NICOTINAMIDE DEAMIDASE IN RAT LIVER AND THE BIOSYNTHESIS OF NAD.

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The biosynthesis of NAD from nicotinic acid has been described by Preiss and Handler (10,11), who showed that nicotinic acid mononucleotide and nicotinic acid-adenine dinucleotide are intermediates in this conversion. Nicotinamide is also a precursor of liver NAD, but the mechanism for this conversion has remained obscure. Recent studies indicate that nicotinamide is deamidated and then reamidated during the synthesis of the NAD molecule (7,9). However, as the authors (7,9) pointed out, the data did not permit any conclusion as to whether the deamidation occurs at the free base, the nucleoside or the nucleotide level. Two objections have been raised in the past to the possibility that the biosynthesis of NAD from nicotinamide in mammalian liver occurs by the deamidation of nicotinamide at the free base level to nicotinic acid which is then converted to NAD via the Preiss-Handler pathway. First, nicotinamide deamidase had been found in several microorganisms (e.g. 3) and in avian species (12), but not in mammalian species (4,12). Secondly, in vivo studies (1,5) indicated that nicotinamide is a better precursor of liver NAD than is nicotinic acid, contrary to what might be expected if nicotinic acid were an intermediate in the conversion of nicotinamide to NAD. The present study overcomes these objections and thus provides a pathway for NAD synthesis from nicotinamide in mammals.

Nicotinamide deamidase has now been found in rat and mouse liver. The activity of the enzyme appears sufficient

to account for the maximum rate of liver NAD synthesis calculated to occur in vivo following the injection of nicotinamide. Some of the properties of the rat liver enzyme which have hindered its detection are described in this report.

Enzyme activity was assayed by measuring the formation of radioactive nicotinic acid from C^{14}-labelled nicotinamide. Except where otherwise indicated, the substrate concentration was 5 mM.

A study of the distribution of deamidase activity among the cell fractions obtained by the differential centrifugation of a 10% homogenate of rat liver in 0.44 M sucrose showed that the enzyme was located primarily in the microsomes. Some activity was also recovered in the mitochondria but very little was found in either the nuclei or supernatant fractions. The possibility that the enzyme is localized in the lysozomes appears unlikely since procedures which rupture the lysozomes did not solubilize the deamidase.

The particulate enzyme was solubilized by deoxycholate, which was then largely removed by precipitation with $MgCl_2$. Treatment of the solubilized enzyme with protamine, ammonium sulfate and charcoal yielded a partially purified enzyme preparation* which catalyzed the formation of about 10 mμmoles of nicotinic acid/hour/mg protein at pH 8.8 when assayed with 5 mM nicotinamide. Activity was proportional to both time and enzyme concentration and showed a broad pH optimum around 9.0. Activity appeared to be unaffected by ATP (10^{-3}M), $MgCl_2$ (10^{-3}M), $HgCl_2$ (10^{-4}M) or p-mercuribenzoate (10^{-5}M).

Studies of the effect of substrate concentration on reaction rate indicated a Km in the range of 1 M for the enzyme in the crude homogenate. However, a decrease in Km occurred during purification of the enzyme. Figure 1 provides evidence that the unusually high Km observed with the crude homogenate is caused by the presence of inhibitory material. Plots of 1/V against 1/S, according to the method of Lineweaver and Burk (8), are shown for the crude homogenate, for the partially purified enzyme and for the partially purified enzyme in the presence of the crude homogenate. Km values calculated from these data are 1020 mM for the crude homogenate, 181 mM

*Details of the purification procedure are available upon request.

for the partially purified enzyme and 910 mM for the partially purified enzyme in the presence of the crude homogenate. Thus, inhibitory material in the crude homogenate markedly increased the Km of the purified enzyme.

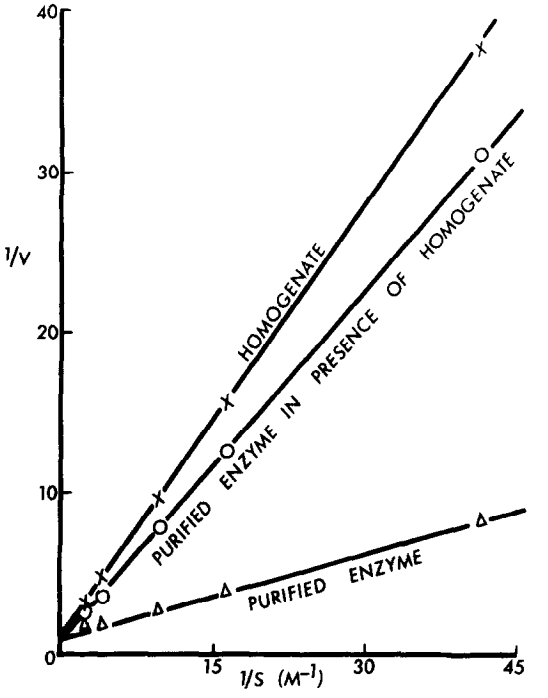

Fig. 1 - Lineweaver-Burk plots showing effect of crude homogenate on Km of partially purified deamidase from rat liver. Incubation tubes (0.5 ml final volume) contained 25 µmoles triethanolamine (TEA) buffer, pH 8.8, from 12 to 200 µmoles of nicotinamide -7-C^{14} and either crude homogenate (50 mg wet weight) or enzyme purified through the charcoal step (1.9 mg protein) or a combination of these amounts of crude homogenate and purified enzyme. (Crude homogenate and purified enzyme were used in amounts designed to give approximately the same maximum velocities.) The tubes were incubated for 2 hours at 38°C; the reaction was terminated by boiling for 2 minutes; the tubes were cooled, and 500 µg of non-radioactive nicotinic acid was added as marker in 0.02 ml of 1 N acetic acid; the tubes were again boiled 2 minutes to insure protein coagulation, the tubes were centrifuged and 0.1 ml of supernatant was subjected to paper electrophoresis in 20 mM TEA, pH 7.5, containing 2 mM EDTA, for 45 minutes at 77 volts-cm^{-1}; the quench spot of nicotinic acid was cut out and counted directly in a Packard Scintillation Counter.

At each substrate concentration, deamidase activity of the purified enzyme in the presence of the crude homogenate was calculated by subtracting the activity in the tube containing crude homogenate alone from the activity found in the tube containing purified enzyme plus crude homogenate. The velocities (V) were expressed as µmoles of nicotinic acid formed per 0.5 ml incubation mixture per 2 hours.

Additional evidence that the enzyme in the crude homogenate is strongly inhibited is provided by the marked stimulation of activity produced by serum albumin (Table 1). In contrast, the partially purified enzyme, after treatment with charcoal, was only slightly stimulated by serum albumin (Table 1) indicating that much inhibitor had been removed during the course of purification. It has also been found that the inhibition of the purified enzyme caused by crude liver homogenate could be prevented by serum albumin. In other experiments, it has been found that the Km of the enzyme in the crude homogenate could be decreased several-fold by the addition of serum albumin, indicating that albumin binds endogenous inhibitor.

Table 1

Activation of deamidase by serum albumin

Incubation tubes (0.5 ml final volume) contained 25 µmoles TEA buffer, pH 8.8, 2.5 µmoles of nicotinamide-7-C^{14}, either crude rat liver homogenate (5 mg wet wt.) or enzyme purified through the charcoal step (3.1 mg protein) and the indicated amounts of crystalline bovine serum albumin (Mann Biochemicals, New York). Other assay conditions as in Figure 1. Specific activity is expressed as mµmoles nicotinic acid formed per hour per mg protein.

Serum Albumin	Crude Homogenate		Purified Enzyme	
mg	Spec.Act.	% of Control	Spec.Act.	% of Control
0	.58	—	8.3	—
4	2.77	478	11.3	136
8	2.63	454	12.2	147

The greater efficacy of nicotinamide than of nicotinic acid for the synthesis of liver NAD *in vivo* can be understood by a comparison of the half-lives of the two compounds. It has been found in the present investigation that the half-life of nicotinamide in rat liver is about 4 1/2 hours compared with a half-life of only about one hour for nicotinic acid*.

*Unpublished experiments.

Thus, an injected dose of nicotinamide remains in the liver for a much longer period of time. This finding, considered together with the observations that high concentrations of nicotinic acid inhibit NAD synthesis *in vivo* from either nicotinamide or nicotinic acid (6,7) suggests that injected nicotinamide functions *in vivo* as a generating system for the maintenance, over a prolonged period of time, of non-inhibitory levels of nicotinic acid.

The nicotinamide deamidase reaction in liver, described in the present communication, appears to be the first enzymatic step in the conversion of nicotinamide to NAD and, in conjunction with other known enzymatic reactions (2,10,11), provides a complete pathway for the biosynthesis of the coenzyme. As a working hypothesis it is proposed that nicotinamide deamidase is the rate-limiting step in the synthesis of NAD from nicotinamide. Moreover, the possibility is suggested that the endogenous inhibitor may control the rate of deamidation *in vivo*, as it does *in vitro*, by altering the Km of the enzyme. The rate of formation of NAD would then be sensitive to control by the inhibitor. The following report indicates that the nicotinamide deamidase reaction may be subject to hormonal control and that this control appears to be mediated through altering the degree of inhibition of the enzyme.

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