CHARACTERISTICS OF QUINOLINATE PHOSPHORIBOSYLTRANSFERASE FROM THE "SHIITAKE" MUSHROOM (*LENTINUS EDODES*)¹

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A highly purified preparation of quinolate phosphoribosyltransferase was obtained from the "Shiitake" mushroom and some characteristics of this enzyme were investigated. Maximum activity was observed at an incubation temperature of 50°C. Monovalent cations generally had no effect, but the divalent and trivalent cations were more or less inhibitory. Fe^{3+} , Ni^{2+} , Zn^{2+} , Al^{3+} and Fe^{3+} strongly inhibited the reaction and Cl^- and NO_3^- were also inhibitory. The PRPP saturation curve of this enzyme was sigmoidal. PRPP may be an allosteric effector to this enzyme. Quinolinic acid analogues (pyridine di- and monocarboxylic acids) exerted no influence on the reaction. Some nucleotides were inhibitory, especially ATP, which was the most potent inhibitor. SH reagents and albocycline did not affect the activity. Possible regulations of the *de novo* biosynthesis of NAD in the mushroom are discussed.

Quinolate phosphoribosyltransferase, an intermediary enzyme in the *de novo* NAD biosynthetic pathway, was extracted until it was chromatographically pure, for the first time, from a non-animal source—the "Shiitake" mushroom. Properties and characteristics of the enzyme were investigated to resolve the mechanism of the *de novo* NAD biosynthetic pathway and of the metabolic regulation of NAD. Some novel aspects of this enzyme preparation were found. Purification and general properties of this enzyme were described in a previous paper (1). This paper describes the characteristics of quinolate phosphoribosyltransferase from the "Shiitake" mushroom.

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Abbreviations used: NAD, nicotinamide adenine dinucleotide; NaMN, nicotinic acid mononucleotide.

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EXPERIMENTS

1. *Materials.* Quinolinate phosphoribosyltransferase was extracted and purified 1,060-fold from the "Shiitake" mushroom by the method described in a previous paper (1). Monoiodoacetic acid was twice crystallized from petroleum ether before use. Albocycline was a gift from Tanabe Seiyaku Co., Ltd., Toda, Saitama, Japan (2).

2. *Assay materials and conditions.* Standard assay materials were: a reaction mixture containing Tris-maleate-NaOH buffer, pH 6.5 (12.5 μ moles); quinolinic acid-2,3,7,8- 14 C (87 m μ moles, 17.4 m μ Ci); PRPP (100 m μ moles); magnesium sulfate (1.5 μ moles) and 4 μ g of purified enzyme in a total volume of 0.5 ml. Incubation was carried out at 37°C for 30 min. Except where otherwise noted, the above assay condition was used. The reaction was stopped by adding perchloric acid. The 14 CO $_2$ which formed as a result of the enzymatic reaction was completely trapped in β -phenylethylamine. Radioactivity was measured with a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 2425. One millimicromole of 14 CO $_2$ formed was equivalent to 1 m μ mole of quinolinic acid-2,3,7,8- 14 C utilized under the enzymatic reaction.

RESULTS

Effect of temperature

The effect of temperature on activity was investigated in an incubation temperature range between 10°C and 65°C. Maximum activity was observed at 50°C as shown in Fig. 1.

Fig. 1. Effect of temperature on quinolinate phosphoribosyltransferase activity. Standard assay conditions were employed, except for various incubation temperatures as indicated.

Effect of metal ions

Many different kinds of metal ions (generally in the form of sulfate salts) were added to the reaction mixture at a final concentration of 1 mM. Monovalent cations had almost no effect, but polyvalent cations were more or less inhibitory. Al^{3+} , Fe^{3+} , Fe^{2+} , Ni^{2+} and Zn^{2+} almost completely inhibited the reaction at a final concentration of 1 mM (Table 1).

Table 1. Inhibition of quinolinate phosphoribosyltransferase activity by metal ions.

The metal ions indicated were each added to the standard assay reaction mixture at a final concentration of 1 mM.

Metal ions	Inhibition (%)
None	0
Fe^{2+}	98.1
Zn^{2+}	95.9
Ni^{2+}	95.5
Hg^{2+}	82.9
Cu^{2+}	86.3
Co^{2+}	63.2
Cd^{2+}	34.4
Mn^{2+}	23.8
Ca^{2+}	18.0
Al^{3+}	99.7
Fe^{3+}	96.3
K^{+}	9.5
Rb^{+}	7.7
Na^{+}	5.1
Tl^{+}	2.0
Cs^{+}	1.6
Li^{+}	0

Effect of anions

The effect of anions (in the form of magnesium salts) on activity was tested at various concentrations. As shown in Fig. 2, acetate and sulfate ions had no effects, but chloride and nitrate ions were inhibitory.

Allosteric property

The PRPP saturation curve of this enzyme is sigmoidal (Fig. 3). The Hill plot ($\log v/V - v$, is plotted against $\log [\text{PRPP}]$) of this data shows a straight line. An "n" value of 1.83 from its slope and a K_m value for PRPP of 2.3×10^{-5} M from its intercept at an abscissa were obtained (Fig. 4).

Effect of quinolinate analogues

Quinolinate analogues, pyridine mono- and dicarboxylic acids were added to

Fig. 2. Effect of anions on quinolate phosphoribosyltransferase activity. Standard assay materials and conditions were used, except each anion was added in the form of magnesium salts at various concentrations as indicated ○—○ Mg (CH₃COO)₂, ●—● MgSO₄, △—△ MgCl₂, ×—× Mg(NO₃)₂.

Fig. 3. Effect of PRPP concentration on quinolate phosphoribosyltransferase activity. Standard assay materials were used, except for PRPP concentrations as indicated.

the standard assay reaction mixture at a final concentration of 1 mM. No analogue, except pyridine-2,3-dicarboxylic acid, had any effect as shown in Table 2. Pyridine-2,3-dicarboxylic acid is quinolinic acid and the apparent inhibition caused by it was the result of the isotope dilution effect, because cold quinolinic acid was added to 1 mM in the standard assay reaction mixture.

Effect of nucleotides

The effect of many kinds of nucleotides was investigated at final concentrations of 1 mM and 5 mM. As shown in Table 3, triphosphates were inhibitory.

Fig. 4. Hill plot of the fractional saturation of quinolinate phosphoribosyltransferase by PRPP. Calculated from the data obtained from Fig. 3.

Table 2. Effect of quinolinate analogues on quinolinate phosphoribosyltransferase activity.

Standard assay materials were used, except that the quinolinate analogues indicated were each added at a final concentration of 1 mM.

Addition	Relative activity
None	100
Quinolinic acid (Pyridine-2,3-dicarboxylic acid)	31
Lutidinic acid (Pyridine-2,4-dicarboxylic acid)	102
<i>iso</i> -Cinchomeric acid (Pyridine-2,5-dicarboxylic acid)	101
Dipicolinic acid (Pyridine-2,6-dicarboxylic acid)	97
Cinchomeric acid (Pyridine-3,4-dicarboxylic acid)	99
Dinicotinic acid (Pyridine-3,5-dicarboxylic acid)	101
Nicotinic acid (Pyridine-3-carboxylic acid)	100
Nicotinamide (Pyridine-3-carboxylic acid amide)	99
<i>iso</i> -Nicotinic acid (Pyridine-4-carboxylic acid)	99
Picolinic acid (Pyridine-2-carboxylic acid)	98

ATP was the strongest inhibitor of the nucleotides tested, but mono- and diphosphates scarcely inhibited the reaction. Figure 5 shows the inhibition of enzyme activity at various concentrations of ATP. At 3 mM ATP, the reaction was inhibited about 50%, and it was completely inhibited at 10 mM ATP.

Table 3. Inhibition of quinolinate phosphoribosyltransferase activity by nucleotides.

The nucleotides indicated were each added to the standard assay reaction mixture at a final concentration of 5 mM.

Nucleotides	Inhibition (%)
None	0
AMP	0
ADP	12.0
ATP	90.0
GMP	1.1
GDP	9.9
GTP	38.9
ITP	49.3
CTP	40.1
UTP	46.5
FAD	4.2
NAD	0
NADH	2.8
NADP	3.6
NADPH	0

Fig. 5. Inhibition of quinolinate phosphoribosyltransferase activity by ATP. Standard assay materials were used, except ATP was added to the reaction mixture at various concentrations as indicated.

Effect of SH reagents

Each SH reagent was first incubated with the enzyme at the final concentrations given in Table 4. After this preliminary incubation at 37°C for 30 min, the enzymatic reaction was continued under standard assay conditions. At various concentrations of SH reagents tested, the enzymatic reaction was never inhibited.

Table 4. Effect of SH reagents on quinolinate phosphoribosyltransferase activity.

Enzyme protein was first incubated with each SH reagent at 37°C for 30 min. After this preliminary incubation, the enzymatic reaction was carried out under standard assay conditions.

SH reagent	Concentration (M)	Relative activity
None	—	100
<i>p</i> -Chloromercuribenzoic acid	10 ⁻⁶	114
	10 ⁻⁵	124
	10 ⁻⁴	119
	10 ⁻³	114
Monoiodoacetic acid	10 ⁻⁵	95
	10 ⁻⁴	100
	10 ⁻³	102
	10 ⁻²	92
<i>N</i> -Ethylmaleimide	10 ⁻⁵	98
	10 ⁻⁴	104
	10 ⁻³	106
	10 ⁻²	96

Effect of albocycline

Albocycline is an antibiotic known to be an inhibitor of the *de novo* NAD biosynthetic pathway (3). The structure of albocycline is shown in Fig. 6. Albocycline was dissolved in *N,N*-dimethylformamide and a 1 mg/ml solution was prepared. This solution (0–50 μ l) was added to the standard assay reaction mixture. As the reference, an equal volume of *N,N*-dimethylformamide was added instead of the albocycline solution. The relative activity shown in Table 5 is a product of the comparison with the reference activity. This antibiotic did not inhibit the enzymatic reaction as shown in Table 5.

Fig. 6. Structure of albocycline.

Table 5. Effect of albocycline on quinolinate phosphoribosyltransferase activity.

Albocycline was dissolved in *N,N*-dimethylformamide, then added to the standard assay reaction mixture at the final concentrations indicated.

Albocycline ($\mu\text{g/ml}$)	Relative activity
0	100
2	99
10	102
20	102
100	103

DISCUSSIONS

“Shiitake” is a most popular edible mushroom in Japan, which is obtainable in all seasons, because it is cultivated. It contains large amounts of the vitamin B group in comparison to other plants. The nicotinamide content is especially high, existing in the form of NAD for the most part (4). Quinolinate phosphoribosyltransferase activity is very high in this mushroom (5), but no extraction and purification of this enzyme from plants including mushrooms has yet been reported. The enzyme was extracted for the first time from the mushroom to the extent of being chromatographically pure. However, in polyacrylamide gel disc electrophoresis, using the method of DAVIS (6) at pH 8.9, the purified preparation appeared as three similar bands. Equal enzyme activity was found in two of the three bands. In the other band, no activity was detected. It was not determined whether the activity-less band is an impure contamination or a denatured protein of quinolinate phosphoribosyltransferase. This enzyme from the “Shiitake” mushroom is relatively heat stable, *i.e.*, its activity did not diminish after heat treatment at 60°C for 10 min without the presence of a substrate or stabilizing agent. But above 60°C in the same treatment, activity rapidly decreased. At 80°C the activity was totally lost. If the incubation temperature is raised above 50°C, the activity decreases because of decomposition of the unstable substrate—PRPP, in addition to the heat denaturation of the enzyme.

The mode of metal ion inhibition of this enzyme is similar to that of the mammalian enzyme (7, 8). Activation by monovalent cations in the mammalian enzyme has been reported, but no activation was observed in the mushroom enzyme. The allosteric property of this enzyme has been demonstrated for the first time in the present study. NAD biosynthesis may be regulated by the PRPP concentration. The effect of quinolinic acid analogues (pyridine carboxylic acids) was investigated. None of the analogues tested, including nicotinic acid and nicotinamide, exerted an influence on the activity. This shows that quinolinic acid can not be replaced as the substrate by its analogues, because if the analogues

combine with the active site of quinolinic acid then radioactivity will be decreased. Many kinds of nucleotides, including purine-, pyrimidine- and coenzyme nucleotides, were added to the standard analysis reaction mixture at final concentrations of 1 mM and 5 mM. Triphosphate nucleotides (ATP, CTP, UTP, ITP) were fairly inhibitory, and diphosphate nucleotides (ADP, GDP) were slightly inhibitory. But mononucleotides (AMP, GMP) did not inhibit the activity. Coenzyme nucleotides (FAD, NAD *etc.*) had almost no effect. Inhibition of enzyme activity by nucleotides has also been reported in beef liver enzyme, and this inhibition was recovered by increasing the magnesium concentration, *i.e.*, the nucleotides acted as chelating agents to the magnesium ion. But in the mushroom enzyme, inhibition by ATP was not completely recovered by increasing the magnesium concentration. Neither was the rate of inhibition by nucleotides always proportional to the stability constants of the magnesium ion with nucleotides (9). These results show that the inhibition is not always due only to the chelating effect of nucleotides. Quinolate phosphoribosyltransferase from the "Shiitake" mushroom is not repressed by NAD, NADP and their reduced forms. This agrees with data on the *Bacillus subtilis* enzyme (10). The mushroom enzyme is not inhibited by several SH reagents even at a high concentration. Therefore, the SH group is not directly essential for revealing enzyme activity. Inhibition by albocycline was not observed. This antibiotic may inhibit the reaction before quinolate is formed in the *de novo* NAD biosynthetic pathway. The *de novo* biosynthesis of NAD in mushrooms may be regulated in various ways, *e.g.*, by metal ions, anions, nucleotides, and PRPP levels.

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