Crystalline Quinolinate Phosphoribosyltransferase from Hog Liver: The Molecular Weight and Some Enzymic Properties[†]

Kazuo IWAI, Katsumi SHIBATA and Hiroshi TAGUCHI*

Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan

**Laboratory of Biochemistry, Faculty of Agriculture,*

Mie University, Tsu, Mie 514, Japan

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The molecular weight of crystalline quinolinate phosphoribosyltransferase from hog liver was re-evaluated at 202,000 and 210,000 by the methods of sedimentation equilibrium and Sephacryl S-200 gel filtration, respectively. Since the molecular weight of its subunit was 34,200, this enzyme was considered to be composed of six identical subunits. The enzyme required Mg^{2+} for its activity but it was found that the crystalline enzyme did not contain any detectable amounts of Mg, Mn, Fe, Cu, Zn and Ca, by using an atomic absorption spectrophotometer. In the liver enzyme reaction, Mn^{2+} could fully replace Mg^{2+} . The reaction mechanism of the enzyme was like ping-pong. The enzyme activity was inhibited by various carboxylic acids, but this inhibition was suppressed by increasing the Mg^{2+} ion concentration. The enzyme activity was inhibited by the addition of glycerol and the inhibition increased as the pH was raised.

QPRTase [EC 2.4.2.19] was first crystallized from hog liver in this laboratory and its properties were reported.^{1~4)} In a previous paper,¹⁾ the molecular weight of hog liver enzyme was reported to be 173,000 by the method of sedimentation velocity and the molecular weight of its subunit to be 34,200 by the method of sedimentation equilibrium. Calculations from these values indicated that this enzyme was composed of five identical subunits. However, Musick proposed that this enzyme is composed of six identical subunits according to X-ray diffraction analysis.⁵⁾ In order to confirm this proposition, the molecular weight was determined by the sedimentation equilibrium method.

Also, metal content of the enzyme and effects on Mn^{2+} ion concentration, carboxylic acids and glycerol, and its reaction mechanism were investigated. As a result, some chara-

cteristic properties were observed.

This paper deals with the molecular weight and some enzymic properties of QPRTase from hog liver.

MATERIALS AND METHODS

Materials. QPRTase was purified from hog liver and crystallized twice.¹⁾ Quinolinic acid-2, 3, 7, 8-¹⁴C was obtained from the Daiichi Pure Chemicals Co., Ltd., Tokyo. PRPP (tetrasodium salt) was obtained from Sigma Chemical Company. Sephacryl S-200 was purchased from Pharmacia Fine Chemicals, Sweden. Other chemicals were acquired from Nakarai Chemicals Ltd., Kyoto.

Standard assay conditions. The enzyme activity was assayed as follows. The reaction mixture contained, in a total volume of 0.5 ml: potassium phosphate buffer at pH 6.0 (25 μ mol); quinolinic acid-2, 3, 7, 8-¹⁴C (0.23 mCi/mmol, 0.153 μ mol); PRPP (1 μ mol); $MgCl_2$ (1 μ mol) and an appropriate amount of the crystalline enzyme. The reaction was stopped by adding 0.8 ml of 4% perchloric acid. The ¹⁴CO₂ that evolved during the shaking of the acidified mixture at 37°C for 1 hr, was completely trapped by 0.2 ml of 25% β -phenylethylamine dissolved in methanol. Radioactivity was counted with a liquid scintillation spectrometer.⁶⁾

Ultracentrifugation. Ultracentrifugal studies were carried out at 20°C using a Hitachi analytical ultra-

[†] The Biosynthesis of Nicotinamide Adenine Dinucleotide. Part X. Part IX, see *Agric. Biol. Chem.*, 43, 345 (1979).

Abbreviations: QPRTase, quinolinate phosphoribosyltransferase; PRPP, 5-phosphoribosyl-1-pyrophosphate; NaMN, nicotinic acid mononucleotide; SDS, sodium dodecyl sulfate.

centrifuge, model UCA-1A. High speed sedimentation equilibrium was performed using an interference cell with sapphire windows. Interference photos were taken after centrifugation at 12,290 rpm for 14 hr.

SDS polyacrylamide gel disc electrophoresis. SDS polyacrylamide gel disc electrophoresis was performed according to the method of Weber and Osborn.⁷⁾ Enzyme protein (80 μ g) treated with SDS was electrophoresed at 8 mA per gel for 5 hr at room temperature. Gel was stained with periodic acid-Schiff reagent for carbohydrate.⁸⁾

RESULTS AND DISCUSSION

Molecular weight

The molecular weight of hog liver QPRTase was reported to be about 173,000 in a previous paper.¹⁾ This enzyme was reported to consist of five identical subunits, based on the molecular weight of the subunit (34,200). It is rare for an enzyme protein molecule to consist of an odd number of subunits. To confirm this, the molecular weight was determined by the sedimentation equilibrium method. As shown in Fig. 1, plots of the

FIG. 1. Molecular Weight Determination of Hog Liver QPRTase by High Speed Sedimentation Equilibrium.

The enzyme concentration was 0.198 mg/ml in 0.05 M potassium phosphate buffer at pH 7.0, containing 0.1 M KCl. Interference photos were taken after centrifugation at 12,290 rpm for 14 hr at 20°C. The figure shows the logarithm of the fringe displacement in μ versus the square of the radial distance in cm.

square of radial distance versus logarithm of fringe displacement was straight. This means that the hog liver enzyme is monodisperse. From the slope of this line, the molecular weight of the hog liver enzyme was estimated

FIG. 2. SDS Polyacrylamide Gel Disc Electrophoresis of Hog Liver QPRTase.

SDS treated enzyme protein (80 μ g) was electrophoresed. Gel was stained with periodic acid-Schiff reagent for carbohydrate.

to be 202,000. This value is incompatible with the previously reported value of 173,000.¹⁾ The hog liver enzyme was found to be glycoprotein as shown in Fig. 2. Sugar content of the enzyme was 1% as mannose by the use of the orcinol-sulfuric acid method.⁹⁾ Since the elution buffer used for the previous gel filtration on a Sephadex G-200 column was 0.05 M potassium phosphate buffer at pH 7.0, some interaction might occur between the enzyme protein as a glycoprotein and Sephadex. As a result, the enzyme may be slowly eluted. Therefore, the molecular weight was estimated by Sephacryl S-200 gel filtration. The elution buffer used was 0.1 M potassium phosphate buffer, pH 7.0, containing 0.5 M KCl in order to avoid ionic bond interaction between the enzyme protein and Sephacryl. From the result shown in Fig. 3, the molecular weight was estimated at 210,000. This value is similar to the molecular weights of crystalline

FIG. 3. Estimation of Molecular Weight of QPRTase by Sephacryl S-200 Gel Filtration.

The column (1.5 × 80 cm) equilibrated with 0.1 M potassium phosphate buffer at pH 7.0, containing 0.5 M KCl was used. The protein was eluted with same buffer at 4°C. The ordinate shows the distribution coefficient (K_d) and the abscissa logarithm of the molecular weight. QPRT, quinolinate phosphoribosyltransferase.

QPRTase from *Alcaligenes eutrophus* nov. subsp. *quinolanicus* (IAM 12305)¹⁰ and hog kidney.¹¹ If the molecular weight of hog liver QPRTase is 202,000, Stokes' radius and frictional ratio of this enzyme are 57.8 Å and 1.49, respectively. It was suggested that this enzyme is composed of six identical subunits. The result coincides with Musick's report⁵ on the X-ray diffraction analysis of crystalline QPRTase from hog liver. Physicochemical properties of this enzyme are summarized in Table I.

TABLE I. SUMMARY OF THE PHYSICOCHEMICAL PROPERTIES OF CRYSTALLINE QPRTASE FROM HOG LIVER

Physicochemical properties	Method	Value
Native enzyme		
Sedimentation coefficient ($s_{20,w}^0$)	Sedimentation velocity	8.00×10^{-13} sec ^a
Diffusion coefficient ($D_{20,w}^0$)	Boundary spreading during centrifugation	4.33×10^{-7} cm ² /sec ^a
Partial specific volume ($\bar{v}$)	Amino acid composition	0.74 ml/g ^a
Molecular weight	$s_{20,w}^0$, $D_{20,w}^0$ and $\bar{v}$	172,000 g/mol ^a
	Sephadex G-200 gel filtration	173,000 g/mol ^a
	Sephacryl S-200 gel filtration	210,000 g/mol
	Sedimentation equilibrium	202,000 g/mol
	$s_{20,w}^0$ and molecular weight (202,000)	57.8 Å
Stokes' radius	$s_{20,w}^0$ and molecular weight (202,000)	1.49
Frictional ratio (f/f_0)	Stokes' radius and molecular weight (202,000)	
Subunits		
Sedimentation coefficient ($s_{20,w}^0$)	Sedimentation velocity	3.20×10^{-13} sec ^a
Molecular weight	SDS disc electrophoresis	33,500 g/mol ^a
	Sedimentation equilibrium (in guanidine HCl)	34,200 g/mol ^a
	$s_{20,w}^0$ and molecular weight (34,200)	24.4 Å ^a
Stokes' radius	$s_{20,w}^0$ and molecular weight (34,200)	1.13 ^a
Frictional ratio (f/f_0)	Stokes' radius and molecular weight (34,200)	

^a Cited reference number 1).

Metal content

The metal content of this enzyme was analyzed with a Hitachi Perkin-Elmer 303 atomic absorption spectrophotometer equipped with a GA-2 graphite atomizer. The result indicated that the contents of Mg, Mn, Fe, Cu, Zn and Ca were insignificant.

Metal ion requirement

The enzymic formation of NaMN from quinolinic acid and PRPP shows an absolute requirement for divalent cations. Mg²⁺ has been reported to be the most effective for the QPRTase catalyzing reaction.² However, as

FIG. 4. Effect of Metal Ion Concentration on QPRTase Activity.

Standard assay conditions were used except for metal ions at various concentrations as indicated. Mn²⁺, ●-●; Mg²⁺, ○-○.

shown in Fig. 4, 0.3 mM of Mn^{2+} could fully replace Mg^{2+} , whereas Mn^{2+} could be partially replaced in QPRTases from "Shiitake" mushroom¹²⁾ and *Alcaligenes* sp.¹⁰⁾

Kinetics

Double reciprocal plots of the initial velocity *versus* the concentration of one substrate at a series of fixed concentrations of the second substrate are presented in Fig. 5. As the resulting double reciprocal plots were linear and parallel, the reaction mechanism of hog liver QPRTase was like ping-pong. This means that the hog liver QPRTase, differing from bacterial¹³⁾ and plant¹⁴⁾ enzymes, does not form a ternary complex (enzyme-quinolinic acid-PRPP) but a binary complex (enzyme-quinolinic acid or enzyme-PRPP).

FIG. 5. Double Reciprocal Plots of Initial Velocity against Substrate Concentration.

Standard assay conditions were varied as given following. PRPP concentrations were 0.06, 0.1, 0.2 and 0.6 mM for Curves A, B, C and D, respectively. Quinolinic acid concentrations were 0.027, 0.0363, 0.0545 and 0.182 mM for Curves E, F, G and H, respectively. The reaction was stopped after 15 min. Each point on the Curves represents the average of three independent reactions.

Effect of carboxylic acids

Carboxyl groups at C-2 and C-3 of pyridine ring play an important role in binding with the enzyme.³⁾ Succinic and maleic acids have two adjacent carboxyl groups like quinolinic acid as a substrate. As shown in Table II, these acids inhibited the enzyme activity. Only two adjacent carboxyl groups might be

TABLE II. EFFECT OF CARBOXYLIC ACIDS ON QPRTASE ACTIVITY

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

Addition (1 mM)	Relative activity (%)
None	100
Formic acid	99
Acetic acid	97
Lactic acid	98
Glutamine	107
Glutamic acid	69
α -Ketoglutaric acid	40
Asparagine	101
Aspartic acid	77
Oxaloacetic acid	44
Succinic acid	49
Maleic acid	66
Fumaric acid	28
L-Malic acid	25
Citric acid	5
Benzoic acid	100

considered to bind with the enzyme protein. To confirm this idea, the effect of various carboxylic acids on the enzyme activity was investigated (Table II). All acids with more than two carboxyl groups also inhibited the enzyme activity independent of these positions. This inhibition was recovered by increasing Mg^{2+} ion concentration like inhibition by ATP.³⁾ Since the inhibition by carboxylic acids has not been reported for bacterial¹³⁾ and plant¹⁴⁾ enzymes, this property may be a characteristic observed only in mammalian enzyme. The mammalian enzyme activity may be regulated by Mg^{2+} or Mn^{2+} ion concentration.

Phthalic acid has been reported to inhibit the enzyme activity³⁾ but its inhibition was not at all dependent on Mg^{2+} ion concentration.

Effect of glycerol

Glycerol is known to cause protein conformational change¹⁵⁾ in some cases. It is reported that glycerol has a stabilizing effect on denaturing QPRTase from *Alcaligenes* sp. during electrophoresis.¹⁶⁾ This bacterial enzyme was activated at pH 6.1 and 6.5 but inhibited by glycerol at pH 7.0 or above.*

* K. Shibata, H. Taguchi and K. Iwai, unpublished data.

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

The standard assay for QPRTase was used except for various pH of McIlvaine type buffer ($\mu=0.02$). Values are expressed as percentage of activity in the presence of 30% glycerol to activity in the absence of glycerol.

pH	Relative activity (%)
4.0	104
5.0	47
6.1	27
7.0	15
8.0	13

Therefore, the effect of glycerol on enzyme activity was investigated at various pH. As shown in Table III, glycerol inhibited enzyme activity, depending on the pH. The extent of inhibition increased as the pH was raised. This inhibition was reversible. The hog liver QPRTase also elicited conformational change by glycerol and its change was found to be dependent on the pH.

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