Note

Immunochemical Studies on Crystalline Quinolate Phosphoribosyltransferases from Hog Liver, Hog Kidney and *Alcaligenes eutrophus* subsp. *quinolinicus*[†]

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NAD is synthesized from tryptophan in animals^{1~3)} or aspartate and glycerol (or closely related compounds) in bacteria^{4~8)} and plants.^{9,10)} In the both biosynthetic pathways, quinolinic acid is the common intermediate and its acid is synthesized to NAD via the same intermediates, nicotinic acid mononucleotide and nicotinic acid adenine dinucleotide. Nicotinic acid mononucleotide, which is the first niacin-active product in *de novo* NAD biosynthetic pathway, is formed from quinolinic acid and 5-phosphoribosyl-1-pyrophosphate in the presence of Mg^{2+} .¹¹⁾ This reaction is catalyzed by QPRTase (Nicotinatenonucleotide pyrophosphorylase (carboxylating); nicotinatenucleotide : pyrophosphate phosphoribosyltransferase (carboxylating), EC 2.4.2.19). This enzyme has been crystallized from three different sources (*Alcaligenes eutrophus* subsp. *quinolinicus*,¹²⁾ hog liver¹³⁾ and hog kidney¹⁴⁾) in our laboratory.

chemically QPRTases from the three different sources. This paper shows that hog kidney and hog liver enzymes have the same immunochemically determinant groups, while *Alcaligenes* and hog kidney enzymes do not.

Each QPRTase was purified and twice crystallized from *Alcaligenes eutrophus* subsp. *quinolinicus* IAM12305,¹²⁾ hog liver¹³⁾ and hog kidney.¹⁴⁾ Large crystals of hog kidney QPRTase are shown in Fig. 1. Quinolinic acid-2,3,7,8-¹⁴C was purchased from Daiichi Pure Chemicals Co., Ltd.,

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Abbreviation; QPRTase, quinolate phosphoribosyltransferase.

FIG. 1. Photograph of Crystalline Hog Kidney QPRTase.

FIG. 2. Precipitin Reaction of Hog Kidney QPRTase Antibody with Hog Kidney QPRTase.

The immunoglobulin and the control globulin (0.29 mg as protein) were incubated with the indicated amounts of hog kidney QPRTase in 0.9% NaCl in 25 mM potassium phosphate buffer, pH 7.0, in a total volume of 0.20 ml, at 4°C for 3 days. The immunoprecipitates were collected by centrifugation at 3000 × g for 30 min and washed twice, each time with 0.25 ml of 0.9% NaCl in 25 mM potassium phosphate buffer, pH 7.0. The amount of protein in precipitin was determined by the method of Lowry *et al.*¹⁵⁾

Tokyo. 5-Phosphoribosyl-1-pyrophosphate was obtained from Sigma Chemical Company. Complete Freund's adjuvant was purchased from Difco Laboratories. Other chemicals were purchased from Nakarai Chemicals Ltd., Kyoto.

Rabbits were immunized with hog kidney QPRTase. The crystalline hog kidney enzyme, 1.31 mg in 1 ml of 0.9% NaCl, was emulsified with an equal volume of complete Freund's adjuvant, and 2 ml of the emulsion was injected in each rabbit, one-half into toe pads and one-half into multiple sites on the back. The injections were repeated

three times at weekly intervals. Blood was taken from the carotid artery 2 weeks after the last injection. Control blood was obtained from an unimmunized rabbit of the same age.

The γ -globulin fraction from immune and control sera was obtained by repeating the precipitation with ammonium sulfate¹⁶ and dissolving in and dialyzing against 25 mM potassium phosphate buffer, pH 7.0, containing 0.15 M NaCl. This dialyzed antibody was used in the following experiments.

Quantitative immunoprecipitation of the hog kidney

FIG. 3. Ouchterlony Double Immunodiffusion of Hog Kidney Crystalline QPRTase Antiserum and Crystalline QPRTase from Hog Liver, Hog Kidney and *Alcaligenes eutrophus* subsp. *quinolanicus*.

A.S. (kidney), antiserum of the hog kidney enzyme, 26.4 μ g of protein; kidney, the hog kidney enzyme, 1.31 μ g; liver, the hog liver enzyme, 1.25 μ g; *Alcaligenes* sp., the *Alcaligenes* enzyme 1.50 μ g.

FIG. 4. Immunoprecipitation of Hog Kidney (A), Hog Liver (B) and *Alcaligenes* sp. (C) QPRTases by the Hog Kidney Enzyme Antiserum.

Each crystalline enzyme (28 μ g as protein) was incubated for 30 min with increasing amounts of antiserum in a total volume of 0.2 ml at 37°C in 25 mM potassium phosphate buffer, pH 7.0, containing 0.15 M NaCl. Mixtures were separated by centrifugation at 3000 $\times g$ for 20 min. Enzyme activity of precipitated proteins (○) and supernatant (●) were assayed for QPRTase.¹²⁻¹⁴⁾

enzyme antibody with the hog kidney enzyme was investigated. The results are presented in Fig. 2. Four molecules of immunoglobulin were found to bind to one molecule of hog kidney QPRTase at the equivalent point, assuming that the molecular weights of hog kidney QPRTase and γ -globulin are 220,000 and 160,000, respectively.

Precipitin reaction of the hog kidney enzyme antibody with hog kidney, hog liver and *Alcaligenes* enzymes were investigated. As Fig. 3-A shows, hog liver enzyme was precipitated by the hog kidney enzyme antibody and the precipitin line fused into that of the hog kidney enzyme. This result indicates that QPRTases from hog liver and hog kidney have the same immunochemically determinant group.

As for the result of precipitin reaction with the hog kidney enzyme antibody and *Alcaligenes* enzyme, the precipitin line did not appear as shown in Fig. 3-B. The result shows that the *Alcaligenes* enzyme and hog kidney enzyme have different immunochemically determinant groups.

The relationship of the enzyme activity and the precipitin reaction was also investigated. As shown in Fig. 4, A and B, precipitated protein (a complex of the enzyme and the antibody) retained the enzyme activity. The reason may be the following that the binding site of the antibody to the enzyme is different from the active site of the enzyme. The *Alcaligenes* enzyme did not react with the hog kidney enzyme antibody and its activity remained in supernatant fraction, as shown in Fig. 4-C.

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