

The Enzymatic Conversion of Quinolate to Nicotinic Acid Mononucleotide in Mammalian Liver*

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It has been known for many years that tryptophan is converted to niacin by mammals (1) and *Neurospora* (2) and that 3-hydroxyanthranilate is an intermediate in this conversion (3). The steps between 3-hydroxyanthranilate and niacin have, however, remained obscure until recently. A very active soluble oxidase present in mammalian kidney and liver converts 3-hydroxyanthranilate to the unstable acyclic compound 2-amino-3-carboxymuconic semialdehyde, which spontaneously cyclizes to form quinolate (4). Considerable evidence showing that quinolate is converted to niacin *in vivo* has been accumulated. In *Neurospora crassa*, one mutant accumulates quinolate in the medium when grown at suboptimal nicotinamide levels, and another mutant utilizes high levels of quinolate in place of niacin (5). In rats, injection of quinolate causes a 3-fold increase in *N*¹-methylnicotinamide excretion (6), and high levels of quinolinic acid can replace niacin in the diet (5). Moreover, the injection of quinolinic acid-³H is followed by excretion of *N*¹-methylnicotinamide-³H in the urine (7). Recently, the conversion of quinolinic acid-³H to niacin-³H in chick embryos has also been demonstrated (8). In spite of this evidence, the low efficiency of quinolinic acid for replacing niacin in nutritional experiments and the failure to observe this conversion *in vitro* have led reviewers in this field to postulate an alternate pathway for niacin formation from 3-hydroxyanthranilate.

Recently, Nishizuka and Hayaishi (9) have reported that quinolate is converted to nicotinic acid mononucleotide in the presence of 5-phosphoribosyl-1-pyrophosphate by a soluble system obtained from rat liver. The same reaction has also been reported to occur in *Escherichia coli* (10). This reaction appears to be analogous to the formation of nicotinic acid mononucleotide from nicotinic acid as described by Imsande and Handler (11). Although the work of Nishizuka and Hayaishi clearly establishes a possible enzymatic basis for the conversion of tryptophan to niacin, it leaves unanswered several basic questions concerning this conversion. For example, are the kinetic parameters of this system such that it could reasonably be expected to account for the conversion of tryptophan to niacin observed *in vivo*, is quinolinic acid ribotide formed as an intermediate, or does addition of the ribosyl 5-phosphate moiety and decarboxylation of quinolate occur in a concerted reaction,

and is this enzyme system distinct from the nicotinic acid mononucleotide pyrophosphorylase described by Imsande and Handler? This study was designed to answer these and other questions relating to the enzymatic formation of nicotinic acid mononucleotide from quinolate.

EXPERIMENTAL PROCEDURE

Materials—Uniformly labeled aniline-HCl-¹⁴C was purchased from New England Nuclear Corporation. Nicotinic acid-7-¹⁴C was purchased from the California Corporation for Biochemical Research. Nicotinic acid mononucleotide-¹⁴C was a gift of Dr. John Imsande of Western Reserve University. PP-ribose-P, ATP, and NAD were obtained from Pabst Laboratories, Inc. Calcium phosphate gel was prepared by the method of Colowick (12).

Methods—Niacin-active material was determined by microbioassay with *Lactobacillus plantarum* (13) as the test organism. The enzymatic reaction in which niacin formation was being measured was stopped by heating in a boiling water bath for 10 minutes. Aliquots were removed, suitably diluted, and sterilized by autoclaving with the bioassay medium.

The enzymatic decarboxylation of quinolinic acid-2,3,7,8-¹⁴C was followed with a modified Thunberg tube. The standard reaction mixture for assaying the enzyme contained the following in micromoles: MgCl₂, 0.6; PP-ribose-P, 0.6; potassium phosphate, pH 6.0, 60; quinolinic acid-2,3,7,8-¹⁴C, 1.0; and 10 to 150 units of enzyme in a total volume of 3.0 ml. The reaction components were placed in the tube, 0.3 ml of 1.0 *N* NaOH was introduced into the side arm, and the tube was evacuated with a water aspirator. After incubation for 1 hour at 37°, the reaction was stopped by introducing 0.2 ml of 30% perchloric acid via the evacuation arm, and the tube was allowed to stand at room temperature while still under partial vacuum. After 1 hour, 0.1-ml aliquots of the NaOH containing ¹⁴CO₂ were removed and assayed in a Packard Tri-Carb liquid scintillation spectrometer. A unit of activity is defined as the amount of enzyme necessary to decarboxylate 1 μ mole of quinolate per hour. Under the conditions of the standard assay, quinolate decarboxylation is linear with time for 1 hour and is directly proportional to enzyme concentration in the range of concentration used. Assay for nicotinic acid mononucleotide pyrophosphorylase activity was performed as described by Imsande and Handler (11). Protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (14).

Synthesis of Quinolinic Acid-2,3,7,8-¹⁴C—Uniformly labeled aniline-¹⁴C was condensed with glycerol by a modification of the

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classical Skraup procedure (15) to form quinoline-¹⁴C, which was then oxidized to quinolinic acid-2,3,7,8-¹⁴C with H₂O₂ in the presence of Cu⁺⁺ (16) (Fig. 1). A 5-ml flask containing 323 mg of uniformly labeled aniline-HCl-¹⁴C (specific activity, 406 μc per mmole), 81 mg of FeSO₄, 0.7 ml of glycerol, 0.15 ml of nitrobenzene, and 0.4 ml of H₂SO₄ was heated under air reflux at 135–140° for 6 hours. The residual nitrobenzene was removed by steam distillation, and the reaction mixture was made basic with 4 ml of 40% KOH and again distilled with steam. To the first 25 ml of this distillate, which contained most of the quinoline-¹⁴C, were added 0.5 ml of glacial acetic acid and 500 mg of cupric acetate. The mixture was then warmed to 65°, and 5 ml of 30% H₂O₂ were added dropwise with stirring while the temperature was maintained at 65 to 70°. The mixture was allowed to cool, and the blue copper quinolinate was removed by filtration and washed with water. The salt was suspended in 25 ml of water and maintained at 60° with stirring while H₂S was bubbled through the suspension for 1 hour. The CuS formed was removed by filtration, the filtrate containing free quinolinic acid was taken to dryness, and the product was recrystallized from 50% ethanol. The over-all yield from aniline-HCl was 35%; the specific activity was 250 μc per mmole. One-fourth of the total radioactivity was located in the α-carboxyl group as shown by pyrolytic decomposition at 190°, and collection and counting of the ¹⁴CO₂ evolved. No radiochemical impurities could be detected by radioautography of paper chromatograms prepared in several solvent systems.

Enzyme Purification Procedure—The procedure for purification of the PP-ribose-P dependent quinolinate decarboxylating activity from beef liver acetone powder is summarized in Table I. This procedure is a modification of the method used by Imsande and Handler (11) for the purification of nicotinic acid mononucleotide pyrophosphorylase. Acetone powder was prepared by extracting for 1 minute 500 g of ground or minced beef liver in a Waring Blendor, containing 2500 ml of acetone precooled to between -15 and -20° with powdered Dry-Ice. The extract was filtered through a Buchner funnel (temperature maintained below -10°), the residue was reextracted with another 2500 ml of cold acetone, refiltered, air-dried, and stored in a desiccator at 0–4°.

Step 1. Acetone powder was extracted with 10 volumes of 0.05 M potassium phosphate buffer, pH 7.4, at room temperature by stirring for 30 minutes. This suspension was centrifuged at 10,000 × g for 10 minutes, and the precipitate was discarded. All subsequent steps were performed at 0–4° unless otherwise stated.

Step 2. The extract was brought to 35% saturation in ammonium sulfate (245.2 g per liter) by slow addition of the solid salt with continuous stirring. After 10 minutes of additional stirring, the mixture was centrifuged as in Step 1, and the supernatant was discarded. The precipitate was dissolved in 0.8 the Step 1 volume of deionized water.

Step 3. The Step 2 solution was adjusted to pH 3.8 with 1.0 N HCl. Stirring was continued for 1 hour at 0°, the mixture was centrifuged as in Step 1, and the precipitate was discarded. The supernatant was adjusted to pH 6.0 with 1.0 N KOH, brought to 32% saturation with ammonium sulfate (208.5 g per liter), and centrifuged; the supernatant was discarded. The precipitate was dissolved in 35 ml of water for each 100 ml of Step 2, and dialyzed against 100 volumes of deionized water for

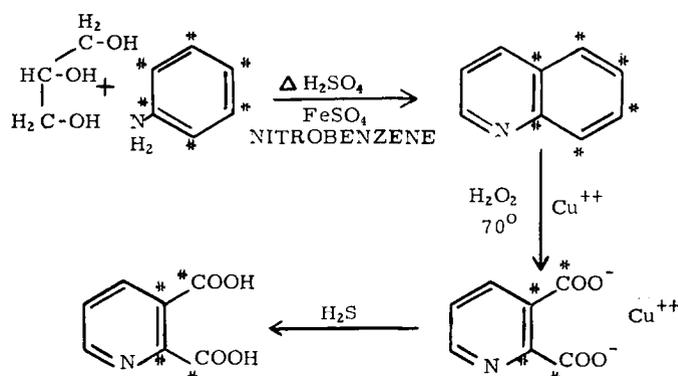


FIG. 1. Synthesis of quinolinic acid-2,3,7,8-¹⁴C

4 hours. Any precipitate which formed during dialysis was removed by centrifugation.

Step 4. Each 35 ml of the product of Step 3 was diluted to 250 ml and made 0.001 M in potassium phosphate, pH 7.3. This solution, after addition of 1 g (dry weight) of calcium phosphate gel, was stirred for 10 minutes and centrifuged; the gel was discarded. The supernatant was brought to 35% saturation by the addition of solid ammonium sulfate as in Step 2 and centrifuged; the supernatant was discarded. The precipitate was dissolved in 12.5 ml of water and dialyzed overnight against 2 liters of deionized water. The precipitate which formed during dialysis was removed by centrifugation and discarded.

Step 5. The enzyme solution obtained in Step 4 was placed on a 2.5- × 10-cm DEAE-cellulose column which had been thoroughly equilibrated with 0.005 M phosphate, pH 7.3. After washing with 60 ml of 0.01 M phosphate, pH 7.3, the column was eluted stepwise with 50 ml of 0.025 M phosphate, pH 7.3, 60 ml of 0.05 M phosphate, pH 7.3, and 80 ml of 0.1 M phosphate, pH 7.3. The fractions containing enzyme activity, which were usually eluted with 0.1 M phosphate, were pooled and dialyzed overnight against 3 liters of deionized water. The DEAE-cellulose eluate exhibited a specific activity slightly more than 5200 times that of the initial extract. However, since Step 3 resulted in a 3.5-fold increase in apparent total activity, the final product is considered to be 1500-fold purified from the original acetone powder extract.

RESULTS

Studies with Microbioassay for Niacin Formation

Initial studies were performed with a microbioassay for niacin formation from quinolinate with *L. plantarum* as assay organism. A preliminary report of some of these studies has been published (17). A PP-ribose-P-dependent formation of niacin-assayable material from quinolinate could be shown in charcoal-treated homogenates of rat liver with this technique. This enzyme activity, which was found to be located in the cytoplasmic fraction of the cell, was purified several fold by ammonium sulfate fractionation and was adsorbed on and eluted from calcium phosphate gel. Further attempts at purification resulted in loss of all activity. Initially, it was erroneously assumed on the basis of the preliminary report by Nishizuka and Hayaishi (9) that the niacin activity being measured was present as nicotinic acid mononucleotide. However, it has been shown (18)¹ that

¹ We wish to thank Dr. Hayaishi for making a copy of this manuscript available to us prior to publication.

TABLE I

Purification of PP-ribose-P-dependent quinolinic acid decarboxylase of beef liver

The starting material was 150 g of acetone powder.

Step	Volume	Units	Protein	Specific activity (A)	Yield	Nicotinic acid mononucleotide pyrophosphorylase specific activity (B)	B:A
	ml		mg	units/mg protein	%	units/mg protein	
1. Extraction.....	1,250	20,750	24,811	0.83	100	0.045	0.053
2. (NH ₄) ₂ SO ₄	1,000	19,000	6,202	3.06	92		
3. pH 3.8.....	350	72,030	785	91.8	347	1.48	0.016
4. Ca ₃ (PO ₄) ₂ gel.....	60	40,620	42.1	963	195	5.22	0.005
5. DEAE-cellulose.....	115	9,016	2.06	4,380	44	7.77	0.0014

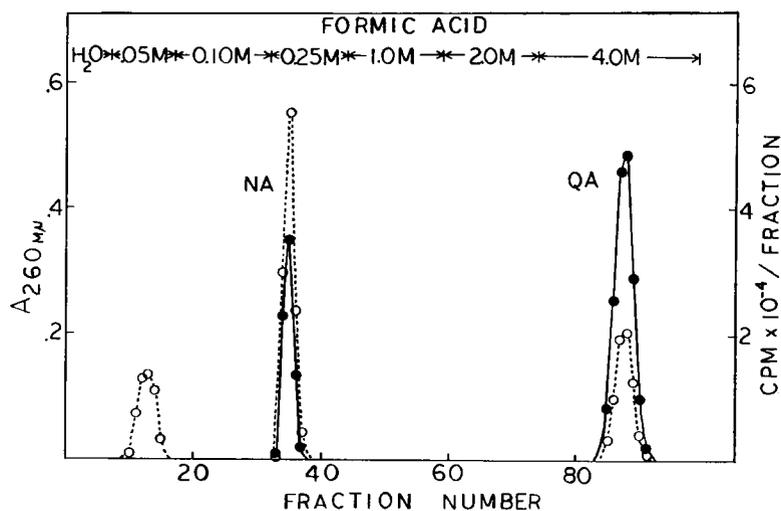


FIG. 2. Chromatographic separation of the products formed from quinolinic acid-2,3,7,8-¹⁴C and PP-ribose-P by beef liver homogenate. The experimental conditions are described in the text. The radioactivity of each fraction (10 ml) was determined with a Packard Tri-Carb liquid scintillation spectrometer. NA, nicotinic acid; QA, quinolinic acid. O---O, absorbance at 260 mμ; ●—●, radioactivity.

the assay organism used does not respond to this compound. In these experiments, the sample to be assayed for niacin was routinely autoclaved with the bioassay medium. Subsequent tests have shown that nicotinic acid mononucleotide is partially hydrolyzed to free niacin under these conditions (autoclaving at 120° for 10 minutes in the bioassay medium resulted in 56% hydrolysis); and, therefore, the results previously reported (17) with rat liver are probably caused by this hydrolysis. On the other hand, crude beef liver homogenates convert quinolinate to free niacin (Fig. 2); therefore, the results reported below are caused by free niacin formation in these preparations.

When beef liver homogenates were found to have several times the apparent niacin-forming activity of rat liver by the microbioassay method, work was begun with this tissue. Table II shows the requirements for niacin formation from quinolinate in a charcoal-treated beef liver homogenate. The beef liver enzyme was prepared by homogenizing fresh or frozen liver at 0–4° in 4 volumes of distilled water in a Waring Blendor, centrifuging at 10,000 × *g* for 30 minutes, and stirring the supernatant with 50 mg of activated charcoal per ml at 0° for 30 minutes to remove the high endogenous levels of niacin-assaying material present in the homogenate. In the experiment shown in Table II, the enzyme preparation contained about 35 μmoles of niacin-assaying material per ml, even after charcoal treatment.

In this system, niacin formation is a linear function of protein concentration in the range of 0.2 to 2.5 mg per ml. Formation of niacin is almost linear with time up to 3 hours. Under optimal conditions, 70 μmoles of niacin per ml of enzyme per hour are formed. The total concentration of pyridine nucleotides in liver has been reported to be about 1.0 to 1.2 μmoles per g of tissue (19). This value is in good agreement with our finding of 200 to 240 μmoles of niacin equivalent per ml of 20% beef liver homogenate by microbioassay. Therefore, the rate of formation of niacin from quinolinate in beef liver is sufficient to replace the total pyridine nucleotide components of this tissue approximately once every 3 hours. An apparent Michaelis constant of 1.5×10^{-5} M for quinolinate was found in this crude system with the microbioassay. Thus, it would appear that the activity of this enzyme system is sufficient to account for the conversion of tryptophan to niacin, which is known to occur *in vivo*.

Studies with Quinolinic Acid-2,3,7,8-¹⁴C

Identification of Reaction Products—The availability of ¹⁴C-labeled quinolinic acid greatly facilitated the identification of the products formed from quinolinate in the presence of PP-ribose-P in beef liver preparations. A reaction system containing quinolinic acid-2,3,7,8-¹⁴C, 1.0 μmole (250 mμ); PP-

ribose-P, 5.0 μ moles; $MgCl_2$, 15 μ moles; $MnCl_2$, 1.5 μ moles; potassium phosphate, pH 7.6, 200 μ moles; and 5 ml of a 20% beef liver homogenate treated with 50 mg of charcoal per ml for 30 minutes was incubated at 37° for 3 hours. The reaction was cooled to 0° and stopped with perchloric acid, and the protein was removed by centrifugation. The supernatant was adjusted to pH 7.0 with KOH, and the precipitate of potassium perchlorate removed. Carrier quinolinic acid and nicotinic acid, 2 μ moles, were added to the supernatant, and the mixture was placed on a 2- \times 25-cm Dowex 1-X8 column, which was then eluted with formic acid as described by Preiss and Handler (20). Fig. 2 shows the elution pattern from such a column. Two major radioactive peaks were found, one corresponding to the adsorption peak caused by quinolinic acid, and the other to that of nicotinic acid. The identification of the nicotinic acid was confirmed by paper chromatography with authentic material in several solvent systems. No radioactivity could be detected in the area where nicotinic acid mononucleotide is eluted in this system. It appears, therefore, that free niacin is the major product formed from quinolinate in beef liver homogenates in contrast to the reported findings in rat liver (9).

On the other hand, in a more purified enzyme system, nicotinic acid mononucleotide is the major product formed, as shown in Fig. 3. When an incubation mixture identical with that used for the experiments depicted in Fig. 2, with the exception that 5 ml of Step 4 enzyme (12 mg of protein) were used as the enzyme source, was passed over a Dowex 1-X8 column, the major radioactive component, other than quinolinic acid, was found to be nicotinic acid mononucleotide. This compound was identified by paper chromatography in several solvent systems as well as by hydrolysis in 0.1 N NaOH at 100° for 30 minutes to yield niacin-¹⁴C, which was identified by paper chromatography.

Incubation of nicotinic acid mononucleotide-¹⁴C with charcoal-treated beef liver homogenate for 3 hours at 37° resulted in complete cleavage of this compound to niacin-¹⁴C. A rat liver homogenate prepared and incubated with quinolinic acid-2,3,7,8-¹⁴C under the conditions described by Nishizuka and Hayaishi (9) formed only nicotinic acid mononucleotide, whereas a beef liver homogenate prepared and incubated under the same con-

TABLE II

Requirements for niacin formation in beef liver homogenate

The complete reaction system contained 0.5 μ mole of quinolinic acid, 0.75 μ mole of PP-ribose-P, 15 μ moles of $MgCl_2$, 1.5 μ moles of $MnCl_2$, 200 μ moles of potassium phosphate (pH 7.6), and 1.0 ml of beef liver enzyme (25 mg of protein) in a total volume of 10.0 ml. The reaction mixture was incubated at 37° for 2 hours, the reaction stopped by heating for 10 minutes at 100°, and aliquots were removed for niacin microbioassay.

Reaction system	Niacin
	μ moles
Complete.....	120
- Quinolinic acid.....	38
- PP-ribose-P.....	37
- Mg^{++}	93
- Mn^{++}	85
- Mg^{++} , - Mn^{++}	34
- Enzyme.....	5
+ Boiled enzyme.....	33

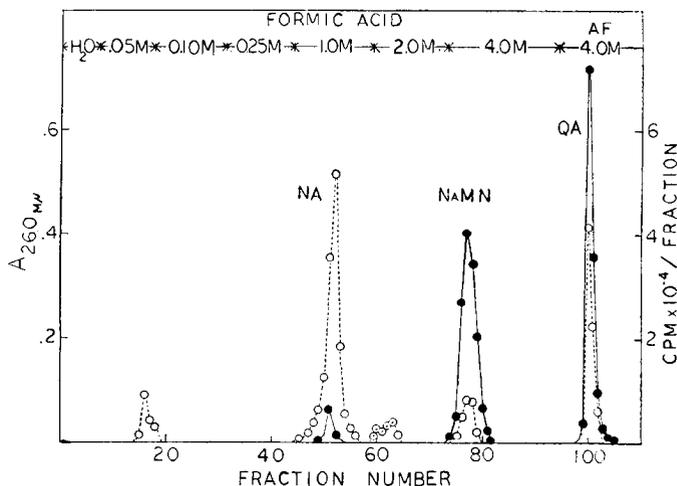


FIG. 3. Chromatographic separation of the products formed from quinolinic acid-2,3,7,8-¹⁴C and PP-ribose-P by a Step 4 enzyme preparation. The experimental conditions are described in the text. The radioactivity of each fraction (10 ml) was determined with a Packard Tri-Carb liquid scintillation spectrometer. NA, nicotinic acid; NaMN, nicotinic acid mononucleotide; QA, quinolinic acid; AF, ammonium formate. O---O, absorbance at 260 mμ; ●—●, radioactivity.

TABLE III

Requirements for nicotinic acid mononucleotide formation by purified enzyme

The complete reaction system contained 0.5 μ mole of quinolinic acid-2,3,7,8-¹⁴C, 0.6 mole of PP-ribose-P, 3.0 μ moles of $MgCl_2$, 60 μ moles of potassium phosphate or potassium acetate, pH 5.8, and Step 4 enzyme in a total volume of 3 ml. The ATP solution was adjusted to pH 5.8 before addition to the system.

Reaction system	Nicotinic acid mononucleotide
	μ moles/hr
Complete.....	40.4
- Enzyme.....	0.0
- Mg^{++}	0.4
- PP-ribose-P.....	1.2
- Mg^{++} , + Mn^{++} , 1×10^{-3} M.....	26.0
- Potassium phosphate + potassium acetate.....	41.9
+ ATP, 1×10^{-4} M.....	32.2
+ ATP, 1×10^{-3} M.....	9.0

ditions converted quinolinate almost entirely to free niacin.² These findings indicate that in beef liver, niacin is formed from quinolinate via nicotinic acid mononucleotide and the enzyme(s) responsible for the degradation of nicotinic acid mononucleotide to niacin are not found in the soluble fraction of rat liver. The enzyme system responsible for the formation of free niacin from nicotinic acid mononucleotide is now being studied.

Studies with Purified Enzyme System—The requirements for the formation of nicotinic acid mononucleotide from quinolinate, as measured by decarboxylation of quinolinic acid, are summarized in Table III. In this experiment, a pH at which both phosphate and acetate have buffering action was used, although it is somewhat lower than the optimal pH for the reaction. A

² J. L. R. Chandler and R. K. Gholson, unpublished observations.

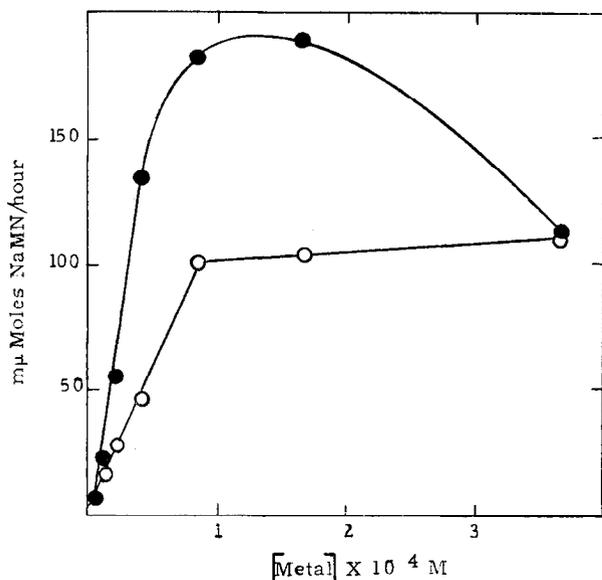


FIG. 4. Effect of Mg^{++} and Mn^{++} concentration on the formation of nicotinic acid mononucleotide. The standard assay system was employed with the Step 4 enzyme preparation. ●—●, $MgCl_2$; ○—○, $MnCl_2$.

TABLE IV

Effects of charcoal treatment, Mn^{++} , and NAD on activity of beef liver homogenate

The standard assay system was used. $MnCl_2$ was added at a final concentration of 3.3×10^{-4} M in the cases indicated. The enzyme source was 0.5 ml of the 10,000 \times g supernatant of a 20% water homogenate of beef liver or the same preparation treated with 50 mg of activated charcoal per ml for 30 minutes.

Reaction system	Nicotinic acid mononucleotide <i>mμ</i> moles/hr
Homogenate.....	2.3
+ Mn^{++}	2.3
+NAD 10^{-3}	1.2
Charcoal-treated homogenate.....	2.0
+ Mn^{++}	28.5
+ Mn^{++} , +NAD, 10^{-5} M.....	27.0
+ Mn^{++} , +NAD, 10^{-4} M.....	7.1
+ Mn^{++} , +NAD, 10^{-3} M.....	5.4

divalent metal ion cofactor and the substrate PP-ribose-P were the only absolute requirements found in addition to quinolate and enzyme. Acetate buffer could be substituted for phosphate buffer without causing any decrease in activity. In contrast, orthophosphate was found to be stimulatory in the synthesis of nicotinic acid mononucleotide from nicotinic acid (11). ATP is not required by the enzyme which converts quinolate to nicotinic acid mononucleotide; and, in fact, this compound strongly inhibits the reaction at a concentration of 1×10^{-3} M. In contrast, nicotinic acid mononucleotide pyrophosphorylase is stimulated by ATP, and in the standard assay for nicotinic acid mononucleotide formation from nicotinic acid, ATP is used at a concentration of 2.5×10^{-3} M (11). The stimulation by $MnCl_2$ (see Table II and Table IV) which is always found in the beef liver homogenate and the Step 1 and 2 preparations by

both the bioassay and the $^{14}CO_2$ assay is not observed in the more purified preparations.

Metal Requirement—The synthesis of nicotinic acid mononucleotide from quinolate and PP-ribose-P shows an absolute requirement for a divalent metal ion as in all known enzymatic reactions in which PP-ribose-P is involved. The relationship between Mg^{++} and Mn^{++} concentration and the rate of quinolate decarboxylation by a Step 5 enzyme preparation is shown in Fig. 4. In this experiment, Mg^{++} was removed from the PP-ribose-P used by passing the magnesium salt of PP-ribose-P through a small Dowex 50- H^+ column. The maximal activity was obtained when Mg^{++} was present at 1 or 2×10^{-4} M, which is approximately equal to the PP-ribose-P concentration used. In this purified enzyme, Mn^{++} is one-half as effective as Mg^{++}

TABLE V

Effect of monovalent cations on nicotinic acid mononucleotide formation from quinolinic acid

The standard assay system was used with the exception that Tris-HCl, 200 μ moles, pH 7.4, or potassium phosphate, 200 μ moles, pH 7.4, was used as the buffer. Monovalent ion concentration was 2×10^{-2} M in all cases. The Step 5 preparation was the enzyme source.

Reaction system	Nicotinic acid mononucleotide <i>mμ</i> moles/hr
Tris-HCl.....	34
+KCl.....	72
+NaCl.....	31
+LiCl.....	62
+ NH_4Cl	94
Potassium phosphate.....	151

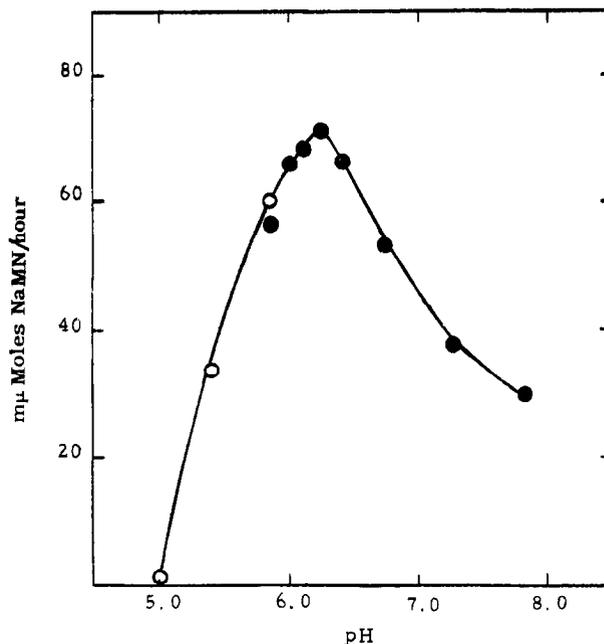


FIG. 5. Influence of pH on the enzymatic synthesis of nicotinic acid mononucleotide from quinolinic acid-2,3,7,8- ^{14}C . The standard assay system was employed with the Step 4 enzyme preparation. ○—○, potassium acetate buffer; ●—●, potassium phosphate buffer. The pH of buffer solutions was measured at 25°.

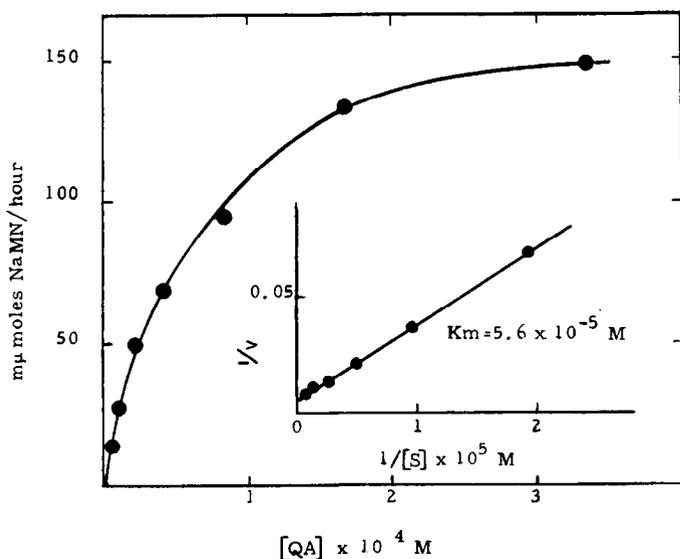


FIG. 6. The rate of nicotinic acid mononucleotide formation as a function of quinolinate (QA) concentration. The standard assay system was employed with the Step 4 enzyme preparation.

at concentrations below 2×10^{-4} M. In the charcoal-treated homogenate at pH 6.0, a striking stimulation by Mn^{++} was observed (Table IV). This stimulation depends on pretreatment of the homogenate with charcoal. A possible interpretation of these results is that two inhibitors of quinolinate decarboxylation are present in the crude homogenate, one of which is removed by charcoal treatment and the other by Mn^{++} .

Evidence that the formation of nicotinic acid mononucleotide from quinolinic acid also requires a monovalent cation is provided by the data shown in Table V. The activity observed in Tris-HCl buffer was markedly stimulated by the addition of K^+ , Li^+ , or NH_4^+ ions. Tris appears to inhibit the reaction, since potassium phosphate buffer is markedly superior to the same concentrations of KCl in Tris-HCl buffer. In contrast, the standard assay for nicotinic acid mononucleotide formation from nicotinic acid is performed in Tris-HCl buffer (11).

pH Optimum—The PP-ribose-P dependent decarboxylation of quinolinic acid shows a sharp pH optimum at pH 6.2 in a purified enzyme system (Fig. 5). In contrast, the synthesis of nicotinic acid mononucleotide from nicotinic acid and PP-ribose-P has a rather broad pH range with an optimum at pH 7.2 (11).

Kinetic Properties—The Michaelis constants for the various components of the reaction were determined in the usual manner (21). The K_m for quinolinate in the Step 4 enzyme at pH 6.0 is approximately 6×10^{-5} M (Fig. 6). The K_m for PP-ribose-P is approximately 5×10^{-5} M. The K_m value for PP-ribose-P is almost the same as that reported for nicotinic acid mononucleotide pyrophosphorylase (11). The K_m for quinolinate is considerably higher than that found for nicotinic acid (1×10^{-6} M) by Imsande and Handler (11), but it is still well within the physiological range. Quinolinate decarboxylation is not inhibited by NMN at 3.3×10^{-3} M or by niacin up to 1.0×10^{-3} M.

Inhibition by NAD—In the experiments with the microbiological assay, beef liver homogenates were treated with charcoal with the object of removing the high levels of niacin-assayable materials present in the crude extract. When the synthesis of quinolinic acid-2,3,7,8-¹⁴C made possible the assay of quino-

linate decarboxylating activity in crude homogenate supernatants, this activity was found to be very low. Treatment of these homogenates with charcoal produced a 5- to 10-fold increase in decarboxylating activity (Table IV), which suggested that the charcoal might be removing an inhibitory substance normally present in the crude homogenate. Several compounds known to be adsorbed on charcoal were tested as possible inhibitors of quinolinic acid decarboxylation. It was found that, among the compounds tested, only NAD had an appreciable inhibitory effect at low concentrations. At 1×10^{-4} M NAD, 75% inhibition of quinolinic acid decarboxylation was observed. However, this inhibitory effect of NAD was lost on aging the crude homogenate or on purification of the enzyme.

DISCUSSION

Since the first postulation (5) that quinolinate is a precursor of niacin, a very considerable body of evidence has accumulated, showing that this conversion can occur *in vivo*. In 1949, it was shown that *N. crassa* mutant 3416 accumulated quinolinate when grown on suboptimal amounts of nicotinamide and mutant 4540 utilized high concentrations of quinolinate as a source of niacin (5). In spite of these observations, it was suggested (22) that quinolinate is a side product of 3-hydroxyanthranilate metabolism rather than an intermediate in niacin biosynthesis, a view which has been prevalent to the present time. The probable conversion of quinolinate to niacin in rats was shown by the 3-fold increase in urinary *N*¹-methylnicotinamide following quinolinate injection. The recent observation that quinolinic acid-³H gives rise to urinary *N*¹-methylnicotinamide-³H (7) and that quinolinic acid-³H is converted to niacin-³H in developing chick embryos (8) has provided unequivocal evidence that this conversion does indeed occur *in vivo*, although these results do not exclude other pathways for niacin biosynthesis. The findings of Nishizuka and Hayaishi (9) provided an enzymatic basis for the conversion of quinolinate to niacin derivatives.

The data presented here, showing the relatively high rate of this reaction and the low K_m value for quinolinate, support the view that this compound is, in fact, an intermediate in the conversion of tryptophan to niacin. The importance of quinolinate in the biosynthesis of NAD is further supported by the observation that, in the acetone powder extract, the total activity of the enzyme forming nicotinic acid mononucleotide from quinolinate is almost 20 times that of the enzyme forming this compound from nicotinic acid (Table I). Since the formation of nicotinic acid mononucleotide seems to be the rate-limiting step in the biosynthesis of NAD from niacin (11), quinolinate should be superior to niacin as a precursor of NAD *in vitro* and also presumably inside the cell. The low efficiency of quinolinate in replacing niacin observed *in vivo* must be caused by a failure of exogenous quinolinate to penetrate the cell, since an active enzyme system for the conversion of quinolinate to NAD is present in liver.

Quinolinic acid also seems to be an intermediate in NAD biosynthesis in organisms which do not convert tryptophan to niacin. Andreoli, Ikeda, Nishizuka, and Hayaishi (10) have shown the PP-ribose-P-dependent formation of nicotinic acid mononucleotide from quinolinate in *E. coli*, and this reaction has also been found to occur in plants (23). Since neither *E. coli* (24) nor plants (25) are able to convert tryptophan to nia-

cin, it may be concluded that in these organisms quinolinic acid arises by a mechanism other than the degradation of tryptophan.

The data presented here show that nicotinic acid mononucleotide pyrophosphorylase and the enzyme catalyzing the PP-ribose-P-dependent decarboxylation of quinolate are probably different proteins. The failure of niacin to inhibit CO₂ release from quinolate, the different response to ATP and orthophosphate by the two enzymes, and their different pH optima support this view. Although the two proteins remain associated during fractionation through several steps, the ratio of the specific activity of nicotinic acid mononucleotide pyrophosphorylase to that of the quinolate-decarboxylating enzyme declined by about 38-fold during the purification procedure.

Nishizuka and Hayaishi (18) have postulated the occurrence of quinolinic acid mononucleotide as an intermediate in the formation of nicotinic acid mononucleotide from quinolate. No evidence for such an intermediate was obtained in our studies, and there was no indication that more than one enzyme fraction is required for the PP-ribose-P-dependent decarboxylation of quinolate.

The formation of nicotinic acid mononucleotide as the major product from quinolate in rat liver homogenates as reported by Nishizuka and Hayaishi (9) has been confirmed. In beef liver homogenates, free niacin is the chief product formed from quinolate, and nicotinic acid mononucleotide does not accumulate until the enzyme system has been partially purified. This difference in products seems to be caused by the presence in beef liver of an enzyme system which degrades nicotinic acid mononucleotide to free niacin.

The stimulation of activity in the crude homogenates by charcoal treatment and its inhibition by NAD led to speculation that the level of NAD in liver may serve to regulate its biosynthesis from quinolate. Such a negative feedback type control is well known in several metabolic pathways in microorganisms (26), but seems to be rare in higher organisms. The failure of NAD to inhibit the purified enzyme may indicate that another factor present in the crude extract and removed during purification is required for the expression of NAD inhibition, or that a compound derived from NAD is the true inhibitor. An understanding of the mechanism of this NAD inhibition will require further study.

SUMMARY

An enzyme that catalyzes the synthesis of nicotinic acid mononucleotide from quinolate and 5-phosphoribosyl-1-pyrophosphate was purified 1500-fold from an extract of beef liver acetone powder. This enzyme seems to be distinct from nicotinic acid mononucleotide pyrophosphorylase. The low Michaelis constant for quinolate and the relatively rapid rate of this reaction are consistent with the assumption that quinolate is an intermediate in the biosynthesis of the pyridine nucleotides

from tryptophan. No evidence was obtained that more than one enzyme is required for this reaction or that quinolinic acid mononucleotide occurs as an intermediate.

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