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Kinetic and Regulatory Properties of Rat Liver Phosphoribosylpyrophosphate Synthetase Complex Are Partly Distinct from Those of Isolated Recombinant Component Catalytic Subunits[1]

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Rat liver phosphoribosylpyrophosphate (PRPP) synthetase exists as complex aggregates composed of two catalytic subunits (PRS I and II, in a ratio of approximately 4:1) and two catalytically inactive PRPP synthetase-associated proteins. To better understand the significance of the complex structure, the properties of the native liver enzyme were compared with those of homologous aggregates of recombinant PRS I and PRS II (rPRS I and rPRS II). (1) The specific activity per catalytic subunits of the liver enzyme was about 2.5 times lower than that of rPRS I over a wide pH range. K_m values for substrates and K_a values for P_i and Mg^{2+} of the three enzymes were similar. (2) Specific activity of the liver enzyme for the reverse reaction was about 2 times lower than those of rPRSs. K_m values for substrates of the three enzymes were comparable. (3) The liver enzyme was more stable than were rPRSs when incubated at a high temperature or in the absence of stabilizing agents. (4) The liver enzyme was markedly less sensitive to inhibition by nucleotides compared to rPRS I. GDP at 1 mM inhibited the liver enzyme and rPRS I by 32 and 93%, respectively. This effect is not ascribable to molecular interaction between rPRS I and II, as reconstitution of the two did not alter the sensitivity to nucleotide inhibition. (5) Our observations suggest that complex aggregation states of the native enzyme not only suppress the activities but also stabilize the catalytic subunits and the associated proteins and remarkably reduce the sensitivity to inhibition by nucleotides.

Key words: complex aggregates, heterologous components, nucleotide inhibition, phosphoribosylpyrophosphate synthetase, phosphoribosylpyrophosphate synthetase-associated proteins.

5-Phosphoribosyl 1-pyrophosphate (PRPP) synthetase (ATP: D-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1) catalyzes the formation of PRPP from ATP and ribose-5-phosphate. PRPP serves as the primary precursor and is also a critical control factor in the biosynthesis of purine and pyrimidine nucleotides (*1-4*). PRPP synthetase has been purified from *Salmonella typhimurium* (*5*), *Escherichia coli* (*6*), *Bacillus subtilis* (*7*), human erythrocytes (*8*), and rat liver (*9, 10*). Mg^{2+} and P_i activate and ADP and other nucleotides inhibit the synthetase activity (*11*).

Native rat liver PRPP synthetase exists in highly aggregated states and has a molecular mass of over 1,000 kDa (*10*). On SDS gel electrophoresis, the enzyme showed three protein bands of 34, 39, and 41 kDa, in a ratio of approximately 3 : 1 : 0.15 (*10*). cDNA cloning (*12-14*) revealed that the 34 kDa catalytic subunit is actually a mixture of two highly homologous isoforms, PRS I and PRS II, in a ratio of approximately 4:1 (*15*). The 39 and 41 kDa components, which we termed PRPP synthetase-associated proteins (PAPs) (*16*), were not separated by conventional purification procedures (*10*). Calculations indicated that the liver enzyme is a heterologous complex of more than 34 mer (PRS I/PRS II/PAP39/PAP41 = 20 : 5 : 8 : 1) (*17*).

We cloned the cDNAs for PAP39 and PAP41 of rat liver (*16, 18*). The deduced amino acid sequences of PAP39 and PAP41 were highly similar to those of PRSs, but with two totally distinct minor sites. Thus the two proteins are part of the PAP subfamily (*18*). PAP39 interacts with the catalytic subunits (*16*). When PAPs were eliminated from the native liver enzyme complex, the enzyme activity of the remaining catalytic subunits increased (*16*). Furthermore, the expressed recombinant isoforms, rPRS I and II, had higher specific activities than the purified rat liver enzyme (*19*). These results indicated a negative regulatory role for PAPs in PRPP synthesis.

It was also likely that heterologous aggregation states of the native rat liver enzyme alter other properties of catalytic subunits. We examined kinetic properties of the

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Abbreviations: PAP39 and PAP41, phosphoribosylpyrophosphate synthetase-associated proteins of 39 and 41 kDa, respectively; PRPP, 5-phosphoribosyl 1-pyrophosphate; PRS I and PRS II, phosphoribosylpyrophosphate synthetase 34 kDa subunits I and II, respectively.

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liver enzyme in comparison with those of the homologous aggregate form of recombinant catalytic subunits rPRS I or rPRS II and also the heterologous complex of rPRS I and II prepared *in vitro*. Our findings will facilitate understanding of the physiological significance of the heterologous complex structure of mammalian PRPP synthetase.

MATERIALS AND METHODS

Materials—ATP, NADH, NADPH, nicotinamide mononucleotide, nicotinic acid mononucleotide, PRPP, 6-azauridine 5′-phosphate, allopurinol riboside 5′-monophosphate, ADP-ribose, and guanosine 5′-diphosphate 3′-diphosphate were obtained from Kyowa Hakko, CMP and NAD from Kojin, dATP, dGTP, and dTTP from Pharmacia, cAMP and adenosine 5′-(β,γ-imido)-triphosphate from Boehringer Mannheim, and orotidine 5′-phosphate from Calbiochem. Nucleotides (sodium salts or free forms) and related compounds, except for those specified above, were purchased from Sigma. Orotidine 5′-phosphate pyrophosphorylase containing orotidine 5′-phosphate decarboxylase (OPRTase-ODCase, yeast) was also obtained from Sigma. PRPP was purified according to Smithers and O'Sullivan (*20*) and assayed by the release of $^{14}CO_2$ from [carboxy-^{14}C]orotic acid in the presence of the OPRTase-ODCase enzyme mixture (*20*). [8-^{14}C]ATP (59 mCi/mmol) was obtained from Amersham and purified by anion exchange chromatography prior to use. [Carboxy-^{14}C]orotic acid (52 mCi/mmol) was from New England Nuclear.

Purification of Enzymes—Rat liver PRPP synthetase was purified from livers of male Sprague-Dawley rats (*10*). Recombinant isoforms of rat liver PRPP synthetase, rPRS I and rPRS II, were expressed in *Escherichia coli* and purified (*19*). All buffers used contained enzyme-stabilizing agents, 0.3 mM ATP, 6 mM $MgCl_2$, 0.1 mM EDTA, and 2.5 mM mercaptoethanol (*10*).

Enzyme Assay—PRPP synthetase was assayed as described (*10*). The reaction mixture (100 μl) contained 50 mM potassium HEPES (pH 7.4, adjusted at 37°C), 10 mM potassium phosphate, 4 mM $MgCl_2$, 1 mM EDTA, 0.4 mM [8-^{14}C]ATP (0.2-0.5 mCi/mmol), 0.2 mM ribose-5-phosphate, 1 mM dithiothreitol, and the enzyme. One unit of enzyme activity was defined as the amount catalyzing formation of 1 μmol of PRPP per min under standard conditions. For determination of the apparent K_m value for ATP, the ribose-5-phosphate concentration was fixed at 0.2 mM, the $MgCl_2$ concentration was 4 mM excess over the concentration of ATP, which was varied. For determination of the K_m for ribose-5-phosphate, the ATP concentration was 0.4 mM. For processing of data, a Lineweaver-Burk plot was used.

For the reverse reaction, the reaction mixture (100 μl) contained 10 mM potassium phosphate, 4 mM $MgCl_2$, 1 mM EDTA, 2.0 mM [*U*-^{14}C]AMP (0.2-0.5 mCi/mmol), 0.25 mM PRPP, 1 mM dithiothreitol, and 50 mM potassium HEPES (pH 7.4, adjusted at 37°C) or 50 mM potassium HEPES-50 mM Tris-50 mM CHES (pH 8.6, adjusted at 37°C). Incubation was for 5 min at 37°C. One unit of enzyme activity was defined as the amount catalyzing formation of 1 μmol of ATP per min.

Preparation of Complexed Forms of rPRS I and rPRS II—rPRS I and rPRS II in various ratios (10 μg of total protein) were maintained in the presence of 1 M $MgCl_2$ at 4°C for 2 h, diluted 50 times with dilution buffer (50 mM potassium phosphate, pH 7.4, containing the enzyme-stabilizing agents except $MgCl_2$), and incubated at 37°C for 30 min and at 4°C for 1 h. Five microliters of each preparation were assayed for enzyme activity.

Other Methods—Protein concentration was determined as described by Bradford (*21*), using bovine serum albumin as a standard. The molecular weight of the complexed form of rPRS I and rPRS II was determined by gel filtration on a TSK G4000SW HPLC column (0.75×60 cm). The running buffer was 50 mM potassium phosphate (pH 7.4) containing the enzyme-stabilizing agents.

RESULTS

Effect of pH on Enzyme Activities—The pH-activity profiles for the native liver enzyme, rPRS I and rPRS II were determined at saturating concentrations of substrates and Mg^{2+}. rPRS I showed maximal activity at pH 8.7 and 66% of the maximal activity at pH 7.4, whereas rPRS II was most active at pH 7.4 and possessed only 20% of the maximal activity at pH 8.7 (Fig. 1). The liver enzyme showed a pH profile similar to that of rPRS I, with the optimum at pH 8.5 and 62% of maximal activity at pH 7.4 (Fig. 1). The specific activity of the native liver enzyme, in terms of the amount of the 34-kDa proteins, was about 2.5 times lower than that of rPRS I not only at neutral pH as reported (*10, 19*) but also over the wider pH range examined here (Fig. 1).

For assay of PRPP synthetase activity, we measured [^{14}C]AMP production from [^{14}C]ATP coupled with PRPP production from ribose-5-phosphate, as described under "MATERIALS AND METHODS." This method can also be used to assay exchange reactions between AMP and ATP,

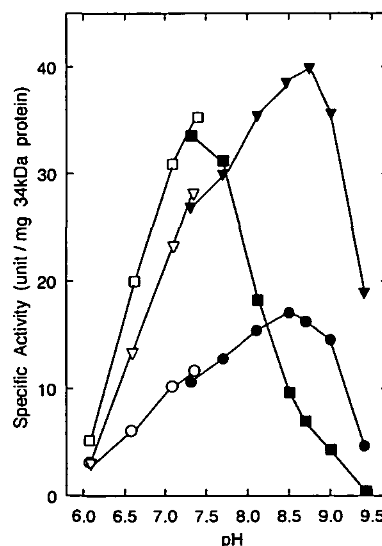

Fig. 1. **Effect of pH on liver PRPP synthetase, rPRS I, and rPRS II.** Specific activities were measured in 50 mM potassium phosphate, 50 mM Tris-acetate, and 50 mM CHES buffer, pH 6.1 to 7.4 (open symbols) or 10 mM potassium phosphate, 40 mM potassium HEPES, 50 mM Tris-acetate, and 50 mM CHES buffer, pH 7.3 to 9.4 (closed symbols). The pH of the reaction mixture containing the substrates and other components was measured at 37°C. ○ and ●, liver PRPP synthetase; ▽ and ▼, rPRS I; □ and ■, rPRS II.

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independent of net PRPP synthesis (*22*). We examined the [^{14}C]AMP-ATP exchange reaction by the liver enzyme under standard assay conditions at pH 7.4, except that 0.4 mM [^{14}C]ATP was replaced with unlabeled ATP and 0.05 mM [^{14}C]AMP was added. The liver enzyme produced 0.034 nmol of [^{14}C]ATP in 5 min, this value being 1.1% of [^{14}C]AMP production from [^{14}C]ATP accompanying PRPP synthesis measured in parallel, which was in accord with the values of rPRS I (1.9%) and rPRS II (0.4%) previously determined (*19*). Thus, the differences in specific activities between the native liver enzyme and the two isoforms were not due to differences in AMP-ATP exchange reactions of the enzyme preparations.

Kinetic Properties of the Enzymes—Kinetic properties of the native liver enzyme were examined in comparison with those of rPRS I and rPRS II (*19*), and the results are summarized in Table I together with documented data. The K_m values for ATP and ribose-5-phosphate of the liver enzyme ranged between those of rPRS I and rPRS II. PRPP synthetase is activated by P_i (*11*). The K_a value for P_i of the liver enzyme was 1.8 mM, equal to the value of rPRS I (*19*).

Mg is required by PRPP synthetase in two ways, *i.e.*, to form a real substrate Mg-ATP, and to bind the enzyme protein as an essential activator (*9, 23-26*). The requirement for free Mg^{2+} of the three enzymes was then studied. An iterative computer program was used to calculate the concentration of free Mg^{2+} in the reaction mixture in which ATP, ribose-5-phosphate, P_i and EDTA are in simultaneous equilibrium with their respective complexes with Mg^{2+}. Activation of the liver enzyme was almost maximal with 50 μM Mg^{2+}, and the concentration that gave half-maximal activation was similar to that of rPRS I and a little lower than that of rPRS II (data not shown). Thus, there were no marked differences in substrates and activator dependency between the native liver enzyme and the two catalytic subunits.

Heat Stability—In previous studies (*19*), a marked difference was found between the heat stabilities of rPRS I and rPRS II. As shown in Table I, the half-life of the liver enzyme at 49°C in the presence of enzyme stabilizers was 250 min, about 2.8- and 500-fold greater than those of rPRS I and rPRS II, respectively (Table I). Stabilities of the three enzyme preparations were further examined at an

TABLE I. **Comparison of properties of liver PRPP synthetase, rPRS I, and rPRS II.** Specific activities of enzymes were determined under saturating substrate conditions and expressed in terms of the amount of the 34-kDa proteins. Apparent K_m values for ATP and R5P and apparent K_a value for P_i were determined as described under "MATERIALS AND METHODS." The half-life of heat inactivation was determined at a protein concentration of 12 μg/ml in 50 mM potassium phosphate buffer (pH 7.4) containing the enzyme-stabilizing agents, as described (*19*). The half-life at 4°C was determined at a protein concentration of 20 μg/ml in 50 mM potassium HEPES buffer (pH 7.4) containing 6 mM $MgCl_2$, 0.1 mM EDTA, and 2.5 mM mercaptoethanol.

	Liver enzyme	rPRS I	rPRS II
pH optimum	8.5	8.7	7.4
Specific activity (units/mg 34 kDa subunit)			
at pH 7.4	10.4[a]	25.7[b]	34.5[b]
at pH 8.6	16.2	39.1	8.3
Apparent K_m at pH 7.4			
ATP (μM)	49	44[b]	60[b]
Ribose-5-phosphate (μM)	64	40[b]	73[b]
Apparent K_a at pH 7.4			
P_i (mM)	1.8	1.8[b]	2.4[b]
Half-life of inactivation			
at 49°C (min)	250	90[b]	0.5[b]
at 4°C without stabilizing agents (h)	21	8	1

[a]Data from Kita *et al.* (*10*). [b]Data from Ishijima *et al.* (*19*).

TABLE II. **Kinetics of the reverse reaction of liver PRPP synthetase, rPRS I and rPRS II.** Assay conditions are described under "MATERIALS AND METHODS." For determination of the apparent K_m values, the PRPP concentration was fixed at 0.25 mM for AMP, and the AMP concentration was fixed at 2.0 mM for PRPP.

	Liver enzyme	rPRS I	rPRS II
Specific activity	(units/mg 34 kDa subunit)		
at pH 7.4	3.59	7.48	8.04
at pH 8.6	2.45	4.50	4.24
Ratio of specific activities of forward to reverse reactions			
at pH 7.4	2.9	3.5	4.4
at pH 8.6	6.6	8.7	2.0
Apparent K_m at pH 7.4		(μM)	
AMP	117	125	123
PRPP	28.9	41.8	40.2

TABLE III. **Inhibition of rat liver PRPP synthetase, rPRS I, and rPRS II by nucleotides and related compounds.** Standard assay conditions (0.4 mM ATP, 0.2 mM ribose-5-phosphate, 4 mM $MgCl_2$, 10 mM potassium phosphate buffer, pH 7.4) were used. All compounds were tested at a final concentration of 1.0 mM with equal amount of $MgCl_2$ added (final $MgCl_2$ concentration, 5 mM).

Compound	Inhibition (%)		
	Liver enzyme	PRS I	PRS II
ADP	94	98	98
AMP	77	66	64
dATP	69	68	57
dADP	55	54	46
dAMP	47	45	30
GTP	13	19	13
GDP	32	93	24
GMP	23	12	2
dGDP	12	10	2
dGMP	14	2	0
XMP	13	1	9
ITP	12	8	0
IDP	28	93	22
Adenosine 5'-(β,γ-imido)-triphosphate	92	86	82
ADP-ribose	13	16	14
6-Mercaptopurine riboside 5'-phosphate	18	15	19
Allopurinol riboside 5'-phosphate	15	8	10
UTP	15	4	13
CTP	11	0	13
TTP	11	0	6
TMP	13	0	0
NAD	12	0	8
NADH	24	25	14
Nicotinic acid mononucleotide	5	19	0
FAD	24	12	8
Pyridoxal-phosphate	38	32	26
Deoxyribose 5-phosphate	15	11	5

Compounds which gave less than 10% inhibition of the three enzymes were as follows: dGTP, XTP, XDP, IMP, cAMP, guanosine 5'-diphosphate 3'-diphosphate, UDP, UMP, dUTP, dUDP, dUMP, CDP, CMP, dCTP, dCDP, dCMP, dTTP, dTDP, dTMP, orotidine 5'-phosphate, 6-azauridine 5'-phosphate, NADP, NADPH, nicotinamide mononucleotide, flavin mononucleotide, ribose 1-phosphate, ribulose 5-phosphate, and 2,3-diphosphoglyceric acid.

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enzyme protein concentration of 20 μg/ml. Final concentrations of the enzyme stabilizing agents, ATP and P_i, were below 1.5 μM and 0.25 mM, respectively. Under these conditions and at 4°C, the half-life of the native liver enzyme was 21 h, whereas half-lives of rPRS I and II were 8 and 1 h, respectively (Table I).

Reverse Reaction of Enzymes—The PRPP synthetase reaction was found to be reversible (*23*). Human erythrocyte PRPP synthetase has been reported to dissociate into aggregates of smaller mass in aged cells, accompanied by an increase in the ratio of the activities of the forward to reverse reactions (*26*). In view of the possible occurrence of similar events for the three enzymes of different compositions, kinetics of the reverse reaction of the liver enzyme was compared with kinetics of the recombinant isoforms. The formation of [^{14}C]ATP from [^{14}C]AMP and PRPP was determined, under the conditions given under "MATERIALS AND METHODS." In contrast to the forward reaction (Fig. 1), the specific activities of the reverse reaction of the liver enzyme and rPRS I were higher at pH 7.4 than at pH 8.6, as was also the case for rPRS II (Table II). The specific activity of the reverse reaction per catalytic subunits of the liver enzyme was about 2 times lower than those of rPRS I and rPRS II at both pH 7.4 and 8.6. The ratio of the specific activities of the forward to reverse reaction showed no large differences between the liver enzyme, rPRS I and rPRS II in pH 7.4 (Table II). The liver enzyme showed similar affinities for AMP and PRPP to the catalytic subunits (Table II). Recently, Nave *et al.* measured the V_{max} and K_m values of the reverse reaction of rPRS I at pH 7.7 (*27*). Those values are in the same range as we have noted here.

Inhibition by Nucleotides—PRPP synthetases from various sources are inhibited by nucleotides, of which ADP is the most potent (*6, 7, 24, 28–30*). For rPRS I and rPRS II, ADP, GDP, and the reaction product AMP were the most effective inhibitors among the nucleotides examined (*19*). rPRS I was far more sensitive to inhibition by ADP and GDP than was rPRS II (*19*). Subsequent preliminary studies showed that the native rat liver enzyme was less sensitive to nucleotide inhibition than was rPRS I, the major component of the liver enzyme. Based on these observations, we compared the sensitivity of the liver enzyme and the rPRS I and II isoforms to inhibition by a wide variety of nucleotides and related compounds. As shown in Table III, among 55 compounds (1 mM) tested, only 5 including ADP and AMP exerted more than 50% inhibition on the native liver enzyme. Only 7 compounds and 4 compounds inhibited by more than 50% rPRS I and rPRS II, respectively. Inhibition of the three enzyme preparations by pyrimidine, pyridine, and flavin nucleotides was negligible or weak under the assay conditions.

It is noteworthy that the native liver enzyme is far less sensitive to inhibition by GDP and IDP compared to rPRS I: the inhibition of liver enzyme and rPRS I by 1 mM GDP was 32 and 93%, respectively, and that by 1 mM IDP was 28 and 93%, respectively (Table III). The inhibitory effect of varying concentrations of ADP, GDP, and IDP, the most effective inhibitors examined, on activities of three enzymes were also compared (Fig. 2). As shown in Fig. 2A, at 0.3 mM ADP, the remaining activity of the liver enzyme was much higher than that of rPRS I and a little lower than that of rPRS II. The characteristic difference in the inhibition of the three enzymes was more clearly observed in the case of GDP (Fig. 2B) and IDP (Fig. 2C), as compared to ADP (Fig. 2A). As ADP inhibition of PRPP synthetase was reported to be partly competitive with respect to ATP, and

TABLE IV. **Inhibition of rPRS I, rPRS II, complex forms of rPRS I and II, and liver PRPP synthetase by ADP and GDP.** Complex forms of PRS I and PRS II were prepared as described under "MATERIALS AND METHODS." Assays were performed with or without 0.3 mM ADP or 1.0 mM GDP, plus equal amounts of $MgCl_2$, respectively, under standard assay conditions. Values in parentheses were calculated based on the assumption that rPRS I and rPRS II are inhibited independently.

Enzyme	% of activity in the presence of nucleotides	
	0.3 mM ADP	1.0 mM GDP
rPRS I	16.2	22.3
rPRS II	43.2	83.5
rPRS I/rPRS II, 4:1	18.8 (21.6)	35.9 (34.5)
rPRS I/rPRS II, 1:1	25.1 (29.7)	55.9 (52.9)
rPRS I/rPRS II, 1:4	39.9 (37.8)	79.3 (71.3)
Liver enzyme	37.5	68.6

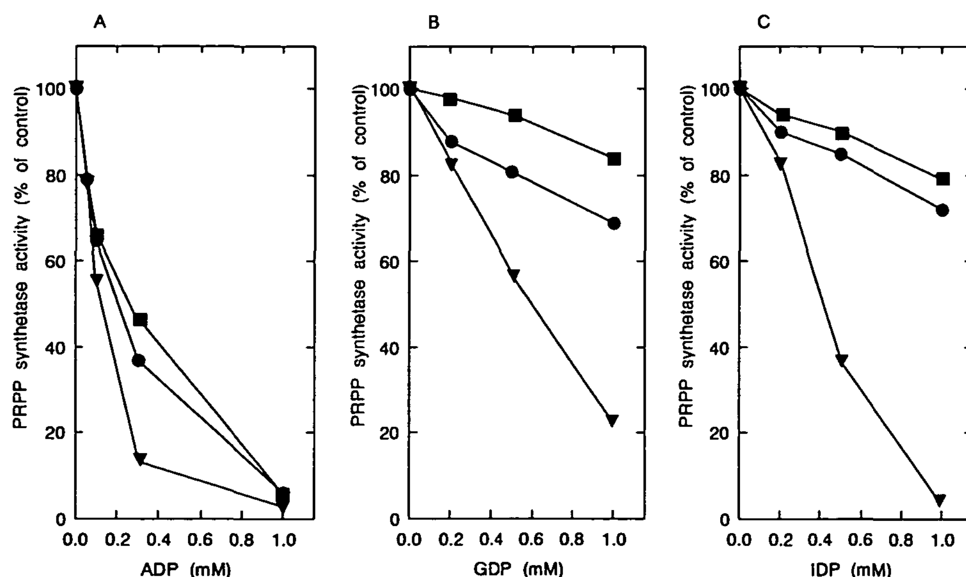

Fig. 2. **Inhibition of liver PRPP synthetase, rPRS I, and rPRS II by ADP (A), GDP (B), and IDP (C).** Standard assay conditions were used. Nucleotides were added with an equal amount of $MgCl_2$. ●, liver PRPP synthetase; ▼, rPRS I; ■, rPRS II.

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GDP and IDP inhibition to be noncompetitive (*24, 29, 30*), the inhibitory effects of these nucleotides on the three enzymes seemed to be different. The liver enzyme was much less sensitive to inhibition by GDP and IDP than was rPRS I, at all concentrations tested. As described above, the catalytic subunits of the native liver enzyme consist of PRS I and PRS II in a quantity ratio of 4:1. The inhibitory properties of the liver enzyme by ADP, GDP, or IDP, however, were distinct from those of rPRS I.

Heterologous Aggregate Forms of rPRS I and rPRS II—To examine the effects of molecular interaction between the two isoforms of catalytic subunits, reconstitution of rPRS I and rPRS II was examined. rPRS I and II were mixed in a quantity ratio of 4:1, 1:1, or 1:4 and treated with 1 M $MgCl_2$, a condition which dissociates the rat liver enzyme complex (*16*). After dilution with buffer and incubation at 37°C for 30 min and 4°C for 1 h to promote reassociation, the preparation (in a quantity ratio of 4:1) was analyzed by HPLC gel filtration on TSK G4000SW. The proteins were eluted as a broad single peak at 500 to 700 kDa (data not shown). As the molecular masses of the homologous aggregate forms of rPRS I and rPRS II are 700 to 1,200 kDa and 550 kDa, respectively (*19*), the reassociated complex of rPRS I and rPRS II described here seemed to be a heterologous aggregate of the two isoforms, although no definitive evidence for this was obtained. The specific activities of the heterologous complex were equal to those calculated assuming their independent activities in the complex (data not shown). The heterologous complex was examined for sensitivity to ADP and GDP inhibition in comparison with rPRS I, rPRS II and the liver enzyme (Table IV). The complexed forms (rPRS I : rPRS II=4 : 1, 1 : 1, or 1 : 4) showed nucleotide sensitivities that were proportional to their subunit compositions and close to the values calculated based on the assumption of independent behavior of rPRS I and rPRS II (Table IV, in the parentheses). It is noteworthy that the inhibition of the native liver enzyme was much less than that of the complex with the same composition of catalytic subunits as that of the liver enzyme (ratio of 4:1). Molecular interactions between the two isoforms of the catalytic subunits might alone be insufficient to induce a conformational change leading to alterations in sensitivity to nucleotides, or the conditions used for subunit dissociation and reassociation may not have been suitable to elicit molecular arrangement present in the native liver enzyme complex. The possibility also remains that the reassociated aggregate of rPRS I and rPRS II is a mixture of homologous aggregates of each isoform.

DISCUSSION

Mammalian PRPP synthetase exists as large molecular mass aggregates composed of two isoforms of catalytic subunits (PRS I and II) and two associated proteins (PAP39 and 41). To better understand the significance of such a complex structure, and the roles played by each component, the kinetic properties of the rat liver native enzyme were compared with those of recombinant PRS I and PRS II.

The pH profile of the native liver enzyme was similar to that of rPRS I with an optimum at pH 8.5 (Fig. 1). The catalytic subunit of the liver enzyme is composed of mainly PRS I (80%), as described above. However, the specific activity of the liver enzyme in terms of the amount of the 34 kDa proteins is about 2.5 times lower than that of rPRS I over a wide range of pH (Fig. 1).

While most kinetic properties of the rat liver enzyme are similar to those of the main component, rPRS I, the native enzyme is much more stable than rPRS I against heat or in the absence of the stabilizing agents (Table I). This means that aggregation states of the native liver enzyme containing associated proteins tend to stabilize the catalytic subunits of the synthetase.

Feedback inhibition by various nucleotides is considered to constitute an important mechanism for regulating mammalian PRPP synthetase. Data presented here, however, indicated that the liver enzyme has a limited sensitivity to nucleotides. Among the 55 compounds tested, only 5 (1 mM) gave more than a 50% inhibition of the liver enzyme (Table III). Pyrimidine, pyridine, and flavin nucleotides were either negligible or weak inhibitors. The assay mixture contained the substrate ATP at 0.4 mM, which is saturating, but a little lower than intracellular physiological concentrations. These results are in accord with the report of Roth and Deuel (*30*), but not with data of Fox and Kelly, who showed inhibition of the human erythrocyte enzyme by a number of nucleotides at relatively high concentrations, using a very low substrate concentration (*24*).

It is noteworthy that the liver enzyme is markedly less sensitive to inhibition by ADP, GDP, and IDP compared to rPRS I (Table III and Fig. 2) and to the heterologous aggregate forms of rPRS I and rPRS II in the ratio of 4:1, the same composition ratio as that of the liver enzyme (Table IV). When the rat liver enzyme was treated mildly with trypsin, which eliminates PAPs (*16*), the sensitivity to nucleotide inhibition increased (42% inhibition by 1 mM GDP) but was lower than that of the aggregate composed of only the catalytic subunits (64% inhibition by 1 mM GDP, Table IV). On the other hand, when the rat liver enzyme complex was dissociated with 1 M $MgCl_2$, and then reaggregated, the sensitivity to nucleotide inhibition also increased (Kita, K. and Tatibana, M., unpublished data). These results suggest that sensitivity to nucleotide inhibition depends partly on the presence of PAPs in the enzyme complex and partly on the conformation of the complex. The aggregation with the associated proteins may produce steric hindrance around the inhibitory site, or induce conformational changes of the catalytic subunits leading to impairment of regulatory functions of the liver enzyme.

PRS II has Val at position 4, whereas PRS I has Lys (*12*). From studies of mutated enzymes with a single point mutation and chimeric enzymes of rPRS I and rPRS II, we have proposed that Lys-4 of PRS I is critical for GDP inhibition with additional involvement of at least four other residues, Val-55 and/or Ala-81, and Arg-242 and/or Cys-264 of PRS I (*31*). The results obtained here raise the possibility that Lys-4 and/or two other residues of PRS I contribute to molecular interactions with associated proteins within the liver enzyme complex. Studies of the expression of PAP39 protein in *E. coli* are proceeding in our laboratory. Reconstitution experiments using recombinant catalytic subunits (rPRS I and rPRS II) and PAP39 will facilitate characterization of the protein–protein association and help to identify amino acid residues involved in the molecular interaction of these proteins.

Two isoforms of catalytic subunits have different regulatory properties and their relative amounts vary with

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tissues (*19*). Furthermore, expression of mRNAs of PAP39 and PAP41 is ubiquitous in rat tissues, and the relative amounts of mRNAs of PAPs to those of PRSs vary with tissues (*16, 18*). These observations suggest that the protein composition of PRPP synthetase complex can vary with tissues, leading to tissue-specific expression of the regulatory properties of the synthetase.

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