith Kline & French Labs., Philadelphia. Microsomal ethanol-oxidizing activity was determined by measuring the production of acetaldehyde semicarbazone (7).

RESULTS

NADPH has been thought to be the only factor to serve as an electron donor for alcohol oxidation in microsomes. In the present study, it was shown that α-NADH has also a property involved in the alcohol-oxidizing system. As indicated in Table 1, α-NADH was about 15% as efficient as NADPH. There was an absolute requirement for oxygen. Replacement of air by nitrogen virtually abolished the activity.

When α-NADH was used as electron donor, the reaction product was identified as α-NAD^+ by the following criteria (1,2); (a) ion-exchange chromatography on Dowex-1 column, (b) absorption spectrum with cyanide, (c) inactivity as a coenzyme of lactate dehydrogenase, and (d) inability to serve as substrate for a Neurospora NAD glycohydrolase. β-NADH was only 2% as efficient as NADPH.

TABLE 1

Cofactor requirement for microsomal ethanol oxidation.

Addition	Acetaldehyde formed
None	0.02
NADPH	16.2
α-NADH	2.4
β-NADH	0.32
$NADP^+$	0
α-NAD^+	0
β-NAD^+	0.09

The reaction mixture (total volume 2.0 ml) contained 80 mM potassium phosphate buffer, pH 7.2, 50 mM ethanol, microsomes (0.6-6.0 mg protein), and pyridine nucleotide (0.3 mM) indicated. Incubations were carried out at 37° for 10 min in sealed center well flasks previously gassed with air. Center wells contained 0.6 ml of 15 mM semicarbazide. Results are mean of duplicate determinations and expressed as nmole per min per mg of microsomal protein.

$NADP^+$, α-NAD^+ and β-NAD^+ all were almost completely inactive.

Both cyanide and azide react with the catalase-H_2O_2 complex to form an inactive compound (8). Addition of cyanide (0.1 mM) and azide (0.1 mM) to the assay medium markedly reduced the activity of ethanol oxidation with α-NADH (Fig. 1), whereas cyanide and azide, at a concentration (0.1 mM) which almost completely abolishes catalase activity (7,9), inhibited NADPH-dependent ethanol-oxidizing activity only slightly (10-20%).

The ethanol oxidation with α-NADH was increased 2-fold in the presence of SKF-525A (Fig. 1), an inhibitor of microsomal mixed-function oxidation processes (10). This is a result quite different from that of rather slight inhibitory effect of SKF-525A on the ethanol oxidation with NADPH.

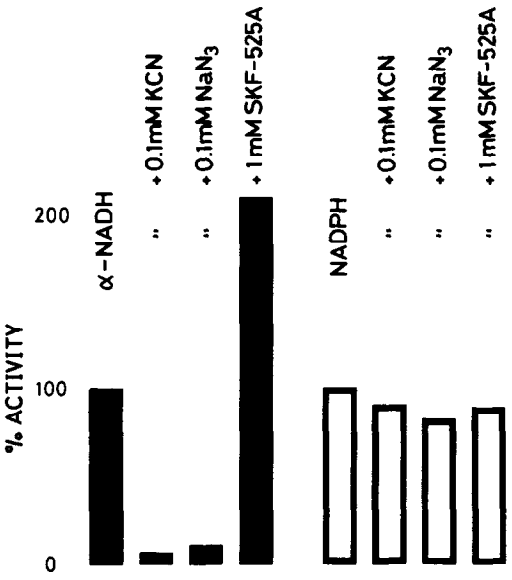

Fig. 1.

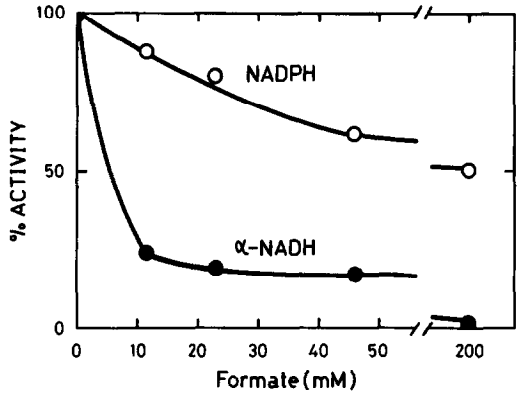

Fig. 2.

Fig. 1. Comparative effects of cyanide, azide and SKF-525A on α-NADH- and NADPH-dependent ethanol oxidation.
Conditions as in Table 1, supplemented with inhibitors at the concentration indicated. The control activities (100%) of α-NADH- and NADPH-dependent ethanol oxidation were 2.1 and 16.0 nmoles of acetaldehyde produced per min per mg of microsomal protein, respectively.

Fig. 2. Effect of concentration of potassium formate on α-NADH- and NADPH-dependent ethanol oxidation.
Conditions as in Table 1.

Formate is a typical peroxidatic substrate for catalase (11). In the presence of potassium formate, as shown in Fig. 2, the ethanol oxidation with α-NADH was more strongly suppressed than that with NADPH.

DISCUSSION

The present work shows that α-NADH can serve as an electron donor for microsomal ethanol oxidation. The α-NADH-dependent ethanol oxidation may be due to a hydrogen peroxide formation

from microsomal α-NADH oxidase system and subsequent peroxidation of ethanol by catalase. Repeated washings of microsomes did not remove completely catalase activity (12), indicating that the microsomes, regardless of the degree of contamination by microbodies, may contain some amount of catalase as an inherent constituent of microsomes. Since hydrogen peroxide per se added to microsomes failed to support the oxidation of ethanol (3,5), whether a continuous supply of hydrogen peroxide or an active species of oxygen such as superoxide anion, $O_2^{\cdot -}$, is effective for the ethanol oxidation remains to be elucidated.

The α-NADH-dependent system seems to be different from the NADPH-dependent ethanol oxidation system described by Lieber and DeCarli (7), because the α-NADH-dependent ethanol oxidation was almost completely inhibited by cyanide or azide and markedly suppressed in the presence of formate. Furthermore, it is of particular interest that the α-NADH-dependent ethanol oxidation was increased by SKF-525A. These results suggest that α-NADH-dependent ethanol oxidation is different not only from the conventional microsomal mixed-function oxidase system but also from the NADPH-dependent peroxidatic ethanol oxidation postulated by Isselbacher and Carter (4) and Thurman et al. (5). The mechanism of increase by SKF-525A remains obscure. However, we are inclined to favor the possibility that the compound may act upon the lipoidal membrane of microsomes, thereby not impeding the access of α-NADH to membrane enzymes.

Although Kaplan et al. (13) have succeeded in isolating α-NAD from liver and yeast and we have already shown that α-NADH is oxidized by several enzyme systems of mammalian origin (1,2), there has so far been no available information concerning the biological significance of α-NAD. The present study provides

evidence that under a certain condition α-NADH can serve as electron donor for some microsomal oxidation system associated with physiological process.

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