Crystalline Quinolate Phosphoribosyltransferase from *Alcaligenes eutrophus* subsp. *quinolinicus*: Effects of Metal Ions, Phosphorous Compounds, Niacin Nucleotides and Glycerol*

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Effects of various substances on crystalline quinolate phosphoribosyl transferase (EC 2.4.2.19) activity from *Alcaligenes eutrophus* subsp. *quinolinicus* were investigated. The enzyme activity was inhibited by divalent cations but not by mono- and trivalent cations. Phosphorous compounds like ATP inhibited the enzyme activity but this inhibition was completely recovered by raising the Mg^{2+} ion concentration. With regard to the effect of reaction products, nicotinic acid mononucleotide and inorganic pyrophosphate inhibited but CO_2 did not. Nicotinic acid mononucleotide inhibited competitively for quinolinic acid and 5-phosphoribosyl-1-pyrophosphate. The effect of glycerol on the enzyme activity was different by pH. Glycerol caused a decrease in K_m values for quinolinic acid and 5-phosphoribosyl-1-pyrophosphate and the turnover rate. The enzyme activity was not affected by 2-mercaptoethanol even at a final concentration of 0.3 M.

NaMN is the first niacin-active compound in *de novo* NAD biosynthetic pathway. NaMN is synthesized from quinolinic acid and PRPP by QPRTase (EC 2.4.2.19).¹⁾

Gopalan and co-workers²⁾ reported that the oral administration of excess L-leucine increased the urinary excretion of quinolinic acid both in normal subjects and in patients suffering from pellagra. The cause is attri-

buted to a decrease of QPRTase activity.³⁾ Ghafoorunissa and Rao⁴⁾ reported that QPRTase is the rate limiting enzyme in tryptophan-NAD pathway and that its levels in livers of rats fed a low protein diet were significantly higher than levels observed in rats fed a high protein diet. Further, Sathanarayana and Rao⁵⁾ have considered that alterations in the activity of QPRTase may be taken as a measure of the efficiency of the conversion of tryptophan to NAD. In castor bean, the level of this enzyme changed according to the stage of development.⁶⁾ The effects of various substances on the enzyme activity were investigated using crystalline hog liver enzyme.^{7,8)} Its activity was inhibited by many kinds of nucleotides, phthalic acid, SH reagents and metal ions.^{7,8)}

As noted above, there is a tendency for mammalian QPRTase activity to be affected by numerous factors. It has also been reported that "Shiitake" mushroom enzyme has an allosteric property for PRPP.⁹⁾ However, the factors which regulate bacterial enzyme activity are not known. We crystallized QPRTase with characteristic properties

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Abbreviations: QPRTase, quinolate phosphoribosyltransferase; NaMN, nicotinic acid mononucleotide; d-NAD, nicotinic acid adenine dinucleotide; PRPP, 5-phosphoribosyl-1-pyrophosphate.

from *Alcaligenes eutrophus* subsp. *quinolinicus* which has an extremely high QPRTase activity.^{10,11)}

This paper describes the effects of various substances on the enzyme activity.

MATERIALS AND METHODS

Enzyme. QPRTase was purified and crystallized twice¹⁰⁾ from *Alcaligenes eutrophus* subsp. *quinolinicus* IAM 12305.¹⁰⁾

Materials. Quinolinic acid-2,3,7,8-¹⁴C was purchased from the Daiichi Pure Chemicals Co., Ltd., Tokyo. NaMN was a gift from the Kyowa Hakko Co., Ltd., Tokyo. NMN, d-NAD, NAD, NADH, NADP, NADPH, AMP, CMP, CDP, CTP, IDP, ITP, GDP, UDP and PRPP were purchased from Sigma Chemical Company. ADP and GMP were products of the Kyowa Hakko Co., Ltd., Tokyo. ATP, IMP, GTP, dTMP, dTDP, dTTP were obtained from Boehringer Mannheim GmbH. All chemicals were of the highest purities obtainable.

Enzyme assay. QPRTase was assayed by counting the radioactivity of the ¹⁴CO₂ released from the PRPP dependent decarboxylation of quinolinic acid-2,3,7,8-¹⁴C.¹²⁾ The standard enzyme assay reaction mixture contained the following, in μ mol, in a total volume of 0.5 ml: Tris-HCl buffer at pH 8.5, 25; quinolinic acid-2,3,7,8-¹⁴C, 0.39; PRPP, 0.5; MgCl₂, 0.75 and 10 μ l of enzyme solution dissolved in 50 mM potassium phosphate buffer containing 10 mM 2-mercaptoethanol at pH 7.0. The reaction was stopped after incubating for 10 min at 37°C by adding 0.8 ml of 4% perchloric acid.

Protein concentration. Protein concentration was determined spectrophotometrically using $E_{1\text{cm}}^{1\%}$ of 3.54 at 280 nm.

RESULTS

Effect of metal ions

Each metal ion was added to the standard assay reaction mixture at a final concentration of 1 mM. The result is shown in Table I. Mono- and trivalent cations had no effect on the enzyme activity. However, divalent cations such as Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Cd²⁺ and Hg²⁺ completely inhibited the enzyme activity.

TABLE I. EFFECT OF METAL IONS ON QPRTASE ACTIVITY

The standard assay for QPRTase was used except for addition of the indicated metal ions (chloride form).

Metal ions (1 mM)	Inhibition (%)
None	0
K ⁺	0
Na ⁺	1
Li ⁺	2
Mn ²⁺	20
Co ²⁺	98
Ni ²⁺	100
Cu ²⁺	94
Zn ²⁺	100
Fe ²⁺	13
Ca ²⁺	52
Cd ²⁺	99
Hg ²⁺	100
Fe ³⁺	0
Al ³⁺	0

Effect of nucleotides

The effect of nucleotides on QPRTase activity was investigated. This result is presented in Table II. Each nucleotides was

TABLE II. EFFECT OF VARIOUS NUCLEOTIDES ON QPRTASE ACTIVITY

The standard assay for QPRTase was used except for addition of the indicated nucleotides.

Addition (1 mM)	Relative activity (%)
None	100
AMP	102
ADP	88
ATP	78
UMP	99
UDP	95
UTP	81
GMP	101
GDP	95
GTP	83
CMP	100
CDP	87
CTP	70
IMP	99
IDP	90
ITP	76
dTMP	100
dTDP	86
dTTP	71

added at a final concentration of 1 mM. Mono- and diphosphates of nucleotides had no effect on the reaction. Triphosphates slightly inhibited the reaction. The enzyme activity was further inhibited as ATP concentration increased. When ATP at a final concentration of 5 mM was added to the reaction mixture, the reaction was completely inhibited. But this inhibition was entirely recovered by raising the Mg^{2+} ion concentration. It might be considered that ATP- Mg^{2+} complex was formed. Optimum Mg^{2+} ion concentration was 1.5 mM in the absence of ATP, but rose to 7 mM in the presence of 5 mM ATP, as shown in Fig. 1.

FIG. 1. Effect of $MgCl_2$ Concentration on QPRTase Activity in the Presence of 5 mM ATP.

The standard assay for QPRTase was used except for $MgCl_2$ concentrations as indicated. ATP was added to the reaction mixture at a final concentration of 0 (—●—) and 5 mM (—○—).

Effect of niacin nucleotides

Table III shows the effect of niacin nucleotides on the enzyme activity. Only NaMN, which is one of the reaction products, inhibited.

Effect of reaction products

i) *NaMN*. NaMN is the most important product from the QPRTase catalyzing reaction. The bacterial enzyme activity was inhibited by NaMN (Table III). The inhibition type of NaMN for quinolinic acid was competitive and its K_i value was 8.38×10^{-5} M, and also, the inhibition type for PRPP was competitive and its K_i value was 2.65×10^{-4} M as shown in Fig. 2.

TABLE III. EFFECT OF NIACIN NUCLEOTIDES ON QPRTASE ACTIVITY

The standard assay for QPRTase was used except for addition of the indicated niacin nucleotides.

Addition (1 mM)	Relative activity (%)
None	100
NaMN	54
NMN	94
d-NAD	93
NAD	95
NADH	95
NADP	96
NADPH	94

FIG. 2. Inhibition of QPRTase Activity by NaMN.

The standard assay for QPRTase was used except for various concentrations of quinolinic acid (A) and PRPP (B) as indicated. NaMN was added to the reaction mixture at a final concentration of 1 (a), 0.5 (b) and 0 mM (c).

ii) *Inorganic pyrophosphate*. Inorganic pyrophosphate was released when quinolinic acid and PRPP were condensed by the enzyme. The enzyme activity was completely inhibited when $Na_4P_2O_4$ at 3 mM was added to the reaction mixture. Even when Mg^{2+} ion concentration was raised, the activity was not recovered fully as shown in Fig. 3.

iii) *Carbon dioxide*. When $NaHCO_3$ at 10 mM was added to the standard reaction mixture, it did not inhibit the enzyme activity.

Effect of glycerol

Glycerol caused to change the K_m values of quinolinic acid and PRPP as shown in Fig. 4. In the absence of glycerol, K_m values of quinolinic acid and PRPP are 9.1×10^{-5} M and 1.28×10^{-4} M, respectively. In the pre-

FIG. 3. Effect of MgCl_2 Concentration on QPRTase Activity in the Presence of 3 mM $\text{Na}_4\text{P}_2\text{O}_7$.

The standard assay for QPRTase was used except for MgCl_2 concentrations as indicated. $\text{Na}_4\text{P}_2\text{O}_7$ was added to the reaction mixture at a final concentration of 0 (—○—) and 3 mM (—●—).

FIG. 4. Effect of Glycerol on K_m Values of quinolinic Acid (A) and PRPP (B).

The standard assay for QPRTase used except for quinolinic acid and PRPP concentrations as indicated. Glycerol was added to the reaction mixture at a final concentration of 0 (—○—) and 30% (—●—).

sence of 30% glycerol, K_m values of quinolinic acid and PRPP are 5.1×10^{-5} M and 5.7×10^{-5} M, respectively. Glycerol decreased both K_m values by about half. In other words, QPRTase has a higher affinity for substrates in the presence of glycerol. The turnover rate of this enzyme was decreased by adding glycerol.

Furthermore the effect of glycerol on the enzyme activity was investigated as a function of pH. As Table IV shows, glycerol markedly activated the enzyme activity at pH 6.1 and 6.5, while it inhibited the activity at pH above 7.0. The inhibition was strongest at pH 9.0. At pH above 9.0, the inhibition

TABLE IV. EFFECT OF GLYCEROL ON QPRTase ACTIVITY AS A FUNCTION OF pH.

The standard assay for QPRTase was used for various pH buffers at constant ionic strength ($\mu=0.02$). Values expressed as percentage of the activity in the presence of 30% glycerol to the activity in the absence of glycerol.

pH (Buffer)	Relative activity (%)
6.1 (McIlvaine)	408
6.5 (McIlvaine)	228
7.0 (McIlvaine)	81
8.0 (McIlvaine)	46
7.5 (Tris-HCl)	61
8.0 (Tris-HCl)	42
8.5 (Tris-HCl)	53
8.5 (Glycine-NaOH)	84
9.0 (Glycine-NaOH)	37
9.5 (Glycine-NaOH)	45
10.0 (Glycine-NaOH)	74
10.5 (Glycine-NaOH)	78

decreased as the pH rose.

Effect of 2-mercaptoethanol

The effect of 2-mercaptoethanol on QPRTase activity was investigated. Even at 300 mM, it did not affect the enzyme activity.

Effect of carboxylic acids

Unlike hog liver QPRTase,¹³⁾ this bacterial QPRTase activity was not inhibited by carboxylic acids.

Effect of amino acids

All amino acids including protein at a final concentration of 1 mM did not inhibit this enzyme activity.

DISCUSSION

Effects of mono- and divalent cations on the bacterial enzyme activity were same as hog liver enzyme⁷⁾ and "Shiitake" mushroom enzyme.⁹⁾ Trivalent cations did not inhibit the bacterial enzyme activity, while they strongly inhibited hog liver⁷⁾ and "Shiitake" mushroom⁹⁾ enzyme activities. One of the inhibitory effects by divalent cations may be the formation of an inactive binary complex

between cation and substrates, quinolinic acid and PRPP. Also, another inhibitory effect by cations might be to attack the enzyme protein directly. With hog liver⁸⁾ and the bacterial enzymes, ATP inhibition was entirely reactivated by raising the Mg^{2+} ion concentration. This inhibition may be the result from lowering the effective Mg^{2+} ion concentration for the enzyme and not from interaction between the enzyme and ATP.

This bacterial enzyme activity was inhibited by inorganic pyrophosphate and NaMN which were both reaction products. In beef liver nicotinate phosphoribosyltransferase,¹⁴⁾ NaMN is known to be a competitive inhibitor for nicotinic acid and PRPP. In QPRTase, however, this is the first observation of NaMN inhibiting competitively for quinolinic acid and PRPP. It is suggested that NAD biosynthesis is regulated by the concentration of NaMN.

This bacterial enzyme showed a multiple profile in polyacrylamide gel disc electrophoresis at pH 9.4 and 8.0.¹⁰⁾ But the enzyme showed a single band when suitable amounts of glycerol and 2-mercaptoethanol were added.¹⁰⁾ The enzyme activity was inhibited by glycerol at pH 8.5, but this inhibition was reversible. The effect of glycerol on the enzyme activity was dependent on pH. Glycerol caused a decrease in both K_m values for quinolinic acid and PRPP at pH 8.5. Recently, Bradbury and Jakoby¹⁵⁾ have reported that aldehyde dehydrogenase from yeast has a higher affinity for its substrate in the presence of glycerol. Therefore, it is probable that this bacterial enzyme is also produced a reversible conformational change by glycerol. As a result, the enzyme seems to

be stabilized from denaturation during electrophoresis. The additional effect of 2-mercaptoethanol during electrophoresis may be due to a protection from the reaction between peroxide, remaining in the polyacrylamide gel, and SH groups having a very important role in exhibition of the enzyme activity.

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