# QUINOLINATE TRANSPHOSPHORIBOSYLASE: A MECHANISM OF NIACIN RIBONUCLEOTIDE FORMATION FROM QUINOLINIC ACID*

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In earlier reports from our laboratory (Nishizuka and Hayaishi, 1963 a, b) 3-hydroxyanthranilic acid was shown to be converted to niacin ribonucleotide in the presence of 5-phosphoribosyl-1-pyrophosphate (PRPP) and a soluble enzyme system obtained from rat and cat liver. In addition, direct evidence has been provided that the site of ribonucleotide formation is quinolinic acid and free niacin is not involved in the PRPP-dependent niacin ribonucleotide formation from quinolinic acid. In order to elucidate the detailed mechanism of this reaction as well as the precise nature of the enzyme concerned, the enzyme has been purified approximately 3,000 to 5,000 fold from beef liver acetone powder. Available evidence indicates that the reaction is catalyzed by a single enzyme and no evidence has so far been obtained indicating that quinolinic acid ribonucleotide is a stable and dissociable intermediate in this conversion. This enzyme will be referred hereafter to as quinolinate transphosphoribosylase.

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These results together with several properties of this enzyme will be described here.

The enzyme activity was assayed by the PRPP-dependent evolution of $C^{14}O_2$ from quinolinic acid-$C^{14}$ 1/ as previously described (Nishizuka and Hayaishi, 1963 a). Beef liver acetone powder (50 g) was extracted for 20 minutes at 25° with 350 ml of distilled water and insoluble material discarded by centrifugation. All subsequent manipulations were carried out in the cold (0-4°). To the crude extract (300 ml), 450 ml of a 0.4 % protamine sulfate solution were added and the precipitate was discarded by centrifugation. Ammonium sulfate, 150 g, was added to the supernatant solution (750 ml) with continuous stirring. After 10 minutes, the mixture was centrifuged and precipitate was dissolved in 100 ml of 0.005 M Tris acetate buffer, pH 7.5. To the ammonium sulfate fraction, 20 ml of calcium phosphate gel (30 mg dry weight per ml) were added dropwise with stirring. After 10 minutes, the mixture was centrifuged and the gel was washed twice with 100 ml of cold distilled water. The enzyme was then eluted twice with 50 ml of 0.05 M potassium phosphate buffer, pH 7.5. To the eluate (100 ml), 16 g of ammonium sulfate were added and the precipitate was discarded by centrifugation. The supernatant solution was further treated with 4 g of ammonium sulfate and the resulting precipitate was collected by centrifugation and dissolved in 5 ml of 0.005 M Tris acetate buffer, pH 7.5. The second ammonium sulfate fraction was warmed rapidly to 65° with continuous stirring. After this temperature was maintained for 3 minutes, the solution was cooled to 0° in an ice bath and the inactive precipitate was removed by centrifugation. The supernatant solution thus obtained was dialyzed for 12 hours at 4° against several liters of 0.005 M Tris

1/ Quinolinic acid, uniformly labeled with $C^{14}$ except for the β-carboxyl carbon, was prepared as previously described (Nishizuka and Hayaishi, 1963 a).

acetate buffer, pH 7.5. The dialyzed enzyme (approximately 3 mg protein) was adsorbed on a DEAE-cellulose column (diameter, 1 cm; length 2 cm). After washing the cellulose column with 30 ml of 0.02 M potassium phosphate buffer, pH 7.5, the enzyme was eluted with 15 ml of 0.10 M phosphate buffer. These procedures represent an overall purification of about 3,000 to 5,000 fold with the yield of 60 - 80 %. The enzyme was stable and could be stored at -20°C for at least one month without appreciable loss of activity.

The enzyme preparation thus purified was essentially homogeneous upon ultracentrifugation[2/] with the sedimentation constant ($S_{20,w}$) of $6.8 \times 10^{-13}$ (cm/sec), and also homogeneous upon electrophoresis[3/].

Although the enzyme is supposed to catalyze the condensation of quinolinic acid with PRPP to produce quinolinic acid ribonucleotide as a primary product, all attempts to demonstrate the latter compound have been totally unsuccessful, and niacin ribonucleotide was consistently formed as an only detectable product in the reaction mixture with concomitant formation of a stoichiometric quantity of $CO_2$. A typical result is shown in Table I. The appearance of niacin ribonucleotide was matched by the disappearance of quinolinic acid and by the amount of $C^{14}O_2$ evolved.

The niacin ribonucleotide produced was isolated from a large scale incubation mixture by Dowex 1-x 8 (formate form; 200 to 400 mesh; diameter, 0.5 cm; length 30 cm) column chromatography and identified by paper chromatography in three different solvent systems and high voltage paper electrophoresis in the same manner as described earlier (Nishizuka and Hayaishi, 1963 a).

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2/ Ultracentrifugation was carried out for 1 hour with the aid of Spinco centrifuge, Model E at 52,640 x g at 20°.

3/ Zone electrophoresis was done on a cyanogum 41 gel for 24 hours at pH 8.5 (Raymond and Weintraub, 1959). The enzyme protein migrated to the anode (4.5 cm).

Table I.

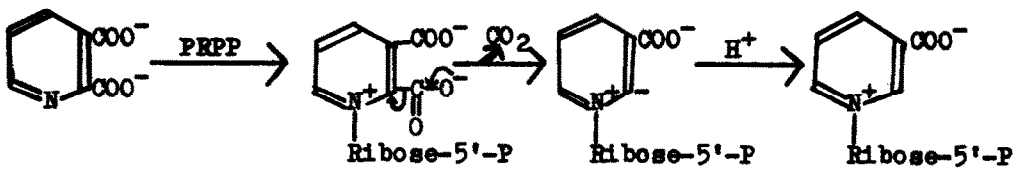
The Stoichiometry of the Reaction

The complete reaction mixture (1.2 ml) containing 92 mμmoles of quinolinic acid-$C^{14}$ (227,000 c.p.m. per μmole), 0.4 μmole of PRPP (Mg free), 1.0 μmole of $MgCl_2$, 300 μmoles of Tris acetate buffer, pH 7.5, and 10 μg of the purified enzyme protein was incubated for 60 minutes at 37°. $C^{14}O_2$ produced was trapped in NaOH, then $Na_2CO_3$ was plated on steel disks as infinitely thin sample and the radioactivity was counted. The reaction product and quinolinic acid remained were determined as described previously (Nishizuka and Hayaishi, 1963 a).

| System | Niacin ribonucleotide | $C^{14}O_2$ | Quinolinic acid |
|---|---|---|---|
| Complete | +44.0 | +47.1 | -45.5 |
| Minus PRPP | 0 | 0 | 0 |
| Minus $MgCl_2$ | 0 | 0 | 0 |
| Complete with boiled enzyme* | 0 | 0 | 0 |

Numbers are expressed in mμmoles.
* Heated in a boiling water bath for 10 minutes.

These results strongly suggest that a single protein is responsible for both the condensation of quinolinic acid with PRPP and the subsequent decarboxylation to yield niacin ribonucleotide. The decarboxylation reaction, however, could be due to nonenzymic reaction since the formation of the ribonucleotide makes the pyridine ring nitrogen quaternary nitrogen. This positively charged nitrogen would raise the electronegativity at the position-2 (Kosower and Skorcz, 1960) and eliminate $CO_2$ from the molecule.



It is noteworthy that the reaction does not require extra ATP, whereas the formation of niacin ribonucleotide from niacin and PRPP requires ATP as an essential cofactor (Preiss and Handler, 1958). The purified enzyme described here does not catalyze the reaction of niacin with PRPP even in the presence of ATP. The Km value for quinolinic acid was calculated to be $7 \times 10^{-6}$ M. A maximum activity of the enzyme was obtained at pH 7.0 and in the presence of $5 \times 10^{-4}$ M $MgCl_2$. The enzyme, however, was markedly inhibited by $MgCl_2$ at a higher concentration (90 - 95 % inhibition at $2 \times 10^{-2}$ M $MgCl_2$). Other divalent metals were more or less inhibitory. $Hg^{++}$, $Co^{++}$, $Cu^{++}$, $Ba^{++}$, $Mn^{++}$ and $Ca^{++}$ inhibited the enzyme by almost 100 % at $1 \times 10^{-3}$ M; $Zn^{++}$ and $Fe^{++}$ by 40 to 70 % at $5 \times 10^{-2}$ M.

The enzyme was found only in the liver and kidney of rat and mouse and could not be detected in other tissues and organs so far tested, including brain, lung, heart, spleen, testicle, intestine, skeletal muscle and blood. On the other hand, niacin ribonucleotide pyrophosphorylase (Preiss and Handler, 1958) was shown to be distributed in all these tissues and organs. The enzyme described in this report, quinolinate transphosphoribosylase, appears to enjoy a wide distribution in several microorganisms, molds and plants, including *Pseudomonas fluorescence*, *Escherichia coli* (Andreoli, et al., 1963), *Neurospora crassa*, yeast and cucumber. These results suggest that quinolinic acid is a common intermediate of NAD biosynthesis both from tryptophan and from succinate and glycerol (Ortega and Brown, 1960), and that free niacin is a degradation product of NAD rather than an obligate intermediate of NAD biosynthesis from the precursors mentioned above.

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