DISTRIBUTION OF QUINOLINATE PHOSPHORIBOSYLTRANSFERASE IN ANIMALS, PLANTS AND MICROORGANISMS

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(Received September 28, 1973)

Distribution of quinolinate phosphoribosyltransferase (an intermediary enzyme of NAD de novo biosynthesis) in animals, plants and microorganisms were investigated by radioassay using quinolinic acid-2,3,7,8-14C as substrate. The enzyme activity was found to be widely distributed in nature. High activities were observed in rat liver, persimmon leaf, cucumber, Shiitake mushroom, Enokitake mushroom, baker’s yeast, Pseudomonas riboflavia and Neurospora crassa. In mammals, significantly high activity was found in liver and kidney, but some activities were detected in brain and spleen. In some of fish livers, plants and microorganisms, the enzyme activity was not detected. The results suggest the possibility that another de novo biosynthetic pathway of NAD, which does not pass through quinolinic acid as an intermediate, may exist in those organisms.

The de novo biosynthesis of NAD has been studied in several laboratories by using various tracer techniques in vivo and some enzymatic skill in vitro. Results of these studies suggest that mainly two separate pathways are present in nature. Namely, the one is derived from tryptophan (all intermediates are well verified) and the other one is from four carbon dicarboxylic acid and glycerol or related compounds (details have not yet been elucidated (1, 2)). It is considered that mammals (3–5), Neurospora crassa (6), aerobically grown yeast (7) have the former pathway and that plants (8) and bacteria (9–12) except Xanthomonas pruni (13) have the latter one. It has also been reported that bacteria (14) and plants (15) lack the tryptophan-NAD pathway.

1 The Biosynthesis of Nicotinamide Adenine Dinucleotide, part I. Presented in part at the Annual Meeting of the Vitamin Society of Japan, Kobe, April 25, 1971.

Abbreviations used: NAD, nicotinamide adenine dinucleotide; PRPP, 5-phosphoribosyl-1-pyrophosphate; EDTA, ethylenediaminetetraacetic acid; POPOP, 2,2’-p-phenylene-bis-(5-phenyloxazole); PPO, 2,5-diphenyloxazole.

岩井和夫, 田口 宽

491
In any pathway quinolinic acid (pyridine-2,3-dicarboxylic acid) has been known as the common intermediate and converted enzymatically to NAD by the subsequent three steps (16) (Fig. 1).

The PRPP-dependent conversion of quinolinic acid to nicotinic acid mononucleotide is the first step in the formation of nicotinic acid-active compounds, and this enzyme has been called quinolinate phosphoribosyltransferase.

![Fig. 1. The de novo biosynthesis of NAD.](image)

Since the growth of *Bacillus subtilis* or *Bacillus megaterium* on media containing $10^{-8} \text{M}$ or higher concentrations of nicotinic acid results in repression of this enzyme (17), quinolinate phosphoribosyltransferase may be an important enzyme in the de novo NAD biosynthetic pathway. At the beginning to elucidate the de novo NAD biosynthetic pathway, the authors gave attention to this enzyme. This paper deals with a detailed investigation on the distribution of quinolinate phosphoribosyltransferase in animals, plants and microorganisms.

**EXPERIMENTAL**

1. **Materials.** Male albino rat of Donryu strain and male mouse of dds strain were kindly supplied by Prof. M. Fujiwara, Faculty of Medicine, Kyoto University. Male white rabbit was generously given by Japan Cage Co., Ltd., Osaka. Raigyo fish (snake head) and Funa fish (crucian carp) were taken from the pond of Uji campus, Kyoto University. Japanese persimmon leaf, sweet potato and okura were obtained from the farm of the Research Institute for Food Science, Kyoto University. Tsukushi (common horsetail) was gathered at Iwakura, Kyoto. Pea seedlings were prepared in the laboratory as follows: air-dried pea seeds (*Pisum sativum* L. var. Alaska) were soaked in distilled water at 25°C for 18 hr and allowed to germinate in the dark at 20°C for 2 days on moist absorbent cotton in a vat covered with wet filter paper. Germ-free *Lemma minor* L. was kindly supplied by Dr. S. Yamashita, Faculty of Agriculture, Nagoya Uni-
distribution of quinolinate phosphoribosyltransferase

The Lemna was cultured aseptically at 20°C for 2 weeks under light in the synthetic medium as follows: NH₄NO₃, 20 mg; K₂HPO₄, 40 mg; CaCO₃, 15 mg; MgSO₄·7H₂O, 50.5 mg; ZnSO₄·7H₂O, 2 mg; FeSO₄·7H₂O, 2.5 mg; H₂BO₃, 1.5 mg; NaMoO₄·2H₂O, 2.5 mg; CuSO₄·5H₂O, 0.5 mg; CoSO₄·7H₂O, 0.1 mg; EDTA, 50 mg; and sucrose 1 g, per 100 ml, pH 6.0. Some mushrooms, i.e., Kiku-rage [Auricularia mesenterica (Dicks.)] and Itachitake [Psathyrella candolleana (Fr.)], were collected in the botanical garden, Faculty of Science, Kyoto University. Animal organs, plants and mushrooms other than those described above were obtained from commercial sources.

Baker's yeast was purchased from Oriental Yeast Co. Ltd., Osaka. Pseudomonas riboflavina (Foster) AKU 0803, Escherichia coli (Crookes Ajinomoto AKU 0001; AKU 0002; AKU 0003; AKU 0006; AKU 0007), Escherichia intermedia A-21, AKU 0010; Neurospora crassa Shear et Dodge, AKU 3552 and Rhizopus oryzae M-21, AKU 3101 were kindly supplied by Prof. K. Ogata, Faculty of Agriculture, Kyoto University. Serratia marcescens (IFO 3048, IFO 3057); Aspergillus niger, IFO 4280 and Penicillium chrysogenum, IFO 5473 were obtained from the Institute for Fermentation, Osaka. Other strains were stock cultures of the laboratory. Lactobacilli were cultured at 37°C for 18 hr in the following medium: yeast extract, 1.0%; peptone, 0.5%; glucose, 1.0%; sodium acetate, 0.5%; KH₂PO₄, 0.2%; tween 80, 0.01%; pH 6.8. Escherichia strains were cultured at 37°C for 18 hr in modified Massen medium (glucose, 3.0%; L-malic acid, 0.07%; asparagine, 1.0%; K₂HPO₄, 0.25%; MgSO₄·7H₂O, 0.04%; Na₂CO₃, 0.25%; CaCl₂, 0.001%; pH 7.0).

Molds were cultured at 25°C for 5 days in Czapek-Dox medium enriched with yeast extract (sucrose, 3.0%; NaNO₃, 0.2%; K₂HPO₄, 0.1%; MgSO₄·7H₂O, 0.05%; FeSO₄·7H₂O, 0.001%; yeast extract, 0.05%; pH 6.0). Quinolinic acid-2,3,7,8-14C was kindly synthesized by Daiichi Pure Chemicals Co., Ltd., Tokyo. PRPP (tetrasodium salt, purity 90-95%) was purchased from Sigma Chemical Company. β-Phenylethylamine was purchased from Tokyo Chemical Industry Co., Ltd., Tokyo. POP and PPO were S.P. grade and other chemicals used were G.R. grade of Nakarai Chemicals, Ltd., Kyoto.

2. Preparation of crude homogenate. Animal organs, plants and mushrooms were homogenized with two to five volumes of isolating medium (0.01 M potassium phosphate buffer, pH 7.0 containing 0.01 M 2-mercaptoethanol) in a Waring blender. The homogenate was squeezed through two layers of gauze and centrifuged at 15,000×g for 20 min at 0-4°C. The supernatant solution was used for crude homogenate.

Yeast cells and mold myceria collected by filtration were ground with sea sand in a porcelain mortar, and extracted with the isolating medium. The extracts were centrifuged at 15,000×g for 20 min in the cold.

Bacterial cells collected by centrifugation (15,000×g for 15 min) were suspended in the isolating medium and sonicated for 10 min at 0°C with Ultra...
Sonic Oscillator 4210, Kaijo Denki. The sonicates were centrifuged at 15,000 × g for 20 min in the cold.

3. Determination of protein. Protein was determined by the method of LOWRY et al. (18) with bovine serum albumin as a standard.

4. Assay conditions. Reaction mixtures contained potassium phosphate buffer (50 μmoles, pH 7.0); quinolinic acid-2,3,7,8-14C (250 mμmoles, 50 mμCi); PRPP (100 mμmoles); magnesium sulfate (250 mμmoles for the crude homogenate from animal organs and microorganisms, 1.5 μmoles for the crude homogenate from plants and mushrooms) and crude homogenate (about one milligram of protein) in a final volume of 0.5 ml.

Reaction tube (15 × 70 mm) and counting vial in which Whatman No. 3MM filterpaper (15 × 77 mm) soaked with 0.2 ml of 25% β-phenylethylamine dissolved in methanol were connected with a thick rubber tube (19) as shown in Fig. 2.

The reaction was initiated by adding certain amount of enzyme with syringe, and incubated at 37°C for 1 hr. The reaction was stopped by injecting 0.8 ml of 4% perchloric acid with syringe through rubber tube.

During shaking at 37°C for 90 min in the shaking incubator (120 strokes per min), the evolved 14CO2 is completely trapped by β-phenylethylamine on filterpaper. Then 10 ml of scintillator (4 g of PPO and 0.1 g of POPOP per liter of toluene) was added to each counting vial, and radioactivity was determined by Packard Tri-Carb Liquid Scintillation Spectrometer Model 2002.

One unit of the enzyme activity is defined as the amount of enzyme to de-
The measurements of the enzyme activity were carried out duplicate and the unit of the activity were expressed as an average value.

RESULTS

Distribution in mammals (Tables 1–3)

Distribution of the enzyme in various organs of rat and mouse was investigated. The results are shown in Tables 1 and 2. The results indicated that the enzyme was mainly localized in liver and kidney, and that the activity was extremely high in livers, which coincided with the report by NISHIZUKA (20), but some activities were also found in brain and spleen. It was noticed that in rabbit and hog the enzyme activity in kidney was significantly higher than in liver (Table 3).

Table 1. Distribution of quinolinate phosphoribosyltransferase in various organs of rat (Donryu, male).

| Organs        | Protein (mg) | Enzyme activity (units) | Specific activity (units/mg) |
|---------------|--------------|-------------------------|-----------------------------|
| Liver         | 52.9         | 266.5                   | 5.0                         |
| Kidney        | 60.0         | 57.2                    | 0.9                         |
| Spleen        | 45.0         | 7.2                     | 0.2                         |
| Brain         | 19.5         | 2.9                     | 0.1                         |
| Thigh muscle  | 43.3         | 5.6                     | 0.1                         |
| Small intestine | 40.0      | trace                   | —                           |
| Lung          | 37.2         | trace                   | —                           |
| Heart         | 18.9         | trace                   | —                           |
| Testicle      | 42.0         | nd (not detectable)     | —                           |

* Data are expressed as values per one gram of fresh weight. One unit is defined as the amount of enzyme required to decarboxylate 1 μmole of quinolinic acid under standard assay conditions.

Table 2. Distribution of quinolinate phosphoribosyltransferase in various organs of mouse (dds, male).

| Organs        | Protein (mg) | Enzyme activity (units) | Specific activity (units/mg) |
|---------------|--------------|-------------------------|-----------------------------|
| Liver         | 62.9         | 194.5                   | 3.1                         |
| Kidney        | 75.6         | 96.3                    | 1.3                         |
| Brain         | 22.6         | 15.8                    | 0.7                         |
| Spleen        | 67.6         | 21.8                    | 0.3                         |
| Stomach       | 18.6         | 6.0                     | 0.3                         |
| Heart         | 20.0         | 6.1                     | 0.3                         |
| Lung          | 52.2         | trace                   | —                           |
| Small intestine | 48.2      | trace                   | —                           |
| Thigh muscle  | 34.6         | trace                   | —                           |
Table 3. Distribution of quinolinate phosphoribosyltransferase in animals.

| Animals | Organs | Protein (mg) | Enzyme activity (units) | Specific activity (units/mg) |
|---------|--------|--------------|-------------------------|----------------------------|
| Rabbit  | Liver  | 28.3         | 14.4                    | 0.5                        |
|         | Kidney | 48.2         | 53.2                    | 1.1                        |
| Hog     | Liver  | 113.4        | 63.6                    | 0.6                        |
|         | Kidney | 51.4         | 94.3                    | 1.8                        |
| Beef    | Liver  | 78.8         | 74.1                    | 0.9                        |
|         | Kidney | 46.8         | 50.5                    | 1.1                        |
| Chicken | Liver  | 65.5         | 34.7                    | 0.5                        |

Table 4. Distribution of quinolinate phosphoribosyltransferase in fish livers.

| Fishes | Protein (mg) | Enzyme activity (units) | Specific activity (units/mg) |
|--------|--------------|-------------------------|----------------------------|
| Eel (Unagi) [Anguilla japonica Temminck & Schlegel] | 59.2 | 42.3 | 0.7 |
| Pike eel (Hamo) [Muraenesox cinereus (Forskal)] | 61.3 | 16.7 | 0.3 |
| Til fish (Amadaï) [Branchiostegus japonicus (Hontsuyn)] | 90.5 | 13.9 | 0.2 |
| Yellow-tail (Buri) [Seriolia quinquera diata Temminck & Schlegel] | 67.2 | 10.3 | 0.2 |
| Bonito (Katsuo) [Euthynnus pelamys (Linné)] | 81.2 | trace | — |
| Sea bream (Tai) [Pagrosomus unicolor (Quoy & Gaimard)] | 51.9 | trace | — |
| Snake head (Raigyo) [Canna argus Cantor] | 48.9 | trace | — |
| Carp (Koi) [Cyprinus carpio Linné] | 106.8 | nd | — |
| Crucian carp (Funa) [Cyprinus carassius Linné] | 99.0 | nd | — |

Distribution in fish livers (Table 4)

Relatively low activities were found in fish livers except in eel. In both carp and crucian carp, the activity was not detected.

Distribution in plants (Table 5)

High activities were observed only in persimmon young leaf and cucumber. In the aseptically grown Lemna minor L. activity was not high. In some plants the enzyme activity was very low or not detectable.

Distribution in mushrooms (Table 6)

Activities were detected in all mushrooms tested, and high activities were present in Shiitake mushroom and Enokitake mushroom.
Table 5. Distribution of quinolinate phosphoribosyltransferase in plants.

| Plants                        | Protein (mg) | Enzyme activity (units) | Specific activity (units/mg) |
|-------------------------------|--------------|-------------------------|-------------------------------|
| Japanese persimmon (young leaf) | 4.2          | 22.2                    | 5.3                           |
| Cucumber (fruit)              | 3.2          | 16.9                    | 5.3                           |
| Parsley (leaf)                | 7.3          | 7.1                     | 1.0                           |
| Common horsetail [Tsukushi]   | 2.8          | 2.7                     | 1.0                           |
| Garden asparagus (green)      | 8.9          | 4.6                     | 0.5                           |
| Turnip (root)                 | 3.7          | 1.8                     | 0.5                           |
| Sweet potato                  | 7.3          | 2.5                     | 0.3                           |
| Tomato (fruit)                | 1.9          | 0.5                     | 0.3                           |
| Pea seedling (2 day-old)      | 37.8         | 10.0                    | 0.3                           |
| Duckweed (Lemna minor L.), aseptically grown | 3.4 | 0.6 | 0.2 |
| Okura (young pod)             | 3.4          | 0.8                     | 0.2                           |
| Trefoil (leaf)                | 11.0         | 2.4                     | 0.2                           |
| Onion (bulb)                  | 4.1          | 0.9                     | 0.2                           |
| Garlic (bulb)                 | 5.2          | 1.1                     | 0.2                           |
| Cabbage                       | 4.2          | trace                   | —                             |
| Spinach (leaf)                | 8.0          | trace                   | —                             |
| Mappé bean sprouts            | 4.6          | nd                      | —                             |
| Carrot (leaf)                 | 2.7          | nd                      | —                             |
| Head lettuce                  | 1.4          | nd                      | —                             |

Table 6. Distribution of quinolinate phosphoribosyltransferase in mushrooms.

| Mushrooms (fruit body)         | Protein (mg) | Enzyme activity (units) | Specific activity (units/mg) |
|--------------------------------|--------------|-------------------------|-------------------------------|
| Shiitake [Lentinus edodes (Berk.) Sing.] | 4.9          | 33.3                    | 6.8                           |
| Enokitake [Flammulina velutipes (Fr.) Sing.] | 3.7          | 23.3                    | 6.3                           |
| Kikurage [Auricularia mesenterica (Dicks.) Pers.] | 8.6          | 17.1                    | 2.0                           |
| Nameko [Pholiota nameko (T. Ito) S. Ito et Imai] | 3.1          | 5.2                     | 1.7                           |
| Hiratake [Pleurotus ostreatus (Fr.) Quel.] | 15.6         | 17.5                    | 1.1                           |
| Itchitake [Psathyrella candolleana (Fr.) A. H. Smith] | 3.8          | 1.2                     | 0.3                           |
| Matsutake [Tricholoma matsutake (S. Ito et Imai) Sing.] | 5.3          | 1.1                     | 0.2                           |
| Seiyomatsutake [Agaricus bisporus (Lange) Sing.] | 8.6          | 1.5                     | 0.2                           |

Distribution in microorganisms (Table 7)

In microorganisms the enzyme activity was relatively low, except in baker’s yeast, *Pseudomonas riboflavina* and *Neurospora crassa*. In *Lactobacillus* the activity was not detected. It is noteworthy that in some bacteria such as *Escherichia coli* Crookes Ajinomoto, AKU 0001 and *Bacillus cereus* IFO 3131, which are auxotroph for nicotinic acid and related compounds, the enzyme activity is trace or not detectable.
DISCUSSION

The radioassay for quinolinate phosphoribosyltransferase using quinolinate-2,3,7,8-\textsuperscript{14}C as substrate was confirmed to be useful for measuring routinely the enzyme activity in various organisms, because of its high specificity, convenience and accuracy. With applying this radioassay the distribution of the enzyme in various animals, plants and microorganisms has been investigated in detail.

This enzyme was found to be widely distributed in nature. In mammals, fairly high enzyme activity was found in rat and mouse livers. In rat and mouse, NAD may be synthesized de novo almost only in liver and kidney, mainly in liver. Other organs scarcely utilize quinolinic acid for the biosynthesis of NAD. These data are agree well with published data (20).

In fish livers, the enzyme activity was generally low. In limnetic fish, \textit{i.e.}, carp and crucian carp, the activity was not detected.

In plants, high enzyme activity was present in persimmon young leaf and cucumber. In other plants the activity was relatively low. Since the enzyme activity was detected even in aseptically grown \textit{Lemna minor} L., the plant by itself might be able to synthesize NAD from quinolinic acid to some extents. In mappé
bean sprouts, carrot leaf and head lettuce, however, the enzyme activity was not detected. These results are not agreed in part with published data. It was interested that relatively high activity was found in some edible mushrooms.

Extremely high enzyme activity was observed in Neurospora crassa which has been known to synthesize NAD from tryptophan (6). High activities were also present in baker's yeast and Pseudomonas riboflavina. On the other hand, the enzyme activity was not detected in Lactobacillus which require nicotinic acid or nicotinamide for the growth, due to the lack of de novo pathway of NAD (21).

It is noteworthy that the enzyme activity is not detectable or trace in some fish livers, bacteria and plants, in spite of their NAD contents. These results suggest that another de novo biosynthetic pathway of NAD, which does not pass through quinolinic acid as an intermediate, may present in those organisms.

The authors are grateful to Prof. Y. Nishizuka, Faculty of Medicine, Kobe University, for supplying the valuable quinolinic acid-2,3,7,8-14C and PRPP which were used during preliminary experiment, and for his continuous encouragement in these studies.

Thanks are also due to Dr. A. Ichiyama, Faculty of Medicine, University of Tokyo, and Dr. S. Nakamura, Faculty of Medicine, Kyoto University, for their helpful discussions and suggestions.

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